

UNIVERSITY OF GHANA, LEGON

COLLEGE OF BASIC AND APPLIED SCIENCES

**QUALITY EVALUATION OF ARTISANALLY PRODUCED HONEY
SOLD ON GHANAIAN MARKETS**

This thesis is submitted to the University of Ghana, Legon

By

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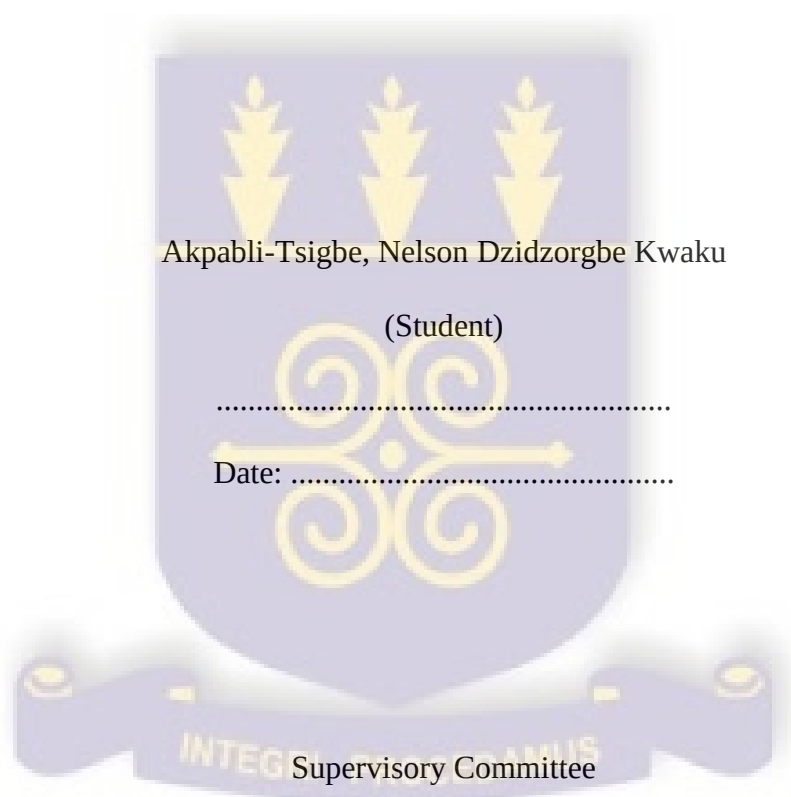
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Degree

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Akpabli-Tsigbe, Nelson Dzidzorgbe Kwaku under the supervision of Professor Kwaku Tano-Debrah and Dr. Angela Parry-Hanson Kunadu towards the award of Master of Philosophy Degree in Food Science in the Department of Nutrition and Food Science, College of Basic and Applied Sciences, School of Graduate Studies, University of Ghana, Legon.



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ABSTRACT

Honey is the most commercialized bee product in Ghana. It is utilized by all age groups and religious bodies with increasing demand. However, the production, packaging and handling has been associated with unsanitary and unhygienic practices with scanty documented information on production practices. Most of the artisanal honey are also mainly retailed in places where environmental conditions and handling practices do not meet fundamental food hygiene standards. This study sought to evaluate the quality of artisanally produced honey sold on Ghanaian markets. The study consisted of two parts: field surveys and laboratory experimentation. Three different surveys (on producers, sellers and consumers) were conducted to collect information on the artisanal production, selling and consumption pattern of artisanally produced honey in Ghana. Samples of artisanally produced honey were procured from some producers and consumer markets in the survey locations for microbial quality, antimicrobial activity and physicochemical analysis to authenticate their quality.

The surveys revealed that apiary and forest honey were the major honey types artisanally produced and sold on the Ghanaian markets. Smoking and/or applying of fire and straining of honey during production were identified critical for desired quality of the final honey product. Majority of the producers, as well as the sellers, package, store and sell honey in recycled packaging materials. Honey adulteration was perceived by the producers, sellers and consumers in the honey value chain. Taste, thickness and flavour were important indices of quality for the sellers and consumers. The survey data showed that the artisanal honey producers' formal educational status ($p = 0.026$) and major occupation ($p = 0.000$) were significantly associated with how honey quality was

controlled during production. Most (87 %) of the artisanal honey producers were subsistence farmers and majority (67 %) had only basic education. However, honey production was a secondary occupation for all.

All the artisanal honey recorded counts for total mesophilic bacteria ($3 \pm 1 \log_{10}\text{CFU/g}$), however, approximately 13 % of the artisanal honey had total mesophilic bacteria counts higher than the established maximum limit ($4.00 \log_{10} \text{CFU/g}$). The rate of detection of *Salmonella* was high among 19 % of the artisanal honey samples. Nevertheless, total coliform, faecal coliform and *Escherichia coli* were not detected in the artisanal honey. All the artisanal honey exhibited high inhibition to *Escherichia coli* and *Staphylococcus aureus*.

Significant ($p = 0.00$) variations occurred in the physicochemical parameters of the artisanal honey. Majority of the artisanal honey were dark amber ($264 \pm 59 \text{ mm}$) indicative of presence of higher levels of polyphenols and mineral contents. They had low pH (4.5 ± 0.5) and showed some level of temperature abuse (with mean Hydroxymethylfurfural = $17 \pm 6 \text{ mg/kg}$). Most of the artisanal honey had high water activity (0.63 ± 0.03) and were produced from nectar without mixture compounds of honeydews and nectar blossom with mean electrical conductivity value of $0.4 \pm 0.4 \text{ mS/cm}$. All the artisanal honey varied from Grade A to D with majority being Grade B (with mean moisture content of $19 \pm 2 \%$). Improvements in hygienic production and post-production handling of artisanal honey as well as proper and hygienic packaging before selling is recommended.

Key words: Artisanal honey, Antimicrobial activity, Microbiological and physicochemical characteristics of honey, honey production, selling and consumption.

DEDICATION

This research is dedicated to the Almighty God for His unending love, favour, mercies, strength and wisdom He accorded me, and made all things possible; to my lovely dad, Mr. Nathaniel K. Tsigbe; sweet lovely mum, Mrs. Dora A. A. Kpamah-Amenuvor; brothers, Mr. Michael H. Tsigbe, Mr. Wisdom K. Tsigbe, sister, Miss Beauty E. Tsigbe and to Professor Kwaku Tano-Debrah for the blessing you have been.



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LIST OF ABBREVIATIONS

AC	Available Carbohydrate
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
BADD	BioWarfare Agent Detection Device
BPW	Buffered Peptone Water
CAA	Código Alimentario Argentino
CFU	Colony Forming Unit
CI	Confidence Interval
EC	Electrical Conductivity
EEC	European Economic Community
EMBA	Eosin Methylene Blue Agar
EU	European Union
FA	Free Acidity
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FDA	Food and Drug Authority
GC/MS	Gas Chromatograph/Mass Spectrometry
GMPs	Good Manufacturing Practices
GT	Greenwich Meridian Time
HMF	Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
IHC	International Honey Commission
IQ	Intelligent Quotient

log ₁₀	Logarithm to base ten
LSD	Least Significant Difference
Max	Maximum
MC	Moisture Content
MERCOSUR	Mercado Común del Sur
MIC	Minimum Inhibition Concentration
Min	Minimum
MT	Metric Tons
nd	Not Detected
PC1	First Principal Component
PC2	Second Principal Component
PC3	Third Principal Component
PCA	Plate Count Agar
PCA	Principal Component Analysis
PDA	Potato Dextrose Agar
RI	Refractive Index
SCs	Sulfite-reducing <i>Clostridium</i> spores
SPSS	Statistical Package for Social Sciences
SSA	<i>Salmonella Shigella</i> Agar
TMB	Total Mesophilic Bacteria
TS	Total solids
TSS	Total Soluble Solids
U.S.	United States
USA	United States of America

USAID	United States Agency for International Development
VRBA	Violet Red Bile Agar
_a W	Water activity

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background statement

Honey is a natural sweet substance, produced by honey bees of the genera *Apis* (specifically *Apis mellifera*) and *Melliponini* from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects (hemipterous insects – aphids, leaf hoppers and scale insects) on the living parts of plants (Arvanitoyannis *et al.*, 2007). The bees collect, transform and combine these materials with specific substances of their own; deposit, dehydrate, store and leave in honey combs to ripen and mature. Honey has numerous benefits as food, medicine and cosmetic. It provides nutrients, boosts energy, metabolizes alcohol, perceived to boost memory, potentially prevents reduction in white blood cell count, prevents cancer and heart diseases, reduces incidence of ulcers and other gastrointestinal disorders (Bardy *et al.*, 2008; Bogdanov *et al.*, 2008; Ajibola *et al.*, 2012). It is also reported to inhibit antibiotic-resistant bacteria, treat wounds, reduce cough and throat irritation, resolve scalp problems and dandruff, relieve seasonal allergies (Khan *et al.*, 2007; Bogdanov *et al.*, 2008; Viuda-Martos *et al.*, 2008; Manyi-Loh *et al.*, 2011; Bagde *et al.*, 2013; Burlando and Cornara, 2013; Vallianous *et al.*, 2014) among others.

In Ghana, honey is a commodity used in several ways as a food, sweetener and flavouring in beverages and confectionary, cosmetic agent and as a medicine. Honey 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. It is also used by pharmaceutical industries in drug formulation. Generally, utilization of honey in Ghana is on the rise (Akangaamkum *et al.*, 2010).

Two types of honey are thought to be produced in Ghana, namely: Apiary honey and Forest honey. Production involves harvesting and extraction of honey from honey combs, straining of the extracted honey to remove some debris and then packaging. In the artisanal process, very simple minimally specialized equipment, such as honey press, bee smoker, “akakatsi” (pieces of dried palm fronds tied together), nylon net (sieve), and plastic/rubber or aluminium containers are used. Hand-squeezing, solar extraction and cold extraction methods are the main extraction methods used. These methods used tend to influence the characteristics of the honey, in terms of physicochemical and microbiological qualities, which influence their acceptability. Harvesting of honey combs with “akakatsi” implies use of naked fire, which for instance darkens the colour of the honey through maillard reactions. The conditions of extraction, straining and packaging most are generally likely to cause physical and microbial contamination. For instance, recycled containers are often used to package honey in the artisanal industry.

There is the general impression that honey on the markets is adulterated. Honey vendors therefore advertise products emphasizing their purity. Adulteration with melted Styrofoam, wax, sugar and water during processing is reported. Adulteration of honey had been common among artisanal honey production and retail honey in Ghana (Ankrah, 1998). Li *et al.* (2012) suggested incidence of adulteration of honey. In honey retailing, it is also common to find in the open markets, some honey sellers displaying honey in uncovered containers in the open, with flies hovering around them in Ghana.

1.2 Justification

Against the background presented, there shall always be the notion that artisanally produced honey sold on Ghanaian markets are of poor quality. They may be adulterated, may have poor physicochemical properties and hence poor functionality, or they may not even be safe. Unfortunately, not much has been reported on the processes and operations of artisanal honey production and quality criteria for artisanally produced honey in Ghana. Some of the few works done on the Ghanaian artisanal honey were on the chemical composition (moisture, nitrogen, reducing sugars, total reducing sugars, sucrose, calcium and iron) by Ankrah (1998) and an overview of the honey industry looking at the value chain (Akangaamkum *et al.*, 2010). Currently, there are no specific national regulatory provisions for the quality of honey in Ghana; such that beekeepers and honey processors rely heavily on their own experience and observation to determine whether their products meet customer expectations. The need to investigate the quality characteristics of honey produced and sold in the Ghanaian markets is thus, necessary to provide basis for any intervention that will improve the artisanal honey industry.

1.3 Purpose of the study

The key research questions the study sought to answer were:

- i. What are the characteristics of the artisanal honey production industry in Ghana?
- ii. What is the status of the quality of artisanally produced honey on the markets?
- iii. Does the quality of Ghanaian artisanally produced honey meet set standards for foods by national and international food regulatory provisions?
- iv. Is there any diversity in the types of Ghanaian artisanally produced honey?

1.4 Study objectives

1.4.1 Main objective

The main objective of this research is to evaluate the quality and characteristic properties of artisanally produced honey sold on the Ghanaian markets.

1.4.2 Specific objectives

The specific objectives of the research were as follows:

- i. Evaluate artisanal production, marketing and utilization of honey in Ghana.
- ii. Determine the microbiological quality of artisanally produced honey on the Ghanaian markets.
- iii. Determine the physicochemical quality of artisanally produced honey on the Ghanaian markets.

1.5 Expected outcome

The main outcomes of the research shall be:

- a) Establishment of the quality of artisanally produced honey on the Ghanaian market.
- b) Establishment of the diversity in honey produced artisanally in Ghana.
- c) Documentation of how honey is artisanally produced, marketed and utilized in Ghana.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Honey as a food commodity

Honey is primarily a high-energy carbohydrate food. Andrew *et al.* (2004) revealed that honey is made up of simple sugars mainly fructose, glucose, maltose and raffinose among others. These sugars provide readily calories when honey is consumed, hence as a food, honey is a good source of energy. Honey is spoken of by all religious books, and accepted by all generations, traditions and civilizations, both ancient and modern (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). According to Akhmazillah *et al.* (2013), honey has been used as food for at least six thousand (6000) years. Jeffrey and Echazarreta (1996) reported that honey is medicine, often regarded as a special tonic food to be eaten during illness. Honey has an important place in traditional food preparation in many societies.

Honey is largely used in baked products, confectionary, candy, marmalades, jams, spreads, breakfast cereals, beverages, milk products and many preserved products (Krell, 1996). Kime and Lee (1987) and Gupta and Sharma (2009) 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. Its many

applications in processed foods and pharmaceuticals make honey versatile and its use on the rise.

2.2 Sources of honey

Two main sources of honey are known: honey from nectarines of flowers and honey from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of the plants (Ouchemoukh *et al.*, 2007). O'Todle and Raw (1991) called the honey from nectarines of flowers blossom honey, nectar honey or floral honey and the honey from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of the plants honeydew honey. Blended or compound honey is a mixture of blossom and honeydew honey. Floral honey are further classified into two: monofloral honey (honey foraged from flowers of one particular plant vegetation) and multifloral honey (honey foraged from flowers of different plants). As the names suggest, monofloral honey has pollen from one plant vegetation while multifloral honey has mixed pollen from different plants.

2.3 Composition of honey

The composition of honey according to Ramírez and Montenegro (2004) is that of a plant product and it is influenced by the sources of materials, the weather and processing of the honey itself. The overall composition of honey is shown in Table 2.0 below.

Table 2.0: Honey Composition (in g/100 g)

Parameter	Blossom honey		Honeydew honey	
	Average	Min - Max	Average	Min - Max
Water	17.2	15 – 20	16.3	15 – 20
Monosaccharides				
Fructose	38.2	30 – 45	31.8	28 – 40
Glucose	31.3	24 – 40	26.1	19 – 32
Disaccharides				
Sucrose	0.7	0.1 – 4.8	0.5	0.1 – 4.7
Others	5	2.0 – 8.0	4	1.0 – 6.0
Trisaccharides				
Melezitose	< 0.1		4	0.3 – 22.0
Erllose	0.8	0.5 – 6.0	1	0.1 – 6.0
Others	0.5	0.5 – 1.0	3	0.1 – 6.0
undetermined oligosaccharides	3.1		10.1	
Total sugars	79.7		80.5	
Minerals	0.2	0.1 – 0.5	0.9	0.6 – 2.0
Amino acids, protein	0.3	0.2 – 0.4	0.6	0.4 – 0.7
Acids	0.5	0.2 – 0.8	1.1	0.8 – 1.5
pH-value	3.9	3.5 – 4.5	5.2	4.5 – 6.5

Source: (White, 1975; Bogdanov et al., 2003; Bogdanov et al., 2008)

2.4 Classification of honey

Fundamentally, there are two main types of honey: apiary honey and forest honey. 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 bees *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 exist. These include blossom honey, honeydew honey, monofloral honey and polyfloral honey (O'Todde and Raw, 1991; Ouchemoukh *et al.*, 2007). Physical characteristics especially viscosity is also used to classify honey: thixotropic honey (gel-like – extremely viscous when standing still and turn liquid when agitated or stirred) and dilatancy honey (viscosity increases with agitation) (Munro, 1943; White, 1975; White, 1978; Yanniotis *et al.*, 2006; Chen *et al.*, 2009).

2.4.1 Physical characteristics of honey

According to Gangwar *et al.* (2010), physical characteristics of honey form the main basis of classification. Their measurement is comparatively simple and has a good information value. The physical characteristics of honey, such as high viscosity and density, consistency and sweetness are due to the high sugar concentration. Previous studies (White, 1978; White and Maher, 1980; Barth, 1989) identified the diversity of the physical characteristics of honey to be dependent on the nectar and pollen of the original plant, colour, flavour, moisture and contents of proteins and sugars. The physical

parameters used to characterize honey are viscosity, density, hygroscopicity, surface tension, thermal properties, colour, crystallization and pH.

2.4.1.1 Viscosity

Viscosity is one of the most important physical and sensory characteristics of honey, which influences the quality of the product (Yanniotis *et al.*, 2006). Honey of high quality is usually viscous. Some authors (James *et al.*, 2009) stated that the viscosity of honey is a function of the composition of its sugars, water and colloid content. It is also influenced by temperature, moisture content, as well as the presence of crystals and colloids in the product (Yanniotis *et al.*, 2006).

Some honey, however, display peculiar viscosity characteristics. For example heather honey (*Calluna vulgaris*), buckwheat honey (*Fagopyrum esculentum*), white clover honey (*Trifolium repens*), New Zealand manuka honey (*Leptospermum scoparium*) and Indian Karvi honey (*Carvia callosa*) have shown thixotropic behaviour (that is they are gel-like – extremely viscous when standing still and turn liquid when agitated or stirred), while dilatancy (meaning viscosity increases with agitation) has been detected in Nigerian honey (*Opuntia engelmanni*) and several eucalyptus honey (example *Eucalyptus fisifolia*) (Munro, 1943; White, 1975; White, 1978; Yanniotis *et al.*, 2006; Chen *et al.*, 2009).

2.4.1.2 Density

The density of honey is an important physical property that influences stratification in honey. Density of honey is greater than the density of water when expressed as specific gravity, and depends on the water content of the honey (White, 1975a; Nanda *et al.*,

2003). Therefore, when honey with different specific gravity are pulled into a storage tank, distinct stratifications may form based on their density variations. These stratifications can be avoided by thorough mixing. The degree of mixing and the final moisture content of honey in storage tanks depend on the individual specific gravity of different types of honey (Moar, 1985).

2.4.1.3 Hygroscopicity

Hygroscopicity is the ability of a substance to absorb and hold moisture from the surrounding. Honey is very hygroscopic. The strongly hygroscopic nature of honey is vital both in processing and for final use. This tendency to absorb and hold moisture is often a desired effect in end products containing honey such as in pastry and bread. However, the same hygroscopicity of honey can become problematic during processing or storage, causing difficulties in preservation and storage due to excessive water content. Normal honey with a moisture content of 18.3 % or less will absorb water (moisture) from the air at a relative humidity of above 60 % (White, 1975a). Hygroscopicity is expressed as a percentage of moisture absorbed by honey.

2.4.1.4 Surface tension

Surface tension is another physical property of honey of great importance and largely applied in cosmetics. Honey has a low surface tension. This property of honey makes it an excellent humectant in cosmetic products. The surface tension varies with the origin of the honey and is probably due to colloidal substances. Together with high viscosity, it is responsible for the foaming characteristics of honey (Krell, 1996).

2.4.1.5 Thermal properties

To design processing plants for honey, the thermal properties of honey have to be taken into account. According to White (1975a), the heat absorbing capacity (specific heat) of honey, varies from 0.56 to 0.73 cal/g/⁰C with its composition and state of crystallization. The same author also reported that the thermal conductivity of honey varies from 118 to 143 x 10⁻³ cal/cm²/sec/⁰C. The relatively low heat conductivity, combined with high viscosity leads to rapid overheating from point-heat sources and thus the need for careful stirring and for heating only in water baths.

2.4.1.6 Colour

Honey colour varies with botanical origin, age and storage conditions, but transparency or clarity depends on the quantity of suspended pollen. According to Hellena and Teresa (1995), liquid honey colour varies from clear and colourless to dark amber or black. Honeydew honey are mostly dark-brown whereas floral honey vary from clear, colourless to brown and may darken if stored for long even at 27 °C (White *et al.*, 1962). Bertoneclicj *et al.* (2007), studied the relationship between colour parameters and antioxidant activity in natural Slovenian honey and reported light honey (example acacia) to be least active and dark honey to be most active against 2,2-diphenyl-1-picryl-hydrazyl (DPPH). Socha *et al.* (2009) also conducted a study on ten (10) herb honey and reported their flavonoid content appearing to be closely connected with colour. Herb honey with strong red or green colours (from raspberry, black chokeberry, thyme) had high flavonoid content, while those with pale yellow or pale green colours (camomile, mint) contained a small amount of these compounds. The various honey colours are fundamentally all nuances of yellow amber, like different concentrations of caramelized sugar, which traditionally has

been used as a colour standard. Less common honey colours are bright yellow (sunflower), reddish undertones (chestnut), greyish (eucalyptus) and greenish (honeydew).

Crystallized honey turns lighter in colour because the glucose crystals are white. Some of the honey reported "as white as milk" in some parts of East Africa are finely crystallized honey which are almost colourless (water white), in their liquid state. Honey colour can be estimated using instrumental analysis and Hunter Lab colorimeter ($L^* a^* b^*$ method) is one of the instruments often used. However, more contemporary methods for honey colour description or measurement are the Pfund scale method and spectral colour absorption method. Pfund scale method is an optical density reading method generally used in international honey trade and often measured in millimetres according to the U.S. Department of Agriculture classifications (White, 1975c; Crane, 1980). Spectral Colour absorption methods are not widely practiced (Aubert and Gonnet, 1983; Rodriguez López, 1985).

The most important aspect of honey colour lies in its value for marketing and determination of its end use. Darker honey are more often for industrial use, while lighter honey are marketed for direct consumption. In many countries with a large honey market, the colour of honey (used as an indication of a preferred flavour) determines consumer preferences, thus, used as an important quality index (Mathew, 2004). The lighter honey are milder in flavour while the darker honey are stronger in flavour and higher in minerals and proteins (Patricia, 1996). Honey colour is the single most important factor determining import and wholesale prices.

2.4.1.7 Crystallization

Crystallization is another vital characteristic for marketing honey, although it is not used as an index for price determination. Conforti *et al.* (2006) identified crystallization of honey as a complex phenomenon, and a matter of interest for beekeepers, honey handlers, and processors. Almost all honey are in liquid form as stored by the bees in the comb, but after beekeepers extract it from the comb, many honey crystallize within a few days or weeks. While some monofloral honey are naturally finely and homogeneously crystallized (example citrus), for most commercial honey, crystallization is a defect. According to Venir *et al.* (2010), spontaneous granulation in honey can lead to undesired coarse crystals formation and may cause quality loss due to phase separation, sedimentation and increment of water activity up to levels which may be suitable for microbial fermentative processes. Studies (White, 1974; Assil *et al.*, 1991; Bhandari *et al.*, 1999) reported that crystallization causes honey to be cloudy when it occurs in an undesired and uncontrolled fashion during storage, and less appealing to the consumer. There is a general consumer perception that, if honey crystallizes, it means that it has gone bad or has been adulterated with sugar.

Glucose and fructose in honey are present in about equal concentrations but the lower solubility of glucose enables it to be the crystallizing sugar. According to Young (1957), crystallization of honey results from the formation of monohydrate glucose (α -D-glucose monohydrate) crystals, which vary in number, shape, dimension and quality with the honey composition and storage conditions. The lower the water and the higher the glucose content of honey, the faster the crystallization. Temperature is very important in honey crystallization. Most honey crystallize at normal storage temperatures in temperate

climates. Venir *et al.* (2010) reported the transition temperature from glucose monohydrate to anhydrous glucose in solutions saturated with fructose to be below 30 °C. The optimum (or maximum) temperature for fast crystallization is around 14 °C (Assil, *et al.*, 1991). The presence of solid particles (example pollen grains) and slow stirring also result in quicker crystallization. Usually, slow crystallization produces bigger and more irregular crystals.

Water is released during crystallization. As a result, the increased water content of the liquid phase increases the risk of fermentation. Also, partially crystallized or reliquified honey is not attractive for retail shelves. This is why controlled and complete crystallization is often induced deliberately.

2.4.1.8 pH

pH, another physical characteristic of honey is of great importance. It influences the texture, stability and shelf-life of honey during extraction and storage (Terrab *et al.*, 2002). 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 (Figures 2.0 and 2.1). According to Bardy *et al.* (2008), most honey are acidic with pH ranging from 3.2 to 4.5 which is inhibitory to most neutrophilic bacteria. IHC (2002) recommended an average of 3.9. Most acids are added by the bees (Echigo and Takenaka, 1974).

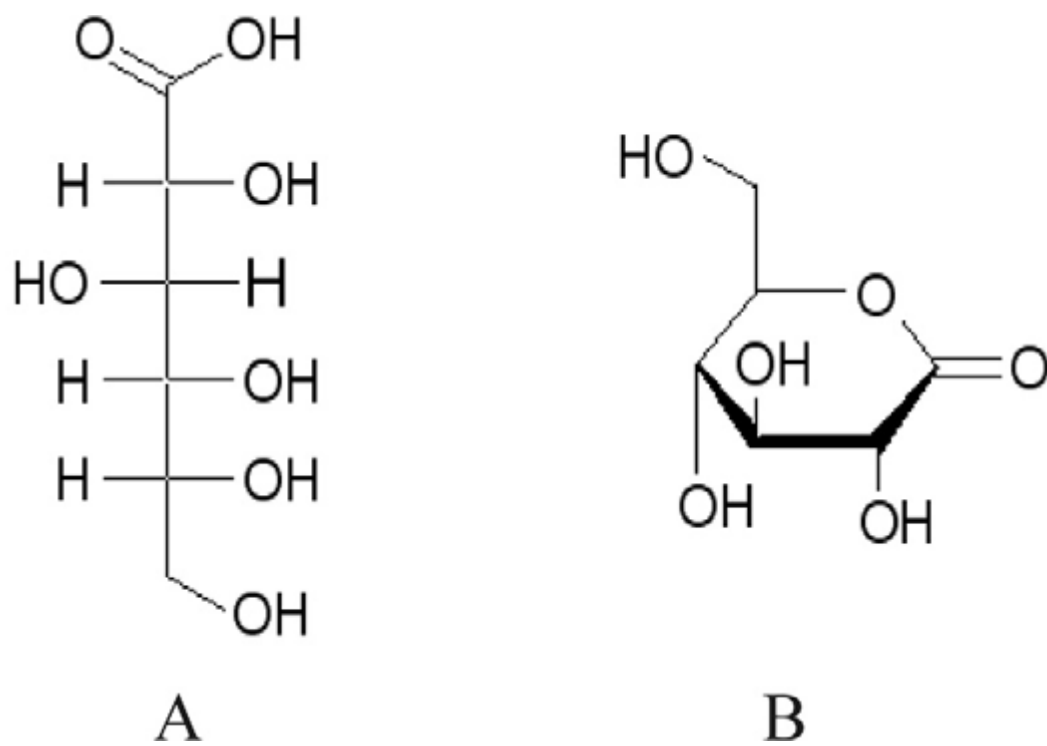


Figure 2.0: Structural of formula of gluconic acid (A) and glucono- δ -lactone (B).

Source: Ramachandran et al. (2006)

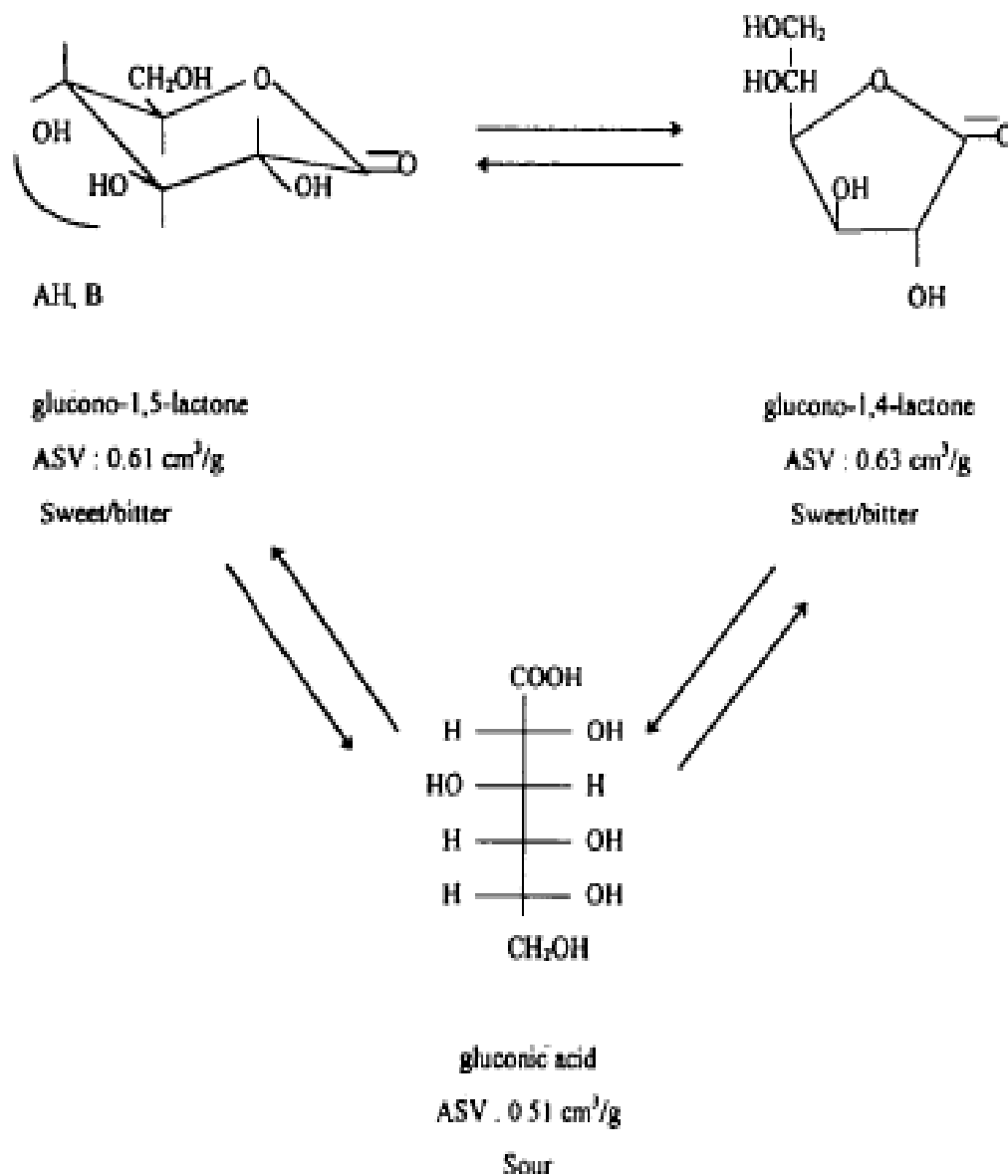


Figure 2.1: D-Glucono-1, 5-lactone, D-gluconic acid and D-glucono-1, 4-lactone system.

Source: Parke et al. (1997)

2.5 Nutritional characteristics of honey

Honey has high nutritional profile and health-related attributes. It is often consumed as a healthy alternative sweetener to sugar in food and beverages. Though, the dietary contributions of individual components to the recommended daily intake are insignificant, their nutritional significance resides in their physiological effects, since they exhibit numerous health-related properties (Alvarez-Suarez *et al.*, 2012; Sant'Ana *et al.*, 2012). Its beneficial role has been attributed to its antimicrobial, anti-inflammatory and anti-oxidant activities as well as boosting of the immune system. Honey clears infection through stimulating the body's immune system to fight infections. Manyi-Loh *et al.* (2011) reported the stimulation of monocytes in cell cultures to release the cytokines TNF-alpha, IL-1 and IL-6, the cell "messengers" that activate the many facets of the immune response to infection. Moreover, honey provides substrates for glycolysis, the major mechanism for energy production in the macrophages, and thus allows them to function in damaged tissue and exudates where the oxygen supply is often poor. It is also rich in micronutrients (Manyi-Loh *et al.*, 2011). In addition, honey provides a supply of glucose, which is essential for the "respiratory burst" in macrophages that produce hydrogen peroxide, the dominant component of their bacteria-destroying activity (Molan, 2001). Honey is also rich in micronutrients. Table 2.1 below shows the nutrients in honey in relation to human requirements.

Table 2.1: Nutrients in honey in relation to human requirements

Nutrient	Unit	Average amount in 100 g honey	Recommended daily intake
Energy equivalent	kcal	304	2800
Vitamins			
A	I.U.	-	5000
B1 (Thiamin)	mg.	0.004 – 0.006	1.5
B2 (Riboflavin)	mg.	0.002 – 0.06	1.7
Nicotinic acid (niacin)	mg.	0.11 – 0.36	20
B6 (Pyridoxine)	mg.	0.008 – 0.32	2.0
Pantothenic acid	mg.	0.02 – 0.11	10
Bc (Folic acid)	mg.	-	0.4
Vitamins			
B12 (Cyanocobaltamine)	mg.	-	6
C (Ascorbic acid)	mg.	2.2 – 2.4	60
D	mg.	-	400
E (Tocopherol)	I.U.	-	30
H (Biotin)	I.U.	-	0.3
Minerals			
Calcium	mg.	4 – 30	1000
Chlorine	mg.	2 – 20	-
Copper	mg.	0.01 – 0.1	2.0

Source: (Crane, 1980; Krell, 1996)

Table 2.1: Continuation

Nutrient	Unit	Average amount in 100 g honey	Recommended daily intake
Minerals			
Iodine	mg.	-	0.15
Iron	mg.	1.0 – 3.4	18
Magnesium	mg.	0.7 – 13	400
Phosphorous	mg.	2 – 60	1000
Potassium	mg.	10 – 470	-
Sodium	mg.	0.6 – 40	-
Zinc	mg	0.2 – 0.5	15

Source: (Crane, 1980; Krell, 1996)

2.6 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.

2.6.1 Source of income for households

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, labour and time. It serves as a source of cash incomes for many households (Shenkute *et al.*, 2012; 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).

2.6.2 Pollination

Honey production plays major role in improving biodiversity and increasing crop production through pollination. In addition to gathering nectar to produce honey, 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, thus fertilizing the plants and enabling them to bear fruit. According to vanEngelsdorp and Meixner (2010), fifty-two (52) of the one hundred and fifteen (115) leading global food commodities depend on honey bee pollination for either fruit or seed set. Almonds, apples, avocados, blueberries, cantaloupes, cherries, cranberries, cucumbers, sunflowers,

watermelon and many other crops all rely on bees for pollination. vanEngelsdorp and Meixner (2010) reported that some honey bee dependant commodities would have greater or equal to ninety per cent ($\geq 90\%$) yield reduction without honey bees.

2.7 Global production, statistics and demand of honey

Generally, honey production has been identified as one of the most lucrative enterprise in many parts of the world, so much in use and consequently in demand that it can be termed a money spinner. 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. According to Famuyide *et al.* (2014), about 109,799,366.60 kg of honey worth \$ 24,200,000.00 is produced in the United States each year. 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; Tornuk *et al.*, 2013). 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.

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 (FAO, 2010; Getachew *et al.*, 2014). According to Serem and 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.

Honey is consumed all over the world. According to Batt and Liu (2012), USA is 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. However, consumption has been relatively stable over the last five (5) years (Batt and Liu, 2012).

2.8 Technology of honey production

Honey production is a complex biological process comprising of two different stages: obtaining of floral nectar by the bees (first stage) and transformation of nectar by the bees and the storage in the combs to ripen into sweet honey (second stage) (Aparna and Rajalakshmi, 1999). The sweet honey, once formed, remains in the combs until is extracted. The extraction of honey involves separation of the honey from the combs either by squeezing or by any other method. Honey extracted from combs obtained from the forest or the apiaries (beekeeping) contains pollens, beeswax, and other undesirable materials including yeast that needs to be removed for better product quality and shelf life. Hence, honey is processed before packaging (in bottles or other containers). The type of equipment used and steps followed in processing depend upon the scale of operation.

Subramanian *et al.* (2007) identified two vital stages of honey processing: filtration and heating. The separation of pollens, beeswax, and other materials are generally done through straining and pressure filtration (Subramanian *et al.*, 2007). The physical separation of undesirable, suspended matter is done before thermal processing of honey. Heat or thermal processing of honey eliminates the microorganisms responsible for spoilage and reduces the moisture content to a level that retards the fermentation process.

In Ghana, there is general lack of records and documentation on technology and/or method(s) of production (honey combs harvesting and honey extraction) of both raw apiary and forest honey and subsequent processing of the raw honey into pure honey. Correspondingly, globally, there is no or very little documented information on technology and/or method(s) of forest honey production in literature as well. However,

there is much literature on the technology and/or method(s) of processing of raw apiary honey into pure honey.

2.8.1 Technology of production of apiary honey

Apiary honey production involves two (2) steps: processing and purification. Generally, honey processing starts from comb selection (elimination of pieces of comb with pollen or even brood), removal of bees and other unwanted materials, comb harvesting and the subsequent thorough mashing of combs. However, in apiary honey processing, uncapping is the first real step consisting of the removal of the thin wax layer that seals the honey cells followed by extraction (using modern methods: solar extraction method, cold extraction method or centrifugal extraction method) of honey from the uncapped honey combs (Krell, 1996). The next step is the removal of impurities (such as wax particles, other debris and air bubbles) incorporated during extraction. Two practical techniques: settling and straining are used for removal of impurities in honey. Figure 2.2 below shows the conventional method of processing apiary honey after obtaining raw honey from the honey comb.

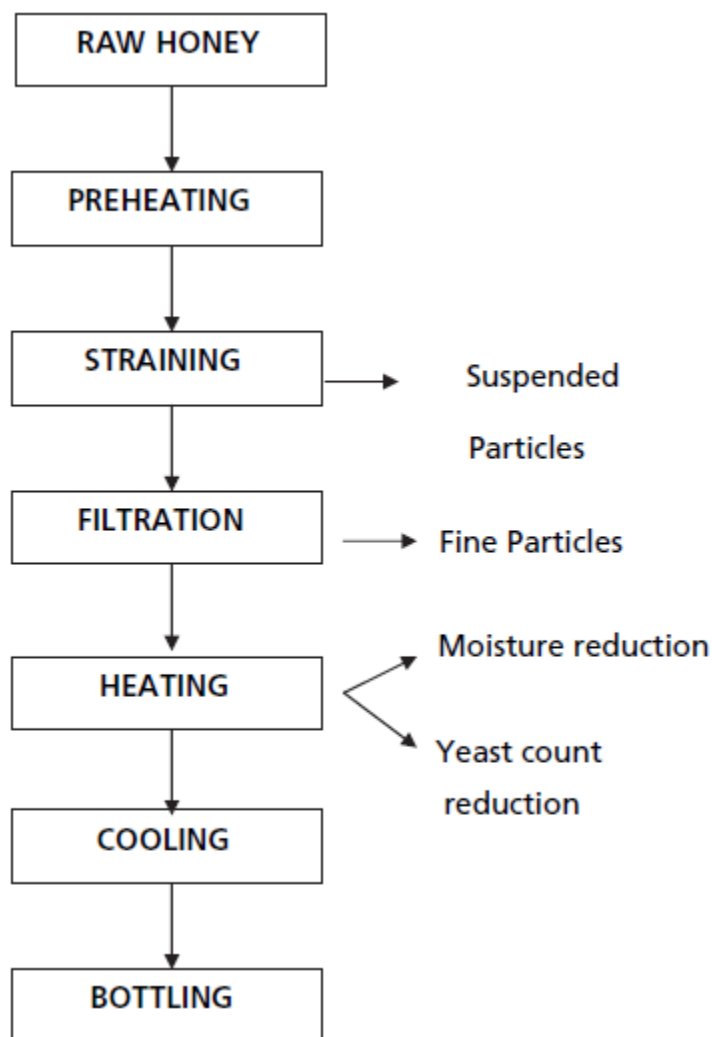


Figure 2.2: Conventional method of processing honey.

(Source: Subramanian et al., 2007)

2.8.1.1 Straining

Honey is strained to remove dead bees and other suspended particles in it. The straining operation to take away suspended solids is done either manually or by mechanical means. The method and the equipment used for straining depend on the size of the operation. In small-scale operations, straining is achieved using cloth or nylon bags, which are often cleaned to take away the suspended particles. In large-scale operations, the straining

operation is combined with the pre-heating (up to 40 °C) operation in a jacketed tank fitted with a stirrer (Subramanian *et al.* (2007).

2.8.1.2 Filtration

Honey is further processed using pressure filters by some industrial processors, an activity uncommon to artisanal honey processing. Characteristically a polypropylene micro-filter of 80 µm is used as a filter medium (Subramanian *et al.*, 2007). The temperature of honey is raised and maintained at 50 – 55 °C, which prevents the melting of the bees wax while filtering (Wakhle and Phadke, 1995). On large-scale production, processors subject honey to coarse filtration, centrifugal clarification, fine filtration, and blending, prior to filling (Subramanian *et al.*, 2007).

2.8.1.3 Heating

Honey is heat processed to modify or delay its tendency to crystallize (Tosi *et al.*, 2002), destroy contaminating microbes (Tosi *et al.*, 2002; Subramanian *et al.*, 2007), and reduces the moisture content to a level that retards the fermentation process (Subramanian *et al.*, 2007). Heating of honey is more often than not carried out in two different ways: in air-ventilated chambers at 45 – 50 °C for 4 – 7 days or in hot water (Fallico *et al.*, 2004; Turkmen *et al.*, 2006).

2.9 Quality indices of honey

Demands for honey, both internationally and locally, have necessitated various studies on quality of honey produced in different countries and setting of standards for quality control. The quality of honey is mainly determined by its sensory, chemical, physical and

microbiological characteristics (Finola *et al.*, 2007; Belay *et al.*, 2013). Honey physicochemical quality criteria are well specified by the EC Directive 2001/110 (EU, 2001) with the main criteria of interest being moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity and hydroxymethylfurfural (HMF) content. However, the Codex Alimentarius Standard for honey quality comprises of moisture content, mineral content, acidity, hydroxymethylfurfural (HMF) content, diastase activity, apparent sugar content, and water insoluble solids content. These analyses help determine the “chemical” and “physical” quality of honey.

Conversely, specifications concerning microbial and hygienic quality of honey are not available, neither in EU legislation nor Codex Alimentarius Standards. Many studies have been reported on the physical and chemical parameters of honey from all over the world but microbial contamination studies are rare and are essentially devoted to *Clostridium botulinum* (Gomes *et al.*, 2010). Current purchasing specifications for microorganisms in honey are often based on microbial specifications for other foods and ingredients. Due to honey’s unique properties, such as its anti-microbial activities, some of those specifications may not be germane to honey. Purchasing specifications currently in use include a standard plate count and tests for coliform bacteria, yeasts, moulds and certain pathogenic bacteria such as *Staphylococcus*, *Salmonella* and *Clostridium* species (Snowdon and Cliver, 1996). Microbiological specifications for honey by some companies are shown in Table 2.2 below.

Table 2.2: Examples of microbial purchasing specifications for honey

Company	Standard plate count	Assay ^a							Rope spore (sic)
		Coliform	<i>E. coli</i>	Yeast	Yeast and Mold	Mold	<i>Staphylococcus</i>	<i>Salmonella</i>	
A	1000	10	Negative	100		100	Negative	Negative	50
B	5000	10	<10	100		100	100		
C ^b	10000	<3	<0.3	100		100	<3	Negative	
D	10000	10	Negative				Negative	Negative	
E	10000	10	Negative		10		Negative	Negative	
F	10000	100	Negative		200		Negative	Negative	
G	1000	10	10	100		100	100		
H	10000	10	Negative	100		100	Negative	Negative	50
I	10000	10	Negative	100		100	Negative	Negative	50
J ^c	5000	10	Negative	10		10			

^aColony forming units per gram.

^bAlso negative for *Clostridium botulinurn* and *C. perfringens*.

^cAlso, 10 cfu/g of mesophilic and thermophilic aerobes and anaerobes.

(Source: Snowdon and Cliver, 1996)

2.9.1 Factors that influence quality of honey

On the whole, the physicochemical and organoleptic quality of honey is influenced by sugar content, maturity (ripe) and presence of active compounds such as aliphatic and aromatic alcohols, aldehydes, acids and their esters (Pucciarelli *et al.*, 2014). These active compounds give special properties and define the honey flavour (Aparna and

Rajalakshmi, 1999; Bhandari *et al.*, 1999). Presence of micro-organisms also influences stability and hygienic quality of honey.

Microbes of concern in honey are fungi and spore-forming bacteria. Amid these microorganisms, the predominant ones are the osmophilic yeast, principally the strains of *Saccharomyces*, *Schizosaccharomyces* and *Torula* (Migdal *et al.*, 2000), responsible for honey fermentation when the moisture content is high (above 21 %). Work done by other authors, (White, 1975a; Zander and Koch, 1975; Frazier and Westhoff, 1978; Kędzia *et al.*, 1996) revealed that aerobic *Bacillus* and anaerobic *Clostridium* spores as well as spores and small fragments of moulds may appear in honey.

2.9.2 Methods for assessing quality of honey

Different methods have been proposed for assessing quality of honey. In spite of that both researchers and Regulatory Authorities are still searching for newer, simpler, more sensitive and more economical procedures (Ruiz-Matute *et al.*, 2010). According to Zhu *et al.* (2010), 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 (Padovan *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

The study was in two phases:

The first phase was a cross-sectional survey on artisanal production, retail and consumption of honey in Ghana. It involved administration of three different sets of semi-structured questionnaires; one to honey producers, one to vendors and the other to consumers. Information on demography, how they farm for or obtain (produce) honey, how they package honey, why they farm/produce honey, honey utilization, marketing volume/scale of operations, consumption, quality evaluation, among others, were solicited depending on the objective of the survey. Two hundred and sixty (260) consumers from Ho (honey producing community) and Accra (non-honey producing community), fifty (50) honey retailers from different markets in Accra (a regional capital with large central markets patronized by several consumers in the Accra Metropolitan Assembly) and thirty (30) honey producers made up of fifteen (15) apiary-type honey producers and 15 forest-type honey producers identified from different honey producing communities (Somanya, Dzolokpuita, Dzologbogame, Ho and Nkwanta) were interviewed. Presented in appendices A1 to A3 are the questionnaires used.

The second phase was on sampling and laboratory analyses of artisanally produced honey sold on Ghanaian markets. Microbiological and physicochemical analyses were done. Microbiological analyses involved determination of general microbial counts (total mesophilic bacteria count, total coliform count, and faecal coliform count) as well as *Escherichia coli* count, *Salmonella* count, sulphite-reducing *Clostridium* spores count and

presence of *Botulinum* toxin in honey samples collected from producers and vendors. In addition, the antimicrobial activity of artisanal honey was evaluated. The physico-chemical analyses involved determination of refractive index, moisture content, water activity, colour, total soluble solids (TSS), total solids (TS), pH, electrical conductivity (E.C.), ash content, hydroxymethylfurfural (HMF) and free acidity.

3.2 Survey

3.2.1 Consumer survey

The number of consumers, n , was determined using the following formula from Moore and McCabe (1993):

$$n = (Z_{\alpha/2}/2m)^2$$

Where:

$$Z_{0.025} = 1.96$$

$$m \text{ (margin of error)} = 6\%$$

$$\alpha = 0.05$$

$$C.I = 95\%$$

Using the indicated margin of error, m , level of significance, α , and confidence interval, C.I, n was calculated as:

$$n = [(1.96/0.12)^2]$$

$$n = 16.3333^2$$

$$n \approx 16^2$$

$$n = 256$$

This number ($n = 256$) was increased to 260 in order that 130 respondents each could be drawn from the two communities (Ho and Accra). The inclusion criteria were that prospective respondents be consumers and do not have any allergic reactions to honey.

3.2.2 Marketing survey

Fifty (50) vendors from informal markets, street hawkers and pharmaceutical shops were selected by convenience and interviewed with semi-structured questionnaires on their methods of marketing or selling, packaging, storage and general handling practices. The interviews were conducted at the market place to enable observation of the methods and practices they describe.

3.2.3 Producer survey

Fifteen (15) producers each of apiary honey producers and forest honey producers were randomly identified by convenience through an association (Federation of Ghana Beekeepers Association) and interviewed with semi-structured questionnaires on their methods of production, packaging, storage, marketing and general honey handling practices. The interviews were conducted in their various homes.

3.3 Sampling for microbiological and physicochemical analysis

Honey samples were obtained randomly both from the markets and directly from honey producers (beekeepers and those that harvest from the wild – specifically from Volta and Eastern part of Ghana) and transported to the laboratory. The samples were collected (as packaged for sale) during the coolest part of the day thus morning (05:30 – 06:30 GT) from honey producers and evening (16:00 – 18:00 GT) from sellers in the markets where

the temperature was around 25 °C to avoid temperature abuse which can facilitate some chemical reactions. Sixteen (16) different honey samples, made up of ten (10) honey samples from 10 different local markets (Madina market, Dome market, Achimota market, Ashaiman market, Nima market, Timber market, Kaneshi market, Malam Ata market, Agbogloboshi market, Makola market – one honey sample from each market) in Accra, the capital of Ghana and 6 honey samples from 6 different producers (3 apiary honey producers and 3 forest honey producers randomly selected) were collected for analyses. Collected samples were stored in a cool place at ambient (normal room) temperature (25 °C) in the laboratory until further analysis. All microbiological analyses were done at least in duplicates and triplicates for all physicochemical analyses. All honey samples were collected in May 2014.

3.4 Analytical methods and statistical analysis

3.4.1 Microbiological analyses

3.4.1.1 Determination of microbiological quality

To assess the microbiological quality of the honey samples collected, the commercial quality parameters (mesophilic microorganisms, yeasts and moulds), the indicators of sanitary quality (faecal coliforms and *Escherichia coli*) and the indicators of safety (sulphite-reducing *Clostridium* spores and *Salmonella*) were analysed using the methods outlined in Nogueira *et al.* (2012) with modifications. All microbial tests were performed in duplicate.

3.4.1.1.1 Sample preparation

Sample preparation was carried out according to Instituto Português da Qualidade (1982). Ten grams (10 g) of each honey sample was weighed on a scale (Mettler PC Model 2000), and homogenized with 90 ml of 0.1 % peptone water (dilution 10^{-1}) (PARK SCIENTIFIC LIMITED; M0216) using Seward Stomacher, Serial No. 44453, Model 400 Circulator, England. For each honey sample, decimal dilutions (10^{-2} and 10^{-3}) were prepared using 0.1 % peptone water as diluents.

3.4.1.1.2 Enumeration of total aerobic mesophilic bacteria

Enumeration of aerobic mesophilic bacteria was made on Standard Plate Count Agar (PCA) (Biolab, MERCK; Art No: HG0000C6.500), incubated at 37 °C for 48 hours (Instituto Português da Qualidade, 2002). Microbial counts were expressed as colony-forming units per gram of honey (cfu/g).

3.4.1.1.3 Enumeration of yeast and moulds

Yeasts and moulds were enumerated on Potato Dextrose Agar (PDA) (OXOID; CM0139), incubated at 25 °C for 3 – 5 days according to a modification of the method suggested by ICMSF (1983). Microbial counts were expressed as cfu/g.

3.4.1.1.4 Enumeration of total coliforms, faecal coliforms and *Escherichia coli*

The presence of total coliforms, faecal coliforms and *E. coli* in honey was determined as follows: Total coliforms were enumerated on standard violet red bile agar (VRBA) (OXOID; CM0107), incubated at 37 °C for 24 hours, according to a modification of the method suggested by ICMSF (1983). Faecal coliforms in each honey sample were

enumerated on Eosin methylene blue agar (EMBA) (OXOID; CM0069), incubated at $(37 \pm 1)^\circ\text{C}$ for 2 hours and increased to $(44 \pm 1)^\circ\text{C}$ for 22 hours, in conformity with standard ISO 7218 (ISO,1996) with modification. *E. coli* enumeration was made on Eosin Methylene Blue (EMB) agar (OXOID; CM0069), incubated at 44°C for 24 hours, according to a modification of the method suggested by Estevinho *et al.* (2012). Microbial counts were expressed as cfu/g.

3.4.1.1.5 Enumeration of sulphite-reducing *Clostridium* spores

The sulphite-reducing *Clostridium* spores were determined according to AOAC (1972) with some modifications. Twenty grams (20 g) of honey sample was weighed into sterile bottle (used All-American Electric Pressure Steam Sterilizer, Serial No. 0014206, Model 25X-2, USA, for the sterilization) and sterile distilled water added to a final volume of 100 ml. The sterile bottle was aseptically swirled to dissolve the honey sample and the content brought to boil rapidly for 5 minutes. The content was cooled in cold water after boiling and the volume made up to 100 ml with sterile distilled water. One hundred microliters was inoculated on perfringes agar base, incubated at 55°C for 24 to 48 hours in anaerobic jar and black colonies counted. Microbial count was expressed as cfu/g.

3.4.1.1.6 Enumeration of *Salmonella*

The enumeration of *Salmonella* in honey was done following the protocol of Ukuku and Sapers (2007) with modification. Twenty-five grams of honey sample was homogenized in 225 ml of buffered peptone water (BPW) and incubated for 16 – 20 hours at 37°C . A volume of 1 ml of the pre-enriched sample was used to inoculate 10 ml of Rappaport-Vassiliadis *Salmonella* Enrichment Broth (MERCK; 61944905001730, B.N.:

JB2JF62024) and incubated for 7 hours at 42 °C. One hundred microliter (100 µl) aliquots each of Rappaport-Vassiliadis medium samples were inoculated on *Salmonella Shigella* Agar (S.S.A) (PARK SCIENTIFIC LIMITED, M0240) with incubation at 35 °C for 48 hours for enumeration. Microbial counts were expressed as colony-forming units per 25 g of honey (cfu/25 g).

3.4.1.2 Determination of *botulinum* toxin

Botulinum toxin was determined qualitatively by the use of a rapid test kit for detection of *Botulinum* toxin (BADD BioWarfare Agent Detection Device, Re-order BOT-E500-4). The determination was done per manufacturer's instruction. The device and the diluents tube were placed on a flat, horizontal surface in front with "C" and "T" towards the top and the small round sample well to the right. The cap of the diluent tube was unscrewed and the swab removed completely. The foam tip was rubbed against the inside walls to extract excess liquid before removing. The absorbent end of the swab was placed into the honey, swirled for 5 seconds and the swab and the collected honey sample returned back to the diluents tube, the cap tightened and shaken gently for approximately 5 seconds. The clear cap from the tip of the diluents tube was removed and the tip broken off and discarded making the tube a dropper for dispensing the honey onto the test device. The tube was inverted to a 45° angle and slowly added 5 drops to the sample well. A timer was set and a maximum of 15 minutes allowed before interpreting the result. A red-dish colour moves across the result window as the test begins to work. A line always appears at the control line "C" indicating that the test is working. Any visible line regardless of intensity (faint or deep) in the test area "T" was viewed as positive result meaning presence of *Botulinum* toxin in sample.

3.4.2 Physicochemical analyses

3.4.2.1 Physical analyses

3.4.2.1.1 Determination of refractive index and moisture content

The determination of moisture (AOAC, 1990; Official Method 969.38) was ascertained by refractometry (using Digital refractometer Atago, Germany). 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.

3.4.2.1.2 Determination of water activity

A small aliquot of honey sample weighing approximately one gram (1 g) was used for determining water activity (a_w) using a Rotronic Hygropalm water activity analyzer (Serial No. 61122743, ART No. HP23-AW-A, and Serial No. 61087777, ART No. HC2-AW, TEKNO 2011B, W.AG, Germany). This equipment measures the water activity of the sample based on its equilibrium relative humidity (ERH). The relationship between a_w and ERH is given as follows:

$$a_w = \frac{ERH \text{ (in percentage)}}{100} \text{ (Aqualab CX2T, Decagon Devices, USA)}$$

(Chirife *et al.*, 2006)

The measurements were performed in triplicates for each sample and the mean was determined.

3.4.2.1.3 Measurement of colour

Honey samples were heated to 50 °C to dissolve sugar crystals, and the colour was determined by spectrophotometric measurement of the absorbance of a 50 % honey solution (w/v) at 635 nm (Spectrophotometer used was CECIL INSTRUMENTS, 3000 Series, Serial No. 127 – 275, Model No. CE 3041, Cambridge England). The honey samples were classified according to the Pfund scale after conversion of the absorbance values:

$$mm\ Pfund = -38.70 + 371.39 \times Abs \text{ (White, 1984)}$$

3.4.2.1.4 Determination of total soluble solids (TSS) and total solids (TS)

The total soluble solids of the honey samples were measured by refractometry using a Digital refractometer (Atago, Germany) and the results were expressed in % Brix. All the measurements were done at ambient temperature and the readings corrected for a standard temperature of 20 °C by adding the correction factor of 0.00023/°C (AOAC, 1990). The total solid content (%) in the honey samples was calculated by using the formula:

$$TS\ (\%) = 100 - moisture\ content \text{ (Amin et al., 1999)}$$

3.4.2.1.5 Determination of pH

Honey pH was measured with HI2210 H2211 Microprocessor-based pH/mV/°C Bench Meter (model pH 210, HANNA instruments Inc., USA), in a solution prepared with 10 g of honey in 75 ml of distilled water (NP 1309/1976; Zenebon and Tiglea, 2008).

3.4.2.1.6 Determination of electrical conductivity (E.C.)

Electrical conductivity was determined by conductometric assay (HANNA instruments, HI 2550 pH/ORP & EC/TDS/NaCl Meter), from a solution containing 10 g of honey in 75 ml of distilled water (Sancho *et al.*, 1992).

3.4.2.1.7 Determination of ash content

Ash content (%) = $0.083 \times \text{conductivity} - 0.092$ as reported by Sancho *et al.*, (1992).

3.4.2.2 Chemical analyses

3.4.2.2.1 Determination of Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural was determined by using the standard method of AOAC (1990) Official Method 980.23 with some modifications. Five grams (5 g) of honey sample was dissolved in 25 ml of distilled water in a 50 ml beaker and quantitatively transferred into a 50 ml volumetric flask. Half millilitre (0.5 ml) of Carrez I solution (150 mg/ml potassium ferrocyanide) was added and thoroughly mixed. Half millilitre (0.5 ml) of Carrez II solution (300 ml/ml zinc acetate) was then added and mixed and made up to the mark with distilled water. The solution was filtered, and the first 10 ml discarded. Five millilitres (5 ml) of the remaining filtrate was transferred into each of two test tubes. A reference solution was prepared by adding 5 ml of 0.20 % sodium bisulphite (NaHSO_3) to one test tube of filtrate. A test sample solution was prepared by adding 5 ml of distilled water to the other test tube of filtrate. Both samples were mixed with a vortex mixer (Vortex-2 Genie, Scientific Industries, INC., Serial No. 2-48077, Model G-560E, Patent No. 4,781,487, Bohemia, N.Y., 11716, USA). The absorbance of the test sample solution and the reference solution was measured at 284 and 336 nm respectively. HMF was

determined by subtracting the absorbance measured at 284 nm for HMF in the test sample solution (honey sample solution) against the absorbance of reference solution (the same honey solution treated with sodium bi-sulphite, 0.2 %) at 336 nm as:

$$HMF/100g \text{ of honey} = (Abs_{284} - Abs_{336}) \times 14.97 \times (5/g \text{ of sample})$$

3.4.2.2.2 Determination of free acidity

Free acidity was determined by potentiometric titration (AOAC, 1990; Official Method 962.19). Honey samples were homogenized in a water bath (Clifton water bath, Serial No. 70594, NICKEL-ELECTRO LTD, WESTON-S-MARE SOMERSET, ENGLAND) and filtered through gauze, prior to analysis. Ten grams (10 g) of honey were then dissolved in 75 ml of distilled water, and alcoholic solution of phenolphthalein added. The solution was titrated with 0.1 N NaOH. Acidity (milliequivalent of acid per kg of honey) was determined as 10 times the volume of NaOH used in titration.

3.4.2.3 Antimicrobial analysis

3.4.2.3.1 Determination of antimicrobial activity of honey

This was determined using the spectrophotometric assay method of Patton *et al.* (2006). The test organisms were *Escherichia coli* and *Staphylococcus aureus*, both obtained from Food Research Institute (Centre for Scientific and Industrial Research), Ghana.

3.4.2.3.2 Preparation of honey solutions

Honey solutions were prepared immediately before testing by diluting honey to the required concentrations (10, 30, 50, 70 and 100 %, v/v). All samples were then incubated for 30 minutes at 37 °C in a shaking water bath that allowed aeration of the solutions.

Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive (White and Subers, 1964).

3.4.2.3.3 Preparation of test organisms

The cultures were pre-identified based on standard microbiological techniques, and sub-cultured in nutrient agar (PARK SCIENTIFIC LIMITED, M0208) slopes at 37 °C for 24 hours. Colonies of fresh cultures of the different microorganisms from overnight growth were picked with sterile inoculating loop and suspended in 3 – 4 ml nutrient broth No. 2 (PARK SCIENTIFIC LIMITED, M0209) contained in sterile test tubes and incubated for 2 - 3 hours at 37 °C. This was diluted with distilled water to set inoculum density (1×10^7) used in this study.

3.4.2.3.4. Minimum Inhibitory Concentration (MIC) determination

Two hundred microliters (200 μ l) of the cell suspension (obtained from the preparation) was inoculated into 4 ml volume of honey concentration in a test tube while inoculation of 4 ml volume of nutrient broth with 0.2 ml of the cell suspension served as control. The optical density (OD) was determined and recorded in a spectrophotometer (CECIL INSTRUMENTS, Serial No. 127-275, Model CE 3041, 3000 series, Cambridge England) at 620 nm before incubation (T_0), after which, the cultures were incubated for 24 hours in the dark at 37 °C (using Clifton water bath, Serial No. 70594, NICKEL-ELECTRO LTD, WESTO-S-MARE SOMERSET, ENGLAND) with constant shaking to prevent adherence and clumping. After 24 hours of incubation, the optical densities were again determined and recorded (T_{24}). The optical density for each replicate at T_0 was subtracted as determined using the formula:

$$\text{Percentage inhibition} = 1 - \frac{OD_{test}}{OD_{control}} \times 100$$

3.4.3 Statistical analysis

Survey data were analysed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 20. STATGRAPHICS Plus version 3.0 was used to conduct analysis of variance (ANOVA) and test of significance at ≤ 0.05 . Test statistics such as principal component analysis (PCA) was applied to variables using Minitab version 15. Means were separated by Fisher's protected LSD at $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Survey on artisanal honey producers

4.1.1 Background information on artisanal honey producers

Table 4.0 shows the demographic characteristics of the artisanal honey producers in the study areas, namely, Somanya (in the Eastern region) and Dzolokpuita, Dzologbogame, Ho and Nkwanta communities in the Volta region of Ghana.

Table 4.0: Background information on artisanal honey producers

Parameters	Category	Frequency (n)*	Percentage (%)
Gender	Male	28	93.3
	Female	2	6.7
Age group (years)	Less than 20	0	0.0
	20 – 29	1	3.3
	30 – 39	4	13.3
	40 – 49	7	23.3
	50 – 59	11	36.7
	60 and above	7	23.3
Ethnicity	Ga-Adangbe	5	16.7
	Ewe	25	83.3
Religion	Christianity	28	93.3
	Islam	2	6.7
Formal educational status	Basic	20	66.7
	Secondary	6	20.0
	Tertiary	4	13.3
Major occupation	Professional	2	6.7
	Artisan	2	6.7
	^a Others	26	86.7

***(N = 30)** Professionals: Agriculturist, Apiculturist Artisans: Electrician, Carpenter

^aOthers: Subsistence farmers, Civil servant (messenger)

Honey production in communities was dominated by men of varying ages, including those above 60 years. Most of these men were natives of the communities. Majority had only basic education but about 13 % had tertiary education. Honey production was a secondary occupation for all. Reasons such as the desire to have extra income to meet the high cost of good living or for sustainable livelihood motivated these producers. The producers however, indicated that they lacked modern logistics and were unable to produce to meet demands with the limited existing equipment they had resulting into low income generation.

4.1.2 Process description of artisanal honey production in the study areas

Artisanal honey production practices have not changed much over the years; the methods and the means of production observed in this study were not different from those reported earlier by Akangaamkum *et al.* (2010). The basic requirements for the production of honey were combs with honey, bee brush, bee knife, container, honey extractor and strainer. These were employed in different ways by each producer. The flow diagrams of the production processes of apiary and forest honey in the study area are shown in Figures 4.0 and 4.1 below respectively.

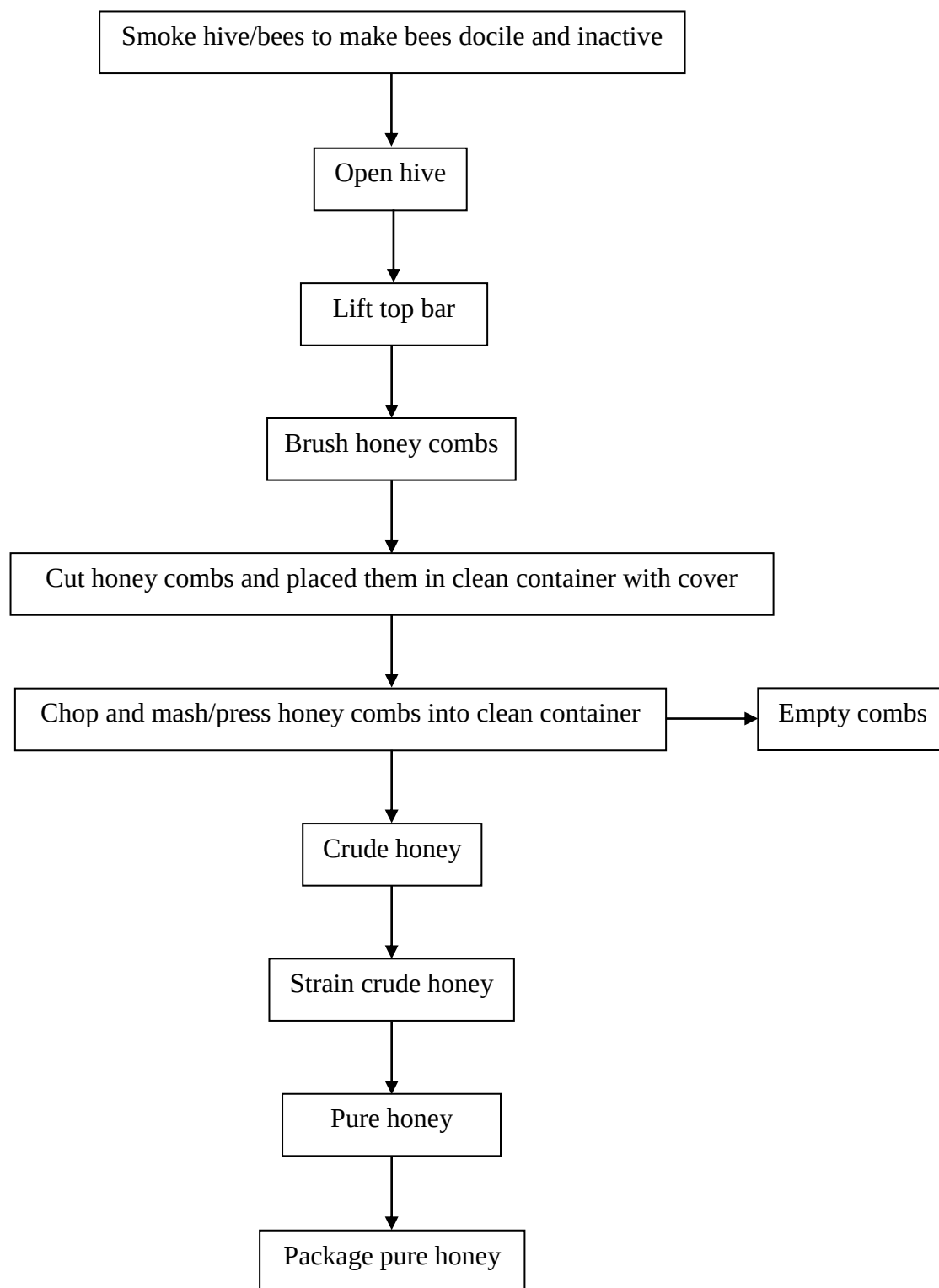


Figure 4.0: Flow diagram of artisanal apiary honey production

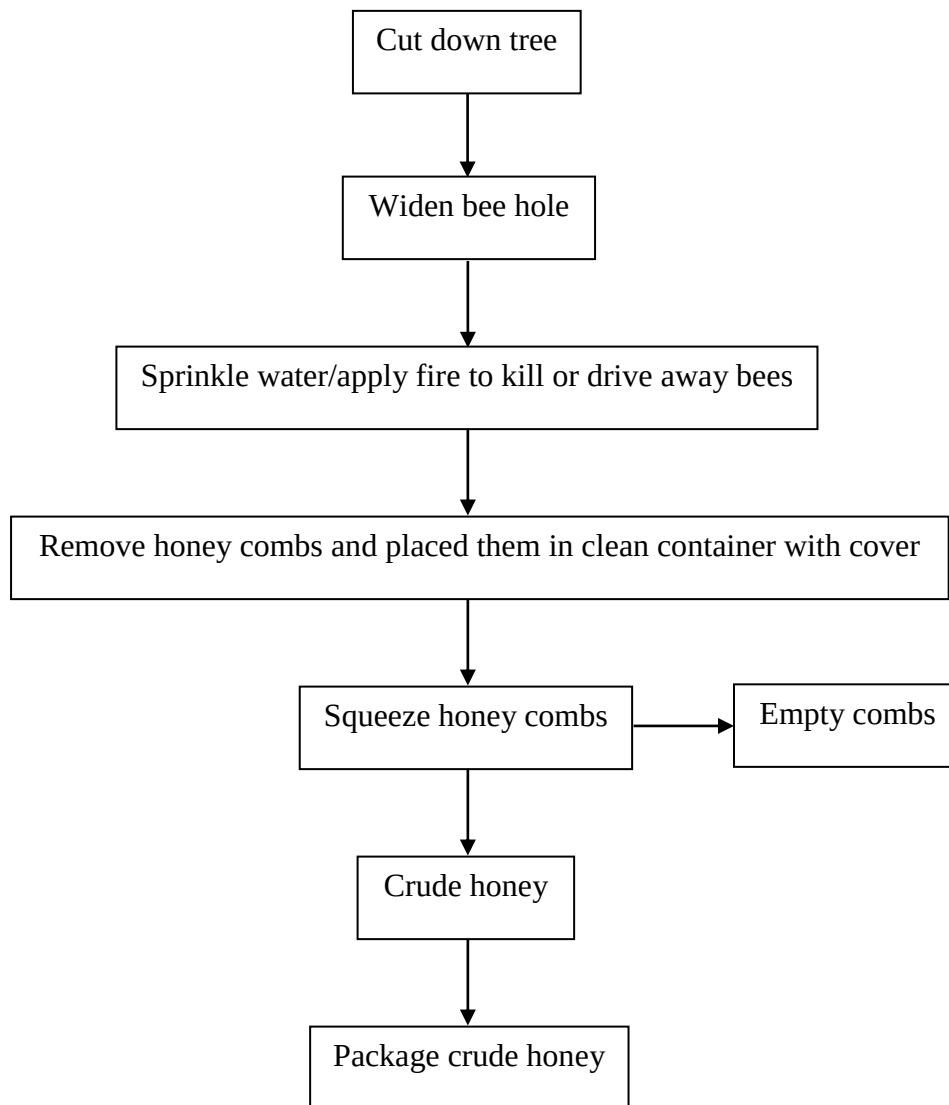


Figure 4.1: Flow diagram of artisanal forest honey production

4.1.2.1 Harvesting of honey combs

The apiary honey producers first smoke hive/bees to make bees docile and inactive, then open hive, lift top bars, brush honey combs off bees and other particles with bee brush, cut brushed honey combs with bee knife into a container and cover them. The smoking of hives/bees is aimed at preventing bees from attacking them and the bee smoker is used to achieve that purpose. The forest honey producers however first cut down the tree on

which they find honey combs with cutlass. They then widen the bee hole with axe, sprinkle water or apply fire to kill or drive away the bees to have access to honey combs. They then hand remove the combs into container and cover.

4.1.2.2 Extraction of honey from honey combs

The apiary honey producers either chopped and mashed honey combs and allowed honey to drain out through a nylon net (sieve) into a clean container for packaging or pressed honey combs with honey press into a clean container for straining and packaging. The forest honey producers on the other hand, hand-squeezed honey combs to obtain honey and packaged it in plastic cooking oil gallons for sale.

4.1.2.3 Straining of honey

This process was done by the apiary honey producers only. They used nylon nets (sieve) with pore size wide enough to allow pollens but prevent dead bees and other particles from passing through to strain their honey to obtain pure honey for sale.

4.1.2.4 Packaging of honey

Both producers used simple inexpensive packaging materials and practiced manual filling. The honey was filled into the packaging materials either by pouring directly into packaging containers or with the help of funnels. The packaging materials used were used cooking oil drums or “jerry cans”, used cooking oil gallons, used plastic mineral water bottles and used alcohol bottles.

The artisanal honey producers used simple traditional and non-standardized methods for honey production. Regardless of the source of honey combs, the method of harvesting

honey combs and the unit operations involved in the production processes were almost the same for both the forest and apiary artisanal honey producers. The honey combs were brushed for about five minutes, cut into clean container, chopped and mashed or machine-pressed for about an hour, or hand-squeezed for about two hours, strained for two to six hours and packaged. Additionally, the entire production from extraction of honey from combs to packaging is done at homes of the producers; no well-structured premises for production. The quantities of honey combs used in a batch production by the respondents in the study areas is shown in Figure 4.2 below. The artisanal honey producers were small scale producers and majority used smaller quantity of honey combs in batch production. All the artisanal honey producers stated that the small quantity of the honey combs they used per batch production was due to lack of logistics and inefficiency of the few they had.

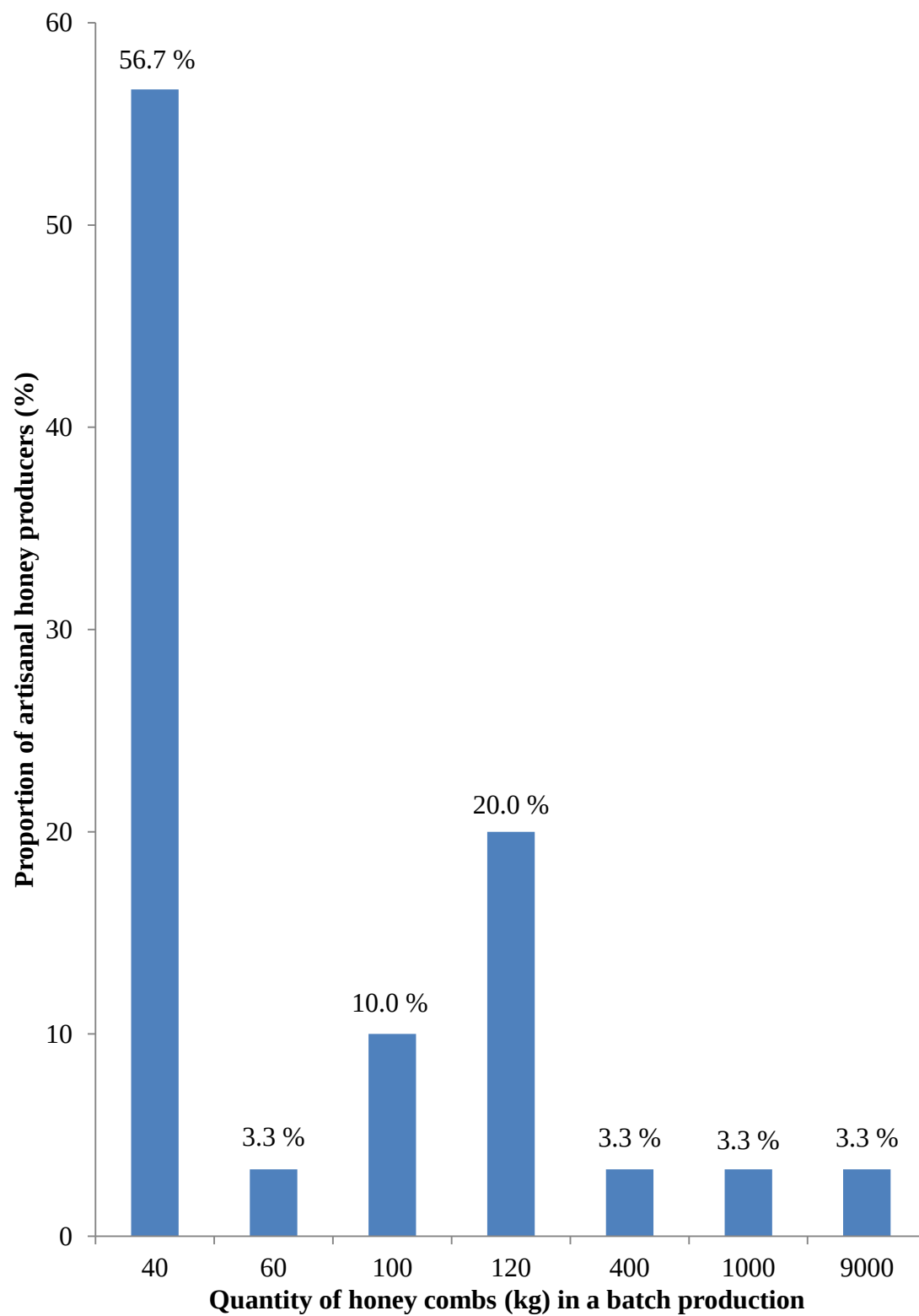


Figure 4.2: Quantity of honey combs used in a batch of honey production

4.1.3 Critical steps for high quality artisanal honey production

Table 4.1 shows that greater number of producers pointed out smoking and/or application of fire and straining of honey in production processes to be critical for achieving the desired good quality honey. Producers make sure that smoking and/or fire application are done gently and gradually for a short period. They also use clean nylon nets with pore size big enough for pollens only to pass through to filter the crude honey so that the desired high quality honey are produced. They indicated that smoke and/or fire can affect the taste and colour of honey negatively if not given much attention. They also stated that straining influences the consistency, level of particles and dust as well as contamination of honey with microorganisms and needs to be done with precaution and under hygienic conditions.

Table 4.1: Measures to control critical steps in honey production

Parameters	Category	Frequency (n)*	Percentage (%)
Control for steps considered important for achieving desired quality in honey during production	Sprinkle little water gently and gradually	3	10.0
	Smoke/apply fire gently and gradually	12	40.0
	Brush gently and softly	3	10.0
	Squeeze gently and gradually	3	10.0
	Use clean net with pore size big enough for only pollens to pass through	9	30.0

(N = 30)

4.1.4 Traditional measurement of quality of artisanal honey

Honey trade is considerably influenced by food quality standard requirements especially in the developed countries. Thus, food safety and quality standards are the determinants

of the volume of trade in honey. Table 4.2 shows how honey produced by artisanal honey producers in Somanya, Dzolokpuita, Dzologbogame, Ho and Nkwanta communities in Ghana are assessed and how quality control is done.

Table 4.2: Assessment and control of honey quality

Parameter	Category	Frequency (n)*	Percentage (%)
How do you assess the quality of your honey?	Do not assess quality	1	3.3
	Visually observe viscosity	7	23.3
	Check moisture content by the use of match stick method	8	26.7
	Visually inspect appearance: colour should be dark amber	1	3.3
	Visually inspect particles: particles should be absent	14	46.7
	Process capped honey combs	2	6.7
	Check brix level	2	6.7
	Hygienic control of production environment	1	3.3
	Observe hygienic practices	24	80.0
	Avoid use of water during production	9	30.0
How do you control the quality during production?	Process capped honey combs	5	16.7
	Strain thoroughly	4	13.3

(N = 30)

*Answers include multiple responses

Artisanal honey produced was considered high quality when it had no dirt and particles, low moisture and was thick (viscous) (Table: 4.2). The survey revealed that physical characteristics are the basis of assessing quality in the study areas. Determinants such as, sensory, chemical, and microbiological characteristics (Finola *et al.*, 2007; Belay *et al.*, 2013) are however, not used. Hygienic practices and avoidance of water during production were considered critical by majority of the producers for the achievement of high quality honey. The respondents indicated that after production, they either sell their honey by hawking or sell in bulk. Furthermore, the respondents stated that they were often unable to sell whole batch of honey on the same day of production hence store in used plastic cooking oil gallons for months until sold out.

4.1.5 Artisanal producers' perceptions of adulterations in honey

Honey adulteration is a complex problem which now has an important economic impact, and undeniable nutritional and organoleptic consequences. It evolved from rudimentary addition of sugar (cane or beet) and water to specially produced syrups for which the chemical composition approximately reproduces the sugar composition and ratios of natural honey (Cordella *et al.*, 2005), thus affecting the quality. Perceptions of honey adulteration are shown in Table 4.3 below.

Table 4.3: Perceptions of adulterations in honey by artisanal honey producers in survey areas

Parameters	Category	Frequency (n)*	Percentage (%)
Do some producers add other ingredients to honey?	Yes	22	73.3
	No	8	26.7
If yes, list ingredient(s) and reasons for adding to honey?	Sugar to increase volume/quantity for more profit	28	93.3
	Foam, do not know	2	6.7
Is honey adulteration a known problem here?	Yes	11	36.7
	No	19	63.3
Do customers complain of adulteration?	Yes	13	43.3
	No	17	56.7

*(N = 30)

The artisanal honey producers admitted the occurrence of honey adulteration in Ghana. About seventy per cent of the producers presumed that other ingredients are added to honey by other producers for different reasons. The addition of sugar to increase quantity for financial gains is reported in literature (Cotte *et al.*, 2003a; Cotte *et al.*, 2007; Li *et al.*, 2012). Majority of the producers however indicated that adulteration of honey was not a known problem in their locations. However, almost fifty-seven per cent of them indicated that customers often complained about honey adulteration and questioned the quality of their honey before buying (Table 4.3). Even though honey adulteration was not a common problem, producers found control methods to combat it to safe guard the quality of their honey to win customer confidence (Table 4.4). The suggested methods specified by the producers for honey adulteration detection were extremely subjective (Table 4.4).

Zhu *et al.* (2010) reported scientific and objective analytical techniques to detect adulteration of honey but these methods are time-consuming, destructive and expensive.

Table 4.4: Methods for detecting adulteration in honey by artisanal producers in survey areas

Parameters	Category	Frequency (n)*	Percentage (%)
How do you detect adulteration in honey	Do not know	14	46.67
	Check colour, if honey colour is dark then its adulterated	1	3.33
	Check ability to foam upon shaking, if honey foams then its adulterated	1	3.33
	Check rate of diffusion in a tumbler of clean cold water, if honey diffuses in a short period then its adulterated	7	23.33
	Check dissolution in sand, if honey soaks up sand then its adulterated	4	13.33
	Check fermentation, if honey ferments then its adulterated	5	16.33
	Check taste, if honey taste different from typical bee honey taste then its adulterated	2	6.67
	Check ability of match stick dipped in honey to ignite fire, inability of a match stick dipped in it to ignite fire means its adulterated	1	3.33
	Check presence of live bees hovering around honey, absence of live bees around it means its adulterated	2	6.67

(N =30)

*Answers include multiple responses

4.1.6 Association between demographic variables and honey production habits of artisanal honey producers in survey areas

Honey production is done using different honey extraction methods: squeezing, solar extraction, cold extraction and centrifugal extraction, and different honey combs harvesting methods involving the use of smoke, fire or water. Apiary honey producers wholly use smoke in honey combs harvesting and cold or centrifugal extraction in honey production. On the contrary, most forest honey producers exclusively use fire in honey combs harvesting and few use water. However, they solely extract honey from honey combs by squeezing. The associations between selected demographic variables and some indicators of honey production habits are shown in Table 4.5 below.

Though there was no significant association between gender, age group, religion, formal educational status and the step(s) followed in honey production, the artisanal honey producers' ethnicity and major occupation were significantly ($p \leq 0.05$) associated with step(s) followed in honey production. This means that the respondents' ethnicity and major occupation determined the step(s) they used in honey production. Also, significant ($p \leq 0.05$) association was found between the artisanal honey producers' ethnicity, formal educational status, major occupation and how they assessed honey quality. Half of the respondents basically assessed honey quality by checking water content. In addition, the artisanal honey producers' formal educational status and major occupation were significantly ($p \leq 0.05$) associated with how they controlled honey quality during production. Most of the respondents controlled honey quality by conforming to hygienic practices. Furthermore, there was significant ($p \leq 0.05$) association between ethnicity, formal educational status, major occupation of the artisanal honey producers and the nature of packaging materials that they used. Majority used recycled packaging materials.

Even though the issue of adulteration is collapsing the honey business sector, it did not have much impact on respondents' honey production habit and are more probable to continue producing honey at high quality as far as ready markets exist.

Table 4.5: Pearson Chi Square test for selected dependent variables on demographic characteristics for responses of honey producers (n = 30)

Cross tabulation		Pearson Chi	Likelihood	p-value
Dependent variable	Demographic variable	Square test	Ratio	
Research question:	Gender	5.510	5.135	0.480
What step do you	Age group	30.139	33.350	0.180
follow in the	Ethnicity	12.857	13.654	0.045*
production of	Religion	5.510	5.135	0.480
honey?	Formal educational status	15.765	14.168	0.202
	Major occupation	40.000	25.287	0.000*
Research question:	Gender	8.571	7.058	0.199
How do you assess	Age group	19.046	20.028	0.750
the quality of your	Ethnicity	17.760	17.531	0.007*
honey?	Religion	1.684	2.084	0.946
	Formal educational status	28.121	25.120	0.005*
	Major occupation	45.000	26.333	0.000*
Research question:	Gender	3.857	2.480	0.277
How do you control	Age group	9.636	10.310	0.648
the quality during	Ethnicity	5.808	5.050	0.121
production?	Religion	3.857	2.480	0.277
	Formal educational status	14.307	9.809	0.026*
	Major occupation	30.277	15.167	0.000*
Research question:	Gender	0.779	1.292	0.377
What is the nature of	Age group	0.397	0.654	0.983
the packages that	Ethnicity	16.500	12.307	0.000*
you use?	Religion	0.779	1.292	0.377
	Formal educational status	9.290	8.975	0.010*
	Major occupation	12.692	12.470	0.002*

4.2 Survey for honey sellers in Accra Metropolis of Ghana

4.2.1 Background information on honey sellers

The market for honey may be classified generally into domestic and export markets. The survey identified two kinds of domestic markets, namely rural and urban (formal and informal). Table 4.6 shows the demographic characteristics of honey sellers in markets in Accra Metropolis of Ghana.

Table 4.6: Background information on honey sellers in Accra Metropolis of Ghana

Parameters	Category	Frequency (n)*	Percentage (%)
Gender	Male	2	4.0
	Female	48	96.0
Age group (years)	Less than 20	0	0.0
	20 – 29	10	20.0
	30 – 39	4	8.0
	40 – 49	16	32.0
	50 – 59	11	22.0
	60 and above	9	18.0
Ethnicity	Ga-Adangbe	6	12.0
	Mole-Dagbani	9	18.0
	Ewe	10	20.0
	Akan	11	22.0
	^b Others	14	28.0
Religion	Christianity	26	52.0
	Islam	24	48.0
Educational status (forma)	No formal education	11	22.0
	Basic	23	46.0
	Secondary	14	28.0
	Tertiary	2	4.0
Major occupation	Professional	2	4.0
	Trader	46	92.0
	Seamstress	2	4.0

***(N = 50)** ^bOthers: Hausa, Colonbi, Banofa

Professionals: Medical counter assistant, Pharmacy attendant

Unlike honey production dominated by men, honey retail was dominated by women. All the sellers were aged between twenty years and above. Honey retail on the markets was

dominated by Akans. Among the sellers, fifty-two per cent were Christians and the rest were Muslims. Most of the sellers had basic education. However, some (22 %) had no formal education (Table 4.6). Almost all the sellers were solely traders. Some indicated that selling of honey was a family trade and they took over the business from their parents or grandparents after their death or in their old age.

4.2.2 Quality of honey on the markets in the Accra Metropolis of Ghana

Honey sellers on the markets usually used four quality characteristics (flavour, taste, colour and thickness) in obtaining honey supply. They believed that these characteristics are holistic in measuring the quality of honey and gives a good judgement of honey quality. More than half of the sellers used taste in obtaining honey supply (Figure 4.3). Also, majority suggested checking moisture contamination by checking ability of a match stick dipped in honey to ignite fire as the method used for assuring quality of honey on the markets (Appendix B6). They stated they used this method often because it measures the moisture content of honey, an important quality index which determines the stability and shelf-life as well as the grade of honey. Majority of sellers ranked thickness as the most important quality characteristics that consumers used in assessing honey quality for purchase (Figure 4.4).

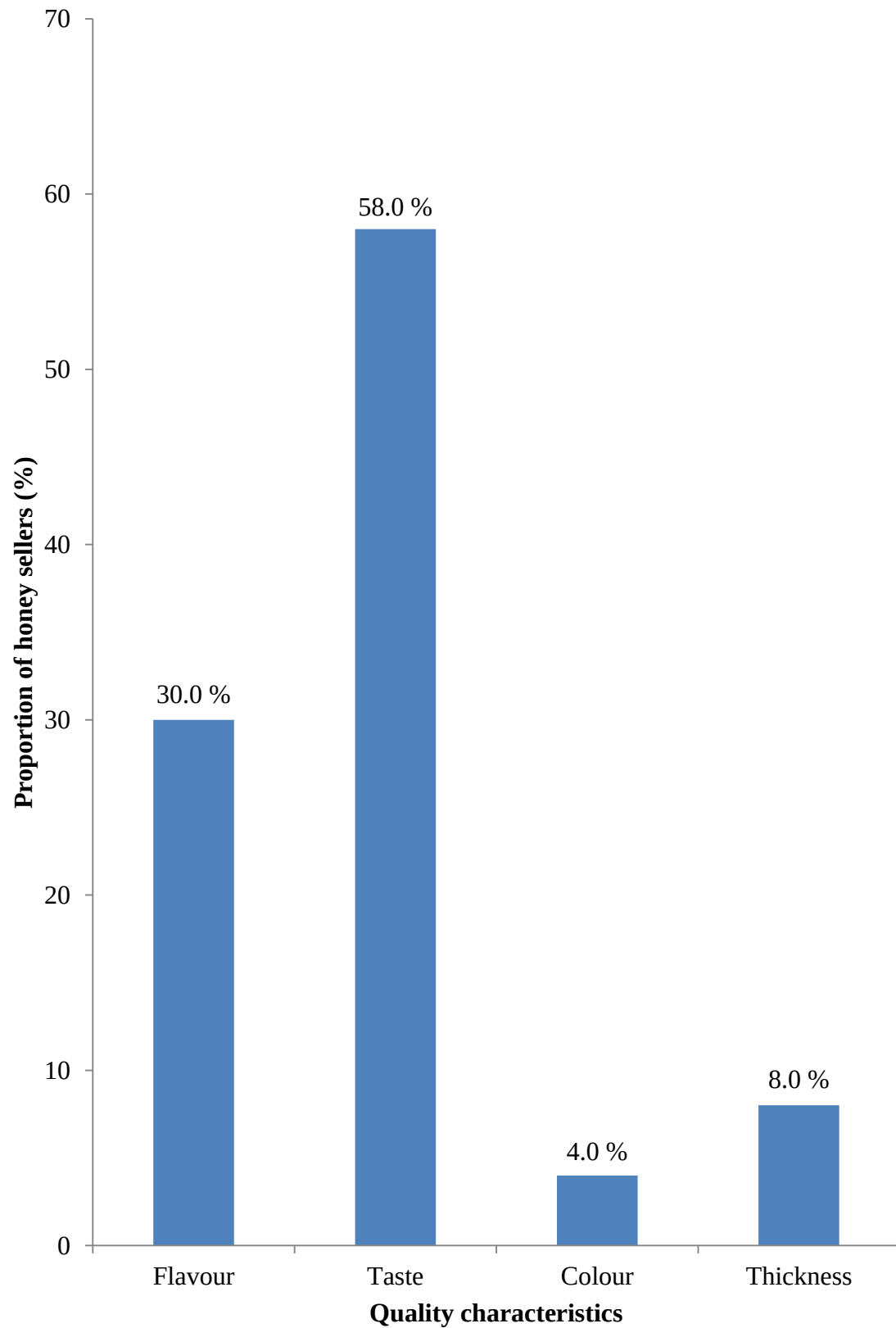


Figure 4.3: Quality characteristics sellers use in obtaining their supply of honey

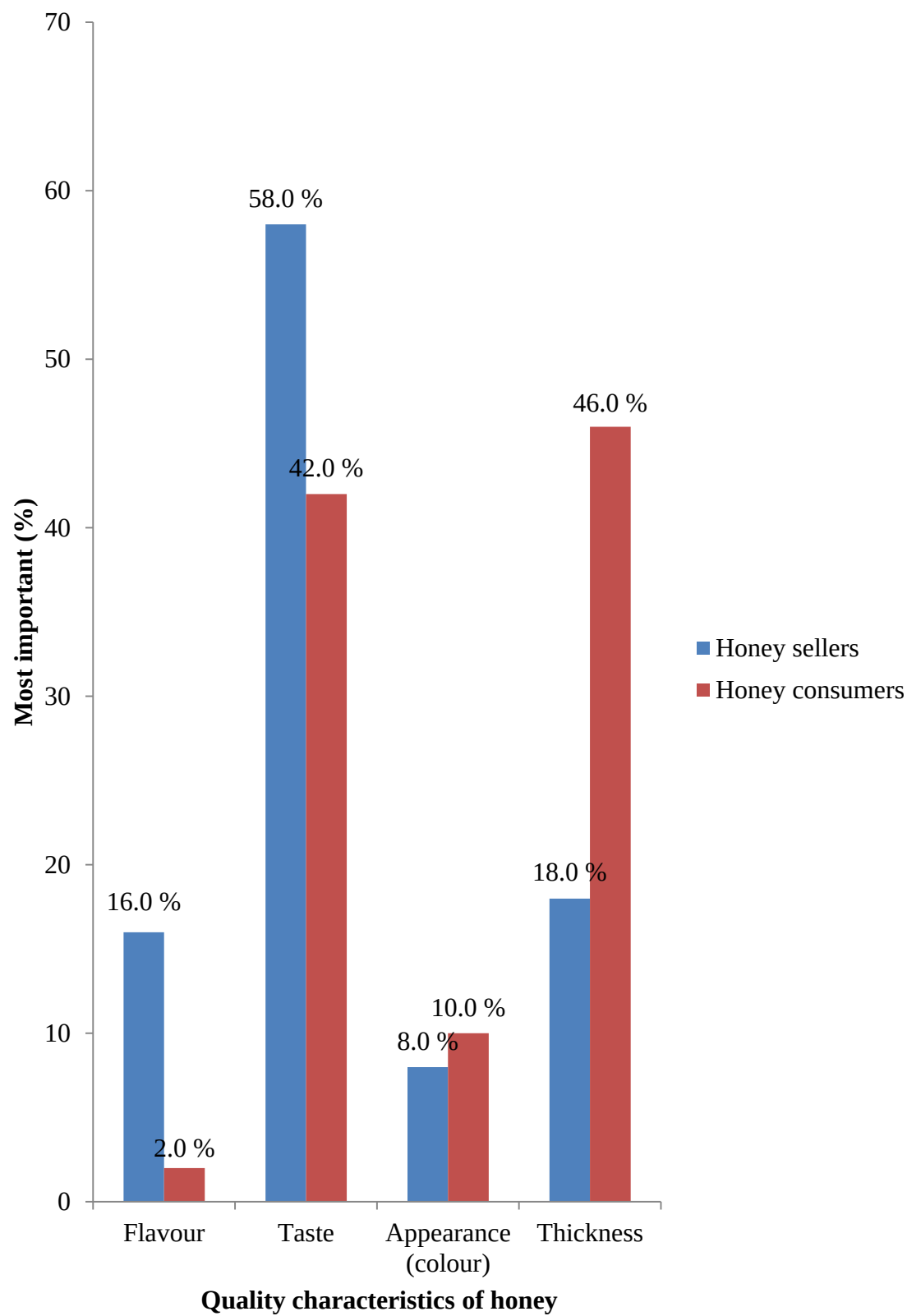


Figure 4.4: Ranking of honey quality characteristics important to honey consumers by honey sellers

Honey sellers purchased honey in large quantities (in drums or “jerry cans” and gallons) and excess kept until the whole consignment was sold out. Majority of the sellers stated that they store the excess honey in drums or “jerry cans”, plastic cooking oil gallons, used plastic mineral water bottles or used soft/alcoholic drinking bottles and indoors (Table 4.7). A greater number of the sellers indicated that they sold out a whole consignment (batch/quantity of honey purchased at a time for sale) within a period less than six months. Nevertheless, they indicated that honey retail is a slow moving business; it takes quite a long time to sell a whole consignment and the turnover is low. This was explained to consumers’ infrequently purchasing honey. Eight per cent of the sellers reported that their honey went bad when stored for a very long time but did not remember the duration. This finding opposed what Prica *et al.* (2014) published, that honey is a durable food, it never deteriorates and has virtually unlimited shelf life. The sellers however, perceived deteriorated honey unfit for consumption and affirmed that they discard their honey when they felt it is deteriorated. The common causes of spoilage of honey during storage gave by the sellers were adulteration and abuse of storage temperature during storage. The sellers stated that they prevented or minimized deterioration of their honey by avoiding adulteration, temperature abuse or purchasing honey from certified honey producers by Food and Drug Authority (FDA).

Table 4.7: Storage and shelf-life of artisanal honey on the markets

Parameters	Category	Frequency (n)*	Percentage (%)
Do you store your honey?	Yes	50	100.0
If yes, how do you store your honey?	Keep in package and store in a room	40	80.0
	Keep in package and store outside	10	20.0
How long do you store your honey?	Less than 6 months	38	76.0
	6 – 12 months	9	18.0
	13 – 18 months	1	2.0
	19 – 24 months	2	4.0
Does your honey spoil when you store for a very long time?	Yes	4	8.0
	No	46	92.0
If yes, how long?	Cannot remember	4	100.0
What are some of the causes of the spoilage during storage?	Addition of sugar	17	34.0
	Addition of water	18	36.0
	Abuse of storage temperature	14	28.0
	Do not know	1	2.0
What are some of the steps you take to prevent or minimize the deterioration of your honey?	Avoid adulteration	22	44.0
	Avoid temperature abuse	8	16.0
	Buy from certified honey producers	3	6.0
	Do nothing	17	34.0
What do you do with the spoilt honey?	Do not use (discard)	50	100.0
(N = 50)			

4.2.3 Association between demographic variables and honey selling habits of honey sellers on the markets

Though the profit margin in honey vending is large, it is a very slow moving business and takes quite a long time for sellers to sell a batch of consignment hence storage is inevitable. As a result, safeguarding its quality during storage and through the period of

sale is very important. Table 4.8 shows the associations between selected demographic variables and some indicators of honey selling habits.

Among all the demographic variables, only ethnicity of honey sellers significantly associated with how honey was stored which meant that only ethnicity of honey sellers determined how honey was stored. Gender ($p = 0.000$) and major occupation ($p = 0.000$) of the honey sellers also associated significantly with how honey was handled and dispensed respectively during the period of sale. Majority of the respondents packaged honey in used plastic mineral water bottles during period of sale. Again, age group, ethnicity and major occupation of the honey sellers showed significant association with how honey quality was assured. Fifty-four per cent of the respondents assured honey quality by checking water content. Additionally, there was significant association between age group of the honey sellers and quality characteristics used to obtain honey supply. Similarly, major occupation of the honey sellers associated significantly with some of the hygienic practices that the sellers observed.

Though there is a very slow turnover due to delay in selling a batch of honey purchased, it did not have much influence on respondents' honey selling habit. Honey sellers are therefore more probable to continue selling honey when made available at high quality.

Table 4.8: Pearson Chi Square test for selected dependent variables on demographic characteristics for responses of honey sellers (n = 50)

Cross Tabulation		Pearson Chi	Likelihood	p-value
Dependent variable	Demographic variable	Square Test	Ratio	
Research question: How do you store your honey, if you do store?	Gender	0.521	0.913	0.470
	Age group	5.114	7.697	0.276
	Ethnicity	10.080	8.479	0.039*
	Religion	1.623	1.666	0.203
	Formal educational status	0.979	1.347	0.806
	Major occupation	1.087	1.870	0.581
Research question: How do you handle your honey during the period of sale?	Gender	32.639	12.975	0.000*
	Age group	20.457	19.107	0.200
	Ethnicity	15.366	14.065	0.498
	Religion	1.255	1.646	0.869
	Formal educational status	17.348	17.048	0.137
	Major occupation	37.888	18.120	0.000*
Research question: How do you dispense your honey for sale?	Gender	23.958	12.707	0.000*
	Age group	8.730	8.178	0.068
	Ethnicity	1.632	2.284	0.803
	Religion	0.921	0.966	0.337
	Formal educational status	6.918	5.394	0.075
	Major occupation	24.008	11.423	0.000*
Research question: How do you assure honey quality?	Gender	26.852	10.515	0.176
	Age group	115.106	91.281	0.014*
	Ethnicity	109.307	93.973	0.033*
	Religion	22.677	30.168	0.361
	Formal educational status	77.975	66.887	0.097
	Major occupation	77.640	27.680	0.001*
Research question: What quality characteristics do you use in obtaining your supply of honey?	Gender	1.509	2.239	0.680
	Age group	24.996	23.490	0.015*
	Ethnicity	18.202	21.165	0.110
	Religion	4.504	4.674	0.212
	Formal educational status	9.773	11.431	0.369
	Major occupation	6.822	5.814	0.338
Research question: What are some of the hygienic practices you observe?	Gender	0.952	0.791	0.329
	Age group	6.844	9.769	0.144
	Ethnicity	2.246	3.500	0.691
	Religion	0.765	0.774	0.382
	Formal educational status	1.717	1.602	0.633
	Major occupation	8.574	7.411	0.014*

4.3 Survey for honey consumers in Accra and Ho Metropolis of Ghana

4.3.1 Background information on honey consumer respondents

The demographic characteristics of honey consumers in Accra and Ho Metropolis of Ghana are shown in Table 4.9 below.

Table 4.9: Background information on honey consumers in Accra and Ho Metropolis of Ghana

Parameters	Category	Frequency (n)*	Percentage (%)
Gender	Male	101	38.8
	Female	159	61.2
Age group (years)	Less than 20	38	14.6
	20 – 29	174	66.9
	30 – 39	20	7.7
	40 – 49	12	4.6
	50 – 59	9	3.5
	60 and above	7	2.7
Ethnicity	Ga-Adangbe	44	16.9
	Mole-Dagbani	6	2.3
	Ewe	91	35.0
	Akan	112	43.1
	^c Others	7	2.7
Religion	Christianity	253	97.3
	Islam	6	2.3
	Buddhism	1	0.4
Educational status (formal)	No formal education	4	1.5
	Basic	22	8.5
	Secondary	69	26.5
	Tertiary	165	63.5
Major occupation	Professionals	43	16.5
	Artisans	12	4.6
	Traders	17	6.5
	Students	188	72.3

***(N = 260)** Professional: Lecturers, Nursing officers, Regulatory officers, Pharmacist
 Artisans: Electricians, Welders ^cOthers: Talensi, Mamprusi, Hausa, Igbo, Kassena, Asona

Consumption of honey spanned through all the classified age groups, even though it appeared more popular with the younger consumers (29 years and below) than the older ones. This could possibly be due to recent trend of health and wellness consciousness among this age group. These were mostly students in tertiary institutions and were possibly aware of the nutritional and physiological benefits of honey in the diet. There is the belief that consumption of honey gives retentive memory or high intelligent quotient (IQ) for excellent academic performance. Majority of the respondents indicated that high price and inaccessibility affected their honey consumption rates. However, greater number of honey consumers purchased honey from supermarkets and distributors or retailers (Table 4.10).

4.3.2 Honey quality indices perceived/important to honey consumers

Food quality evaluation is commonly used by consumers to assess acceptability of sensory characteristics of a food. It is also the index used by government and international bodies to regulate safety of food product. These sensory properties are important determinants of whether or not a food product will be purchased. The sensory characteristics considered most important to consumers in this study were taste, thickness, flavour and appearance (colour) (Figure 4.5).

Table 4.10: Honey consumption history of consumers

Parameters	Category	Frequency (n)*	Percentage (%)
What is your status?	Consumer	258	99.2
	Both consumer and seller	2	0.8
How often do you eat honey?	More than once a week	31	11.9
	Once a week	22	8.5
	Every two weeks	4	1.5
	Every month	36	13.8
	Rarely	167	64.2
Where do you buy your honey?	Super markets	87	33.5
	Producers	77	29.6
	Distributers	87	33.5
	Pharmaceutical shops	9	3.5
Why do you buy honey from this or these source(s)	Quality	149	57.3
	Affordability	21	8.1
	Accessibility	47	18.1
	Convenience	14	5.4
	Reliability	4	1.5
	Quality and affordability	6	2.3
	Quality and accessibility	3	1.2
	Quality and convenience	2	0.8
	Quality and reliability	3	1.2
	Affordability and accessibility	3	1.2
	Affordability and reliability	1	0.4
	Affordability and convenience	6	2.3
	Accessibility and convenience	1	0.4
	Accessibility and reliability		

(N = 260)

Majority of the consumers perceived taste as the most important quality index. They indicated that it is the surest quality index that determines sugar adulteration of honey precisely without any cost and it is fast.

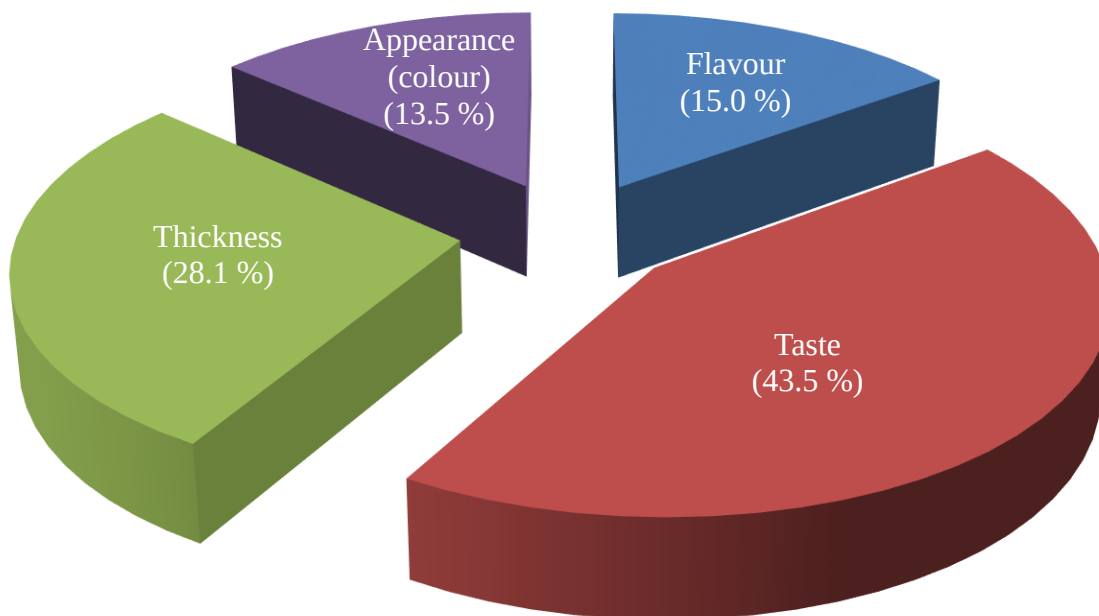


Figure 4.5: Quality characteristics consumers look out for in honey

4.3.3 Association between demographic variables and honey consumption habits of honey consumers

Despite the numerous physiological benefits of honey (specified in chapter two under nutritional characteristics of honey), it is not consumed on regular basis. Majority of honey consumers rarely consume honey and consume as and when they get it. Correspondingly, most of the honey consumers are people mindful of their health and wellness and are aware of the benefits of honey or incorporating it in their diets. The

associations between selected demographic variables and some indicators of honey consumption habits are shown in Table 4.11 below.

Gender, age group, formal educational status and major occupation of the honey consumers significantly associated with their frequency of honey consumption. More than half of the respondents rarely consumed honey. Amid all the demographic variables, only ethnicity of the honey consumers significantly associated with the quality characteristics they checked in honey before buying. There was also significant association between religion, formal educational status and major concerns about honey. The major concern the respondents had was about adulteration. Additionally, ethnicity, formal educational status and major occupation showed significant association with the packages in which honey was bought.

The limitation to the consumption of honey was due to adulteration (with Styrofoam, candle, bee wax, sugar and water during production or retail). Although many consumers complained about honey adulteration, it did not have much influence on their consumption of honey. The survey showed that consumers are more likely to continue eating honey when made available at affordable prices with high quality.

Table 4.11: Pearson Chi Square test for selected dependent variables on demographic characteristics for responses of honey consumers (n = 260)

Cross tabulation		Pearson Chi	Likelihood	p-value
Dependent variable	Demographic variable	Square test	Ratio	
Research question:	Gender	10.339	10.176	0.035*
How often do you eat honey?	Age group	34.675	30.332	0.022*
	Ethnicity	11.182	14.039	0.798
	Religion	2.503	4.220	0.962
	Formal educational status	29.710	19.223	0.003*
	Major occupation	40.289	36.821	0.000*
Research question:	Gender	3.524	3.499	0.318
What are the quality characteristics you look out for in honey you are going to buy?	Age group	23.671	20.494	0.071
	Ethnicity	25.456	26.087	0.013*
	Religion	1.559	1.930	0.956
	Formal educational status	8.533	9.596	0.481
	Major occupation	9.246	9.168	0.415
	Gender	84.649	112.976	0.369
	Age group	296.443	181.313	1.000
	Ethnicity	324.489	210.728	0.482
	Religion	253.146	42.690	0.000*
What are the major concerns you have about honey?	Formal educational status	290.530	161.048	0.020*
	Major occupation	159.780	121.807	1.000
	Gender	1.337	1.688	0.855
Research question: In what package do you buy your honey?	Age group	18.926	20.478	0.527
	Ethnicity	28.650	34.141	0.026*
	Religion	5.944	6.009	0.654
	Formal educational status	28.814	24.781	0.004*
	Major occupation	43.617	35.878	0.000*

4.4 Microbiological status of artisanal honey on the markets in the Accra Metropolis of Ghana

In general, based on the samples analysed, the microbiological quality of artisanal honey on the markets was acceptable according to international standards. The few samples that were contaminated might be due to handling and packaging practices, which were not aseptic. Total mesophilic bacteria, yeast and moulds and *Salmonella* were counted at varying levels with some exceeding standard values (Table 4.12). However, no total coliforms, faecal coliforms, *Escherichia coli*, sulphite-reducing *Clostridia* spores and *Botulinum* toxin were detected in the artisanal honey samples. The generally low microbial counts of the artisanal honey samples were due to the intrinsic properties (low pH, low moisture content, low water activity, hygroscopicity, hydrogen peroxide – antimicrobial property among others) of honey which inhibits microbial growth and survival.

The artisanal honey samples showed a wide range of total mesophilic bacteria counts from 0.00 to 4.78 log₁₀CFU/g, with an average of 3 log₁₀CFU/g and a standard deviation ± 1 log₁₀CFU/g (Table 4.12) which falls within the legislative limits (1×10^4 CFU/g or 4.00 log₁₀CFU/g) set by Mercado Común del Sur (MERCOSUR) and Código Alimentario Argentino (CAA) (Pucciarelli *et al.*, 2014). However, approximately 13 % of the artisanal honey samples (2 out of 16) had total mesophilic bacteria counts higher than the established maximum limit (1×10^4 CFU/g or 4.00 log₁₀CFU/g). On the whole, the overall total mesophilic bacteria populations enumerated in all the artisanal honeys were not statistically ($p > 0.05$) significant (Table 4.12). Mesophilic bacteria (likewise yeast and moulds) are commercial quality parameters hence their presence alone can affect the

stability, shelf-life and resistance of artisanal honey to microbial spoilage during storage (Prisca *et al.*, 2014).

The yeast and moulds values enumerated in artisanal honey samples A, D, E, F, H and J were far higher than the maximum limit value set by MERCOSUR (1×10^2 CFU/g or $2.00 \log_{10}$ CFU/g) (Finola *et al.*, 2007). Approximately 44 % of the artisanal honey samples (7 out of 16) had counts for yeast and moulds (Table 4.12). In addition, the overall counts of yeast and moulds in all the artisanal honey were statistically ($p < 0.001$) significant (Table 4.12). The subsistence of yeast and moulds in honey is due to their ability to grow under high sugar concentrations even with limited available water (Snowdon and Cliver, 1996). But from microbiological point of view, the high count of yeast and moulds than the maximum limit value (1×10^2 CFU/g or $2.00 \log_{10}$ CFU/g), is indicative of inappropriate management of apiaries, unsatisfactory sanitation, manufacturing practices, general hygiene or non-conformance to acceptable conditions for processing premises. The high count of yeast and moulds can affect the stability and shelf-life of artisanal honey.

The result also showed that three (3) out of sixteen (16) (approximately 19 %) artisanal honey samples contained *Salmonella* species (Table 4.12). *Salmonella* population enumerated in all the artisanal honey samples was statistically ($p < 0.001$) significant (Table 4.12). Work done by Gomes *et al.* (2010), Iglesias *et al.* (2012), Pota and Aruna (2013) and Pucciarelli *et al.* (2014) did not detect *Salmonella* in honey samples. However, Musa *et al.* (2014) detected *Salmonella* in two (2) honey samples out of a total of nine (9) market honey samples sourced from different markets in Khartoum, Sudan. Some species or strains of *Salmonella* is a food borne pathogen and causative agent of

many gastrointestinal tract infections (Pota and Aruna, 2013). Hence, its presence in artisanal honey makes them unsafe for consumption.

4.5 Antimicrobial activity of the artisanally produced honey

The result showed varying degrees of *in vitro* growth inhibition activity of artisanal honey against the tested organisms. The lower concentrations (30 – 70 %) of the artisanal honey had greater inhibitory effect on microbial cultures compared to 100 % concentration. Honey contains three main enzymes namely diastase (amylase); which decomposes starch or glycogen into smaller sugar units; invertase (sucrase, α -glucosidase), which decomposes sucrose into fructose and glucose, as well as glucose oxidase which produces hydrogen peroxide alongside gluconic acid from glucose in the presence of water (Bogdanov *et al.*, 2008). The lower concentrations (30 – 70 %) were dilutions and probably contained just enough water needed for glucose oxidase activity to produce peroxide and gluconic acid (resulting in low pH) giving higher inhibition factor as obtained. On the other hand the 10 % concentrations contained too much water while the 100 % concentrations contained little water needed for effective and efficient activity of glucose oxidase. This might be the reason for the difference in the inhibition factors of the different sample concentrations.

Table 4.12: Average microbial count and detection of *Botulinum* toxin of artisanal honey from different sources in Ghana

Honey sample	Average count in log ₁₀ (CFU/g)							
	TMB	Total coliform	Faecal coliform	<i>Escherichia coli</i>	Y&M	<i>Salmonella</i>	SCs	<i>Botulinum</i> toxin
Honey A	4.00 ^b	nd	nd	nd	5.36 ^d	nd	nd	
Honey B	2.00 ^{ab}	nd	nd	nd	nd	nd	nd	nd
Honey C	2.00 ^{ab}	nd	nd	nd	nd	nd	nd	
Honey D	3.80 ^b	nd	nd	nd	5.60 ^d	nd	nd	nd
Honey E	4.20 ^b	nd	nd	nd	5.45 ^d	5.70 ^c	nd	
Honey F	4.78 ^b	nd	nd	nd	5.50 ^d	5.70 ^d	nd	
Honey G	2.00 ^{ab}	nd	nd	nd	nd	nd	nd	nd
Honey H	4.00 ^b	nd	nd	nd	5.10 ^{cd}	5 ^b	nd	
Honey I	3.80 ^b	nd	nd	nd	nd	nd	nd	
Honey J	nd	nd	nd	nd	3.80 ^c	nd	nd	nd
Honey K	nd	nd	nd	nd	nd	nd	nd	
Honey L	2.00 ^{ab}	nd	nd	nd	nd	nd	nd	
Honey M	3.80 ^b	nd	nd	nd	2.00 ^b	nd	nd	
Honey N	3.80 ^b	nd	nd	nd	nd	nd	nd	nd
Honey O	2.00 ^{ab}	nd	nd	nd	nd	nd	nd	
Honey P	4.00 ^b	nd	nd	nd	nd	nd	nd	
p-value	0.200				< 0.001	< 0.001		

Values in column with the same superscript are not significantly different

CFU: Colony Forming Unit

nd: not detected

Y&M: Yeast and Mould TMB: Total Mesophilic Bacteria

SCs: Sulfite-reducing *Clostridium* spores

Among all the artisanal honey samples, A, D, and E had relatively lower antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Similarly, artisanal honey sample N had relatively lower antimicrobial activity against *Staphylococcus aureus*. Seventy-five per cent of the artisanal honey samples had minimum inhibition concentration (MIC) of 85 % and above against *Staphylococcus aureus* while approximately eighty-one per cent had MIC of 91.76 % and above against *Escherichia coli*. Artisanal honey samples K and P had the highest antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* respectively.

The high antimicrobial activity of the artisanal honey samples might be due to the osmotic effect, the effect of low pH, and the sensitivity of these organisms to hydrogen peroxide which are unsuitable for bacterial growth, represented as an inhibition factor in honey (Postmes *et al.*, 1993; Minisha and Shyamapada, 2011). According to Gomes *et al.* (2010), the concentration of honey which inhibits 50 % the specific microbial growth rate (IC 50 %) of microorganisms is used as the parameter of the significant inhibitory effects induced by honey in the growth of microorganisms. All the different concentrations (10, 30, 50, 70 and 100 % v/v) of the artisanal honey samples showed growth inhibition against *Escherichia coli* and *Staphylococcus aureus* (Table 4.13). The different concentrations of all the artisanal honey samples had good growth inhibitory effect (more than 50 % Minimum Inhibition Concentration, MIC) on the tested microorganisms (Table 4.13). Similar results were reported by Agbaje *et al.* (2006) for *E. coli* and *K. pneumonia*, Hern *et al.* (2009) for *A. baumannii*, Mohapatra *et al.* (2011) for *E. coli* and *P. aeruginosa* and Alqurashi *et al.* (2013) for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*.

The antimicrobial activity of the artisanal honey against *Escherichia coli* was relatively higher than that of *Staphylococcus aureus*. This result contradicts the findings of Hegazi (2011), Hegazi and Fyrouz (2012) and Khairy *et al.* (2013) who reported less growth inhibition activity of honey against *Escherichia coli* than other bacteria. Variations in antimicrobial activity are due to changes in the level of hydrogen peroxide achieved and in some cases to the level of non-peroxide factors. According to Molan and Russell (1988), the content of non-peroxide factors clearly relates to the floral source and sometimes accounts for the major part of the antibacterial activity in honey. Molan and Cooper (2000) also reported that the difference in antimicrobial potency among different honey can be more than hundred fold (100-fold), depending on its geographical, seasonal and botanical source. These reasons could have accounted for the slight differences in the antimicrobial activity of the artisanal honey samples obtained. Antimicrobial activity is very important therapeutically, especially in situation where the body's immune response is insufficient to clear infection (Manyi-Loh *et al.*, 2011). This study therefore provided an insight on the antibacterial activity of artisanal honey of Ghana and proved that many Ghanaian artisanal honey have the potential for therapeutic use as antibacterial agents.

Table 4.13: MICs of honeys at different concentrations against *Escherichia coli* and *Staphylococcus aureus*

Honey sample	Concentration (%)	<i>Escherichia coli</i> MIC (%)	<i>Staphylococcus aureus</i> MIC (%)	Honey sample	Concentration (%)	<i>Escherichia coli</i> MIC (%)	<i>Staphylococcus aureus</i> MIC (%)
Honey A	10	66.36 ± 0.09	53.54 ± 0.09	Honey C	10	95.80 ± 0.04	98.2±0.1
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	90.66 ± 0.09	91.11 ± 0.08		100	98.88 ± 0.06	99.53±0.04
Honey B	10	97.9 ± 0.0	98.01 ± 0.09	Honey D	10	76.84 ± 0.03	68.16±0.07
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	98.9 ± 0.1	99.9 ± 0.0		100	89.07 ± 0.07	81.37±0.04

MIC: Minimum Inhibition Concentration

Keys

Honey A = Apiary honey from Nkwanta (North Volta)

Honey B = Apiary honey from Ho (West Volta)

Honey C = Apiary honey from Nkwanta (North Volta)

Honey D = Forest honey from Dzolokpuita (West Volta)

Table 4.13: Continuation

Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
		MIC (%)	MIC (%)			MIC (%)	MIC (%)
Honey E	10	78.76 ± 0.09	57.97 ± 0.04	Honey G	10	91.76 ± 0.05	85.2±0.0
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	96.45 ± 0.07	90.08 ± 0.09		100	96.9 ± 0.0	91.67±0.04
Honey F	10	93.00 ± 0.05	87.5 ± 0.1	Honey H	10	92.87 ± 0.08	89.4±0.0
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	96.8 ± 0.0	99.79 ± 0.07		100	93.7 ± 0.2	92.5±0.0

MIC: Minimum Inhibition Concentration

Keys

Honey E = Forest honey from Dzolokpuita (West Volta)

Honey F = Forest honey from Somanya (Eastern Region)

Honey G = Honey from Mallam Atta market

Honey H = Honey from Achimota market

Table 4.13: Continuation

Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
		MIC (%)	MIC (%)			MIC (%)	MIC (%)
Honey I	10	98.1 ± 0.0	95.3 ± 0.0	Honey K	10	99.3 ± 0.0	97.0±0.0
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	100.0 ± 0.0	99.82 ± 0.08		100	100.0 ± 0.0	100.0±0.0
Honey J	10	98.4 ± 0.0	98.8 ± 0.0	Honey L	10	98.2 ± 0.0	97.3±0.0
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	99.4 ± 0.0	100.0 ± 0.0		100	100.0 ± 0.0	98.7±0.0

MIC: Minimum Inhibition Concentration

Keys

Honey I = Honey from Dome market

Honey J = Honey from Nima market

Honey K = Honey from Agbogbloshie market

Honey L = Honey from Kaneshie market

Table 4.13: Continuation

Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
		MIC (%)	MIC (%)			MIC (%)	MIC (%)
Honey M	10	91.9 ± 0.8	90.4 ± 0.7	Honey O	10	98.4 ± 0.0	85.0±0.0
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	95.8 ± 0.0	93.8 ± 0.0		100	99.8 ± 0.0	93.9±0.0
Honey N	10	98.0 ± 0.0	79.4 ± 0.0	Honey P	10	98.5 ± 0.0	99.4±0.0
	30	> 100	> 100		30	> 100	>100
	50	> 100	> 100		50	> 100	>100
	70	> 100	> 100		70	> 100	>100
	100	100.0 ± 0.0	86.3 ± 0.0		100	100.0 ± 0.0	100.0±0.0

MIC: Minimum Inhibition Concentration

Keys

Honey M = Honey from Makola market

Honey N = Honey from Timber market

Honey O = Honey from Ashaiman market

Honey P = Honey from Madina market

4.6 Physicochemical quality of the artisanal honey

The mean scores of the 16 samples and basic statistics obtained for refractive index, moisture content, total solids, total soluble solids, pH, water activity, electrical conductivity, ash, hydroxymethylfurfural and free acidity are summarized in Table 4.14 below. There was significant difference ($p < 0.05$) in the physicochemical parameters of all the artisanal honey samples analysed (Table 4.14).

4.6.1 Refractive index (RI)

The refractive index of the samples ranged from 1.481 to 1.497 with an average of 1.489 ± 0.005 . The measured values agree with those reported by Youssef and El-Gadawy (1973), Al-khalifa and Al-Arif (1999), 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, 1975a). The less variation in the refractive index values of the artisanal honey analysed confirms less variation in their moisture content. This helped prevent microbial contamination and activities stated on page 71 above. The artisanal honey samples in this study therefore might have a stable shelf life without crystallization and fermentation.

4.6.2 Moisture content (MC)

Moisture contents ranged from 15.8 to 22.2 %, with an average of 19 ± 2 % (Table 4.14). The difference in moisture content was significant ($p < 0.05$) between all the samples analysed. 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

(Acquarone *et al.*, 2007). High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow. It also greatly affects the rate of fermentation, its shelf life span and processing characteristics of honey (Getachew *et al.*, 2014).

A regulation in Serbia cited by Sl. list SCG (2003), which is harmonized with the EU Directive (EU Council, 2002), fixes a maximum moisture content in honey sold in the market at 20 %. Most of the artisanal honey samples analysed had lower percentage moisture content than the limit (20 %) set by the Codex Alimentarius Standard (Serem and Bester, 2012) and EU Directive (EU Council, 2002) for honey. 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). Higher moisture content could lead to undesirable honey fermentation during storage caused by the action of osmotolerant yeasts resulting in the formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized to acetic acid and water resulting in a sour taste (Saxena *et al.*, 2010).

Fasasi (2012), reported that the chemical composition of honey dictates the grades and the market value of honey, either Grade A (14 – 16 % moisture content), Grade B (17 – 18 % moisture content), Grade C (19 – 21 % moisture content) or Grade D (> 21 % moisture content), depending on the physicochemical parameters such as the moisture content, hydroxymethylfurfural, diastase activities and pH. Based on this, the classification of all the artisanal honey samples analysed cut across all these grades. By

Ethiopian Standards, the moisture content of honey is grouped into three grades: Grade A (17.50 – 19.00 g/100 g), Grade B (19.10 – 20.00 g/100 g) and Grade C (20.10 – 21.00 g/100 g) (Belay *et al.*, 2013). The internationally set limit (20 %) for honey moisture content indicates the degree of maturity of honey against possible fermentation. The moisture content of some of the samples analysed (D, E, F, G and M) thus, suggest that some honey on the Ghanaian markets showed insufficient maturity in the honey combs and could ferment during storage.

4.6.3 Total solids (TS)

The average total solids value of the artisanal honey samples analysed in the present study was 81 ± 2 g/100 g (Table 4.14), ranging from 77.8 g/100 g to 84.2 g/100 g. Fifty-six per cent (56 %) of the artisanal honey samples (A, B, C, D, G, H, N, O and P) had total solids values that agreed with those (78.4 – 82.8 %) reported by Saxena *et al.* (2010) while forty-four per cent (44 %) of samples (C, I, J, K, L, N and P) had total solids values that agreed with those (81.73 – 84.33 %) reported by Al-Khalifa and Al-Arif (1999). Total solids relate to moisture content. The high the total solid content, the low the moisture content and vice versa. Generally, all the artisanal honey analysed 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).

4.6.4 Total soluble solids (TSS)

Total soluble solids (sugar content) of the artisanal honey sample ranged from 76.3 % to 82.9 % for the samples (Table 4.19). The mean TSS was 79 ± 2 %. Some of the differences were statistically ($p < 0.05$) significant. 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). The high sugar content of the artisanal honey analysed 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 artisanal honey analysed might make them vital both in processing and for final use and antimicrobial in nature, contributing to their high antimicrobial activity exhibited as stated on page 75 above.

4.6.5 pH

All artisanal honey samples analysed were acidic in nature, with pH values ranging from 3.79 to 5.39 and the mean of 4.5 ± 0.5 (Table 4.14). 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). Low pH of honey is attributed to the presence of organic acids such as gluconic, pyruvic, malic and citric acids (Bogdanov *et al.*, 2004), and the variation in pH of different honey samples is described to be due to floristic composition and floral diversity (Fahim *et al.*, 2014). The low pH of honey inhibits the presence and growth of microorganisms. This parameter has great importance during the extraction and storage of honey, as it influences the texture, stability and shelf life of honey (Gomes *et al.*, 2010). Gomes *et al.* (2010) also reported that pH of honey has not been legislated. However, Olugbemi *et al.*

(2013) stated 3.7 – 4.2 to be the pH limits of international standard and IHC (2002) recommends pH of 3.2 to 4.5.

4.6.6 Water activity (a_w)

Water activity in all the sixteen (16) artisanal honey samples studied ranged from 0.591 to 0.690 with an average of 0.63 ± 0.03 (Table 4.14). The difference in water activity was significant ($p < 0.05$) between all the samples analysed (Table 4.14). These results were comparable to those (0.57 – 0.70) reported by Saxena *et al.* (2010) and quite similar to those of Greek honey for which the water activity values ranged from 0.53 to 0.67 (Lazaridou *et al.*, 2004). Like pH of honey, water activity of honey is also not legislated (Gomes *et al.*, 2010). However, water activity is an important factor, which governs food stability by preventing or limiting microbial growth. In several cases water activity is the primary parameter responsible for food stability, modulating microbial response and determining the type of microorganisms encountered in food (Chirife *et al.*, 2006). Fernández-Salguero *et al.*, (1993) reported that food stability usually decreases with increasing water activity and when it is greater than 0.950, bacteria make up the predominant flora in most types of food. Nevertheless, when water activity is below 0.950, yeasts and moulds are the cause of most biological alterations of foods (Hocking, 1988). Osmotolerant yeasts are able to grow at a minimal water activity of 0.6 (Chirife *et al.*, 2006). Almost all the artisanal honey samples had higher water activity above the minimum required for the growth of osmotolerant yeasts, whose activities could affect the stability and shelf life of the samples.

Table 4.14: Physicochemical quality of artisanal honey from different sources in Ghana

Honey sample	RI	MC (%)	TS (g/100g)	TSS (%)	pH	_a W	EC (mS/cm)	Ash (%)	HMF (mg/kg)	FA (mEq. Acid/Kg)	Colour*
Honey A	1.487 ^{de}	19.7 ^h	80.3 ^d	79.1 ^e	4.32 ^f	0.644 ⁱ	0.5956 ⁿ	0.04180 ⁿ	12.2 ^d	56.3 ^l	Dark amber
Honey B	1.491 ^{fg}	18.4 ^e	81.6 ^g	80.0 ^h	4.58 ^j	0.629 ^h	0.3046 ^f	0.01764 ^f	27.1 ⁿ	20.3 ^{bc}	Dark amber
Honey C	1.493 ^{gh}	17.6 ^c	82.4 ⁱ	80.9 ^j	4.42 ^h	0.620 ^e	0.3053 ^g	0.01770 ^g	16.9 ^h	25.3 ^e	Dark amber
Honey D	1.483 ^{ab}	21.4 ⁱ	78.6 ^c	77.0 ^c	4.30 ^f	0.677 ^m	0.4749 ^k	0.03178 ^k	8.9 ^b	49.7 ^j	Dark amber
Honey E	1.481 ^a	22.2 ^k	77.8 ^a	76.3 ^a	4.12 ^e	0.680 ⁿ	0.5045 ^l	0.03424 ^l	9.7 ^c	53.0 ^k	Dark amber
Honey F	1.482 ^{ab}	21.8 ^j	78.2 ^b	76.6 ^b	3.79 ^a	0.682 ^o	0.5683 ^m	0.03953 ^m	18.9 ⁱ	41.3 ⁱ	Dark amber
Honey G	1.486 ^{cd}	21.4 ⁱ	78.6 ^c	81.0 ^j	4.76 ^l	0.617 ^d	0.3638 ^j	0.02256 ^j	15.7 ^g	30.0 ^g	Dark amber
Honey H	1.489 ^{def}	19.2 ^g	80.8 ^e	79.2 ^f	4.34 ^g	0.645 ^j	0.6520 ^o	0.04648 ^o	22.5 ^l	52.0 ^k	Dark amber
Honey I	1.497 ⁱ	15.8 ^a	84.2 ^k	82.9 ^l	5.39 ^o	0.591 ^a	0.2838 ^e	0.01592 ^e	14.4 ^{ef}	19.7 ^b	Dark amber

* - USDA Colour Standard

*Values in column with the same superscript are not significantly different*Keys

Honey A = Apiary honey from Nkwanta (North Volta)

Honey B = Apiary honey from Ho (West Volta)

Honey C = Apiary honey from Nkwanta (North Volta)

Honey D = Forest honey from Dzolokpuita (West Volta)

Honey E = Forest honey from Dzolokpuita (West Volta)

Honey F = Forest honey from Somanya (Eastern Region)

Honey G = Honey from Mallam Atta market

Honey H = Honey from Achimota market

Honey I = Honey from Dome market

Table 4.14: Continuation

* - USDA Colour Standard			Values in column with the same superscript are not significantly different								
Honey sample	RI	MC (%)	TS (g/100g)	TSS (%)	pH	_a W	EC (mS/cm)	Ash (%)	HMF (mg/kg)	FA (mEq. Acid/Kg)	Colour*
Honey J	1.494 ^h	17.0 ^b	83.0 ^j	81.4 ^k	5.19 ⁿ	0.648 ^k	0.1723 ^b	0.00666 ^b	14.4 ^e	8.7 ^a	Light amber
Honey K	1.484 ^{bc}	17.0 ^b	83.0 ^j	77.4 ^d	3.81 ^b	0.665 ^l	0.1345 ^a	0.00352 ^a	22.8 ^m	21.3 ^c	Amber
Honey L	1.494 ^h	17.0 ^b	83.0 ^j	81.3 ^k	5.01 ^m	0.610 ^b	0.1924 ^c	0.00834 ^c	28.5 ^o	20.7 ^{bc}	Dark amber
Honey M	1.482 ^{ab}	21.8 ^j	78.2 ^b	76.7 ^b	4.10 ^d	0.690 ^p	0.8822 ^p	0.06559 ^p	7.0 ^a	50.3 ^j	Dark amber
Honey N	1.492 ^{fg}	18.2 ^d	81.8 ^h	80.0 ^{hi}	4.53 ⁱ	0.623 ^f	0.2513 ^d	0.01322 ^d	22.0 ^k	23.7 ^d	Dark amber
Honey O	1.490 ^{ef}	18.8 ^f	81.2 ^f	79.6 ^g	3.98 ^c	0.615 ^c	0.3438 ^h	0.02090 ^h	14.7 ^f	37.0 ^h	Dark amber
Honey P	1.491 ^{fg}	18.2 ^d	81.8 ^h	80.1 ⁱ	4.71 ^k	0.624 ^g	0.3473 ⁱ	0.02119 ⁱ	19.9 ^j	28.0 ^f	Dark amber
p-values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
RI – Refractive Index			MC – Moisture Content			TS – Total Solids			TSS – Total Soluble Solids		
EC – Electrical Conductivity			HMF – Hydroxymethylfurfural			FA – Free Acidity			_a W – Water activity		

Keys

Honey K = Honey from Agboglobloshie market

Honey J = Honey from Nima market

Honey L = Honey from Kaneshie market

Honey M = Honey from Makola market

Honey N = Honey from Timber market

Honey O = Honey from Ashaiman market

Honey P = Honey from Madina market

4.6.7 Electrical conductivity (EC)

The electrical conductivity of the artisanal honey samples varied in the range of 0.1345 – 0.8822 mS/cm (Table 4.14), with the mean of 0.4 ± 0.4 mS/cm. The values were significantly different from each other ($p = 0.00$) (Table 4.14). These results compared well with the findings (0.414 – 1.748 mS/cm) of Karabagias *et al.* (2014), 0.224 – 1.168 mS/cm of Escuredo *et al.* (2012) and 0.1 to 0.4 mS/cm of Kirs *et al.* (2011). According to Soria *et al.* (2003), artisanal honey may have a minimum electrical conductivity value of 0.119 mS/cm and a maximum value of 1.515 mS/cm. Electrical conductivity depends on ash, organic acids, proteins, some complex sugars and polyols contents, and varies with botanical origin (Belay *et al.*, 2013). It also depends on the ash content in honey: the higher the ash and acid content, the higher the resulting conductivity (Kirs *et al.*, 2011). Electrical conductivity is widely used for discrimination between honeydew and blossom honey and also for the characterization of unifloral honey (Chefrour *et al.*, 2009). Codex Alimentarius Committee on Sugars (2001) and European Commission (2002) stated that the botanical origin of honey is often classified into two classes: blossom and honeydew honey. Classifying honey into blossom or honeydew origin is based on the measurement of electrical conductivity.

According to Bogdanov (2007), honey with electrical conductivity values higher than 0.8 mS/cm are classified as honeydew honey, whilst those with electrical conductivity values lower than 0.8 mS/cm are classified as blossom honey or blends of blossom with honeydew honey. Honey with an electrical conductivity value ranging from 0 to 0.8 mS/cm are classified as honey produced from nectar without mixture compounds of honeydews and nectar blossom (Belay *et al.*, 2013). Accordingly, all the artisanal honey samples studied with the exception of artisanal honey sample M could be classified as

honey produced from nectar without mixture compounds of honeydews and nectar blossom.

4.6.8 Ash

The percentage ash content is an indicator of the mineral content. It is considered as a quality criterion indicating the possible botanical origin of honey. Its value in the analysed artisanal honey samples ranged from 0.00352 to 0.06559 % with a mean of 0.03 ± 0.02 % (Table 4.14). Generally, the ash content of blossom honey is ≤ 0.6 % as compared to honeydew honey or blends of honeydew and blossom honey which is ≥ 1.2 % (Ouchemoukh *et al.*, 2007). Thus based on this criterion, the analysed artisanal honey samples could be regarded blossom honey. The ash content values of the artisanal honey sample are comparable to commercial Indian honey brands (0.03 to 0.43 %) reported by Saxena *et al.* (2010), the blossom honey of Romania (0.03 to 0.40 %) (Al *et al.*, 2009) and also with Algerian honey (0.06 – 0.54 %) (Ouchemoukh *et al.*, 2007). The variability in the ash content of the artisanal honey of this study could be due to harvesting processes, and the material collected by the bees during foraging on the flora, as suggested by Finola *et al.* (2007).

4.6.9 Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural is a breakdown product of fructose that is formed slowly and naturally during the storage of honey and much more quickly when honey is heated. The hydroxymethylfurfural range of the samples was 7.0 mg/kg to 28.20 mg/kg with the mean value of 17 ± 6 mg/kg. These results were comparable to results published by Kahraman *et al.* (2010) who reported a range of 7.68 to 52.6 mg/kg for retailed honey

and Juszczak *et al.* (2009) who reported a range of 5.6 mg/kg to 37.3 mg/kg for herb honey. The hydroxymethylfurfural content of all the samples analysed in this study, was however lower than the value (40 mg/kg) recommended by the Codex Alimentarius (Codex Alimentarius, 2001).

The hydroxymethylfurfural content is widely recognized as a parameter of honey freshness (de Rodríguez *et al.*, 2004; Gomes *et al.*, 2010; Kahraman *et al.*, 2010), an index of heat treatment of honey (Izhar-ul-Haq *et al.*, 2010) and therefore establishes shelf-life of honey (Belay *et al.*, 2013). It is absent in fresh honey and tends to increase during processing and/or aging of honey. The higher the hydroxymethylfurfural value the lower the quality of the honey is considered to be. Several factors such as temperature and time of heating, storage conditions, pH and floral source, influence the levels of hydroxymethylfurfural in honey, thus it provides an indication of overheating and storage in poor conditions (Fallico *et al.*, 2006). Even though all the artisanal honey samples showed levels of hydroxymethylfurfural lower than the allowed limit of 40 mg/kg, their levels above zero (0) are indicative of temperature abuse during processing (at high temperature above 75 °C) and/or bad storage (above 27 °C for months) practices as suggested by Rodgers (1979) and Izhar-ul-Haq *et al.* (2010). It also indicates that the artisanal honey analysed were not fresh.

4.6.10 Free acidity (FA)

The mean free acidity content of the artisanal honey samples was 34 ± 15 mEq/kg (8.7 – 56.3 mEq/kg), (Table 4.14). These results were within the range (12.0 – 134.5 mEq/kg) reported by Alqarni *et al.* (2012) but not all conformed to international standard. The allowed free acidity value limits is 50 mEq/kg (The Council of the European Union,

2002). The free acidity values of almost all the artisanal honey samples of this study were above the allowed limit. Free acidity of honey is explained in view of the presence of organic acids in equilibrium with their corresponding lactones or internal esters, and some inorganic ions such as phosphate and chloride. Acidity measurement is useful for evaluation of honey fermentation, authentication of unifloral honey and differentiating nectar from honeydew honey (Nanda *et al.*, 2003). High acidity can be indicative of fermentation of sugars into organic acids. Values for free acidity of artisanal honey samples A, E, H and M exceeding the EU limit may be taken as indicative of presence of undesirable fermentation.

4.6.11 Colour

The colours of all the artisanal honey samples analysed varied between light amber to dark amber (Table 4.14), which were similar to that (extra-light amber to dark amber) observed by Fasasi (2012) and amber to dark amber reported by Fahim *et al.* (2014), and within the prescribed colours by international standards (EEC, 1974; Codex Alimentarius, 2001a). Colour is the main optical property of food considered by consumers in evaluating its quality (Barbosa-Cánovas *et al.*, 2004/Rev.2006). The visual appearance of products is a critical determinant of consumer response and product success (Bloch, 1995). On the international market, colour of honey is the second most important factor determining import and wholesale prices (USAID, 2012). Darker honey are more often sold for large-scale commercial purposes, while lighter honey are marketed for direct consumption and demand a price premium over the darker ones.

The colour of natural honey is often an indication of their origin. Natural honey vary greatly in colour, ranging from nearly colourless (example clover honey) to very dark

(example buckwheat or honeydew honey). The dark honey are known to contain more minerals than the lighter ones (White, 1975a). Colour is affected by the harvesting method, storage time, and exposure to elevated temperature (Juszczak *et al.*, 2009). Batrusaityte *et al.* (2007) reported that the colour of honey is usually related to the mineral, pollen and phenolic contents of honey. Juszczak *et al.* (2009) also reported that the colour of honey is closely related to its chemical composition, primarily to the presence of pigments such as chlorophylls, carotenoids, flavonoids and derivatives of tannins and polyphenols. According to Jasicka-Misiak *et al.* (2012), higher levels of polyphenols in honey result in darker colour. In honeydew honey, the dark strong colour also results from the higher mineral contents and the presence of algae and green algae forming part of the flora of forest trees, and in herb honey, colour depends on the raw material composition of the food fed to bees, example is the addition of intensely red fruit juices (Juszczak *et al.*, 2009). The variations in the colour of the artisanal honey studied may probably be due to the nectar sources, age in the honey combs during storage by the honey bees, prolonged and continuous usage of honey comb cells for storage by honey bees, chemical composition, harvesting method, storage time after processing, and exposure to elevated temperatures as suggested by Fasasi (2012) and Juszczak *et al.* (2009).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The producers use simple traditional and non-standardized methods for production. Majority of the producers as well as the sellers, package, store and sell honey in recycled packaging materials. Adulteration of honey is perceived by the producers, sellers and consumers in the honey value chain. Taste, thickness, flavour and appearance (colour) are important indices of quality for the sellers and consumers of honey.

The microbiological quality of the samples was generally acceptable according to international standards. All the artisanal honey showed high sanitary quality. They had no coliforms (faecal and *Escherichia coli*). Handling and packaging practices, which are not aseptic, could be the reasons of the occurrence of total mesophilic bacteria, yeasts and moulds and *Salmonella* in the few contaminated samples. The occurrence of *Salmonella* in the samples, suggests that artisanally produced honey could be a vehicle for the transmission of pathogens to consumers.

The artisanal honey have good growth inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*, showing high antimicrobial activity. The antimicrobial activity of the artisanal honey against *Escherichia coli* is relatively higher than against *Staphylococcus aureus*.

Significant ($p < 0.05$) variations occur in the physicochemical parameters of the artisanal honey. The honey on the markets are of different grades, ranging from A to D based on a grading system which depends on the moisture content with Grade A as the best. The

majority of them are of Grade B (44 %) and only a few are of Grade A (6 %). They have low pH and also show some level of temperature abuse (with mean HMF = 17 ± 6 mg/kg). Furthermore, all the honey are blossom honey based on their ash content and most are dark amber in colour.

5.2 Recommendations

Further studies should be conducted on:

- Melissopalynology (study of pollens) of Ghanaian artisanally produced honey to authenticate their botanical, floral and geological origin.
- The sugar profile and fructose/glucose ratio of artisanally produced honey in Ghana.
- The enzymes, their mechanisms in relation to stability and shelf life and free amino acid composition of Ghanaian artisanally produced honey.
- The bioactive properties of honey artisanally produced honey in Ghana.
- The pesticide residue contamination of honey artisanally produced honey in Ghana.

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APPENDICES

APPENDIX A: SURVEY QUESTIONNAIRES

Appendix A1: Survey questionnaire for artisanal (local) honey producers

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

SCHOOL OF GRADUATE STUDIES

UNIVERSITY OF GHANA

QUALITY EVALUATION OF ARTISANALLY PRODUCED HONEY SOLD ON GHANAIAAN MARKETS

Dear respondent, this questionnaire seeks to solicit some information on the artisanal production of honey in Ghana, as part of an MPhil Food Science Thesis on the topic above. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you.

Date: _____

Region: _____

Area: _____

Type of honey produce: _____

Producer Code: _____

Kindly tick (✓) or circle the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

RESPONDENT'S BIOGRAPHY

1. Gender:.....Male/Female

2. Ethnicity:.....

3. Religion:.....

4. Occupation:.....

5. 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

6. Educational status (formal)

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

PRODUCTION OF HONEY

7. Local name of honey

8. How long have you been processing honey?

- a) Less than a year b) 1 – 5 years c) 6 – 10 years d) Longer than 10 years

9. What is the source of your honey for processing?

- a) Through culturing of bees b) From the wild/forest c) Crude honey from a processor

10. How did you acquire the skills of honey processing?

- a) From a relative who processes honey b) Through apprenticeship under a processor
c) Other, specify.....

11. Is honey processing your main occupation? (a) Yes (b) No

12. If no, what do you do in addition to the honey processing?

TECHNOLOGY

13. What steps do you follow in the processing of honey?

- a) f)
b) g)
c) h)
d) i)
e) j)

14. Please describe what you do at each step, equipment use and how these affect the yield and quality of the honey? (Indicate the time spent in each step). Where you use honey comb, please indicate the proportion of honey comb, pre-treatment and conditions of operations such as heating temperatures, squeezing, and pressing pressure you use? (if known)

Action at step	Equipment use	Effect on yield and quality	Time spent
a)			
b)			
c)			
d)			
e)			
f)			
g)			

Proportion of honey comb	Pre-treatment	Conditions of operations
.....		
.....		
.....		
.....		

15. Which of the steps listed in 14 do you consider important in achieving the desired quality in honey? (Give reasons)

Steps	Reasons
a)	
b)	
c)	

- d)
e)

16. How do you control the steps listed in 14 stated above?

Steps	Control
a)	
b)	
c)	
d)	
e)	

17. In which of these steps listed in 14 do you think improvement is necessary to improve yield and quality and ease of operation?

- | | |
|----------|----------|
| a) | e) |
| b) | f) |
| c) | g) |
| d) | h) |

18. Suggest any improvement you will want to see in the steps listed in 18?

Step	Improvement
a)	
b)	
c)	
d)	
e)	

19. Describe any other honey extraction process you know, as in 14. Indicate the variation with the previously described process and your reasons for a preference.

Extraction process	Variation from your process	Reasons for your preference
.....		
.....		
.....		
.....		
.....		
.....		

20. What quantities of honey do you process in a batch?

21. How do you assess the quality of your honey?

.....

.....

.....

22. Do you clarify your honey? (a) Yes (b) No

23. Is there any variation in your processing method(s) due to seasonal variations?

(a) Yes (b) No

24. If yes, do these variations affect the yield and quality of your honey?

(a) Yes (b) No

25. How do you control the quality during processing?

.....

.....

.....

EQUIPMENTS

26. List the equipments and instruments used for processing?

Equipment (Local/Foreign)	Uses
.....	
.....	
.....	
.....	
.....	
.....	
.....	

27. Which of the equipment do you own? (Indicate cost and when purchase)

Equipment	Cost	Date purchased
.....		
.....		
.....		
.....		
.....		

28. Which of these equipments do you rent?

.....

.....

29. Which of the equipments do you pay for services?

Equipment	Charges per batch of process
.....	
.....	

.....

.....

.....

HONEY QUALITY AND MARKETING

30. Are there any grading systems for processed (or marketing) honey? (a) Yes (b) No

31. If yes, what criteria are used for grading?

a) Intended uses

b) Colour

c) Thickness

d) Flavour

e) Taste

f) Other

32. How many grades are there and what are their characteristics?

Grade

Characteristics

.....	
.....	
.....	
.....	

33. What grade of product do you produce and why?

.....

.....

.....

34. Where do you market your honey?

35. How do you market your honey?

36. What are your units of measures and the prices that could be obtained for them?

Unit	Prices
.....	
.....	
.....	

37. Do you receive complaints from customers? (a) Yes (b) No

38. If yes, are the complaints recorded? (a) Yes (b) No

39. Is any action taken about customers' complaint? (a) Yes (b) No

40. Please describe the actions that might be taken with the kind of complaint?

Complaint	Action
.....	
.....	
.....	
.....	

41. Do you find it a problem to market your honey? (a) Yes (b) No

42. Describe the problems?

.....

.....

.....

43. Whom do you sell your honey to?

44. What is the average stable price per unit quantity?

BY-PRODUCTS

45. What kind of by-products do you produce?

46. What do you do with them?

.....

.....

.....

PACKAGING AND ADULTERATION

47. Do you use any specialized form of packaging? a) Yes b) No
48. What packaging materials do you use? a) Glass b) Plastic/Rubber c) Metal
49. What is the nature of the packages that you use?
- a) New packages b) Recycled packages
50. Do some processors add other ingredients to honey? a) Yes b) No
51. If yes, please list the ingredients and reasons for the addition.

Ingredient	Reasons for addition
a)	
b)	
c)	
d)	
e)	

52. Is adulteration of honey a known problem here? a) Yes b) No
53. Do buyers/customers complain of adulteration? a) Yes b) No
54. How do you detect adulteration in honey?
-
-

Thank you for your collaboration.

Appendix A2: Survey questionnaire for honey vendors (sellers)

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

SCHOOL OF GRADUATE STUDIES

UNIVERSITY OF GHANA

**QUALITY EVALUATION OF ARTISANALLY PRODUCED HONEY SOLD ON
GHANAIAI MARKETS**

Dear respondent, this questionnaire seeks to solicit some information on selling of artisanally produced honey in Ghana, as part of an MPhil Food Science Thesis on the topic above. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you.

Date: _____ Region: _____

Area/Market: _____ Vendor Code: _____

Kindly tick (✓) or circle the responses that apply to you. Where appropriate, write out your own responses in the spaces provided

RESPONDENT'S BIOGRAPHY

1. Gender:.....Male/Female
2. Ethnicity:.....
3. Religion:.....
4. Major Occupation:.....
5. 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

6. Educational status (formal)

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

SELLING OF HONEY

7. What is your status? a) Seller b) Both processor and seller

8. Is selling of honey your primary business? a) Yes b) No

9. If no, what do you do in addition?

10. How long have you been selling honey?

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

11. Where do you obtain your honey? (Please indicate the region)

- a) Direct from farm b) Wholesale point/Distributors c) Other retailers.....

Region

12. What volume/quantity do you buy at a time?

13. Do you store your honey? a) Yes b) No

14. If yes, how do you store your honey?

- a) Keep in package and store in a room b) Keep in package and store outside
c) Keep in open container in a room d) Keep in open container outside
e) Other, specify.....

15. How long do you store your honey?

16. Does your honey get bad/spoil when you store for a very long time? a) Yes b) No

17. If yes, how long?

18. What are some of the causes of the spoilage during storage?

.....
.....
.....

19. What are some of the steps you take to prevent or minimize the deterioration of your honey?

.....

.....

.....

.....

.....

20. How do you know that your honey is not good (spoiled) for consumption?

.....

.....

.....

21. What do you do with the spoilt honey? a) Mix with new batch and sell b) Do not use

22. How do you handle your honey during the period of sale?

- a) Packaged in aseptically 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

23. How long does it take you to sell a batch of honey?

24. Do you go in for fresh batch only when an old batch is completely sold out?

- a) Yes b) No

25. How do you dispense your honey for sale?

- a) Plastic bags b) Unused container c) Used container d) Already packaged
- e) Other, specify.....

26. Do you reprocess your honey before selling? a) Yes b) No

27 If yes, what do you do?

.....

.....

.....

28. Please give reasons for reprocessing?

.....

.....

.....

29. Do you sometimes see the need to add something to honey to improve its quality before selling? a) Yes b) No

30. If yes, what do you think can be added to honey to improve its quality and why?.....

.....

Ingredient	Reasons for addition
a)	
b)	
c)	
d)	
e)	

31. What **are the major problems** you encounter in selling of honey?

1.
2.
3.
4.
5.

32. What do you think can be done about these problems?

1.
2.
3.
4.
5.

HONEY QUALITY

33. How do you assure honey quality?

.....

.....

.....

34. What quality characteristics do you use in obtaining your supply of honey? (You can tick/circle more than one)

- a) Flavour b) Taste c) Colour d) Thickness e) Other, specify.....

35. Please rank these quality characteristics in order of importance? (**1 = Most important to 4 = Least important**)

- a) Flavour
- b) Taste
- c) Appearance (colour)
- d) Thickness

36. Rank these quality characteristics in the order that informs consumer judgment of your honey. (**1 = Most important to 4 = Least important**)

- a) Flavour
- b) Taste

c) Appearance (colour)

d) Thickness

37. What makes your honey competitive to other vendor's honey?

.....

.....

.....

38. Do you think consumers purchase your honey based on perceived quality?

a) Yes b) No

39. Do you think consumers will be willing to pay more for quality? a) Yes b) No

40. Have you ever rejected/refuse to buy a consignment or a batch of honey?

a) Yes b) No

41. What are some of the hygienic practices you observe?

a)

b)

c)

d)

e)

42. Do you know of processors or sellers that add other ingredients to honey?

a) Yes b) No

43. If yes, please list the ingredients and reasons for the addition

Ingredient

Reasons for addition

a)

b)

c)

d)

e)

44. Is adulteration of honey a known problem here? a) Yes b) No

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

BENEFITS OF HONEY

46. What makes you choose to sell honey?

.....

.....

47. Please list the benefits of honey?

Nutritional Benefits

Medicinal Benefits

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

Thank you for your collaboration.

Appendix A3: Survey questionnaire for honey consumers

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

SCHOOL OF GRADUATE STUDIES

UNIVERSITY OF GHANA

**QUALITY EVALUATION OF ARTISANALLY PRODUCED HONEY SOLD ON
GHANAIAI MARKETS**

Dear respondent, this questionnaire seeks to solicit some information on consumption of artisanally produced honey in Ghana, as part of an MPhil Food Science Thesis on the topic above. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you.

Date: _____ Region: _____
Area: _____ Consumer Code: _____

Kindly tick (✓) or circle the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

RESPONDENT'S BIOGRAPHY

1. Gender:.....Male/Female 2. Hometown:..... 3. Ethnicity:.....
4. Religion:..... 5. Major Occupation:.....
6. 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 year
 - e) 50 – 59 years f) 60 years and above

7. Educational status (formal)

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

CONSUMPTION OF HONEY

8. Do you eat honey? a) Yes b) No

9. What is your status?

- a) Consumer b) Both consumer and seller b) Both processor and consumer

10. How often do you eat honey?

- a) More than once a week b) Once a week c) Every two weeks d) Every month
e) Rarely f) Other, specify.....

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

- a) Super markets b) Processors c) Distributors d) Pharmaceutical shops
e) Other, specify.....

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

.....
.....

13. What are the quality characteristics you look out for in the honey that you are going to buy?

- a) Flavour b) Taste c) Thickness d) Appearance (colour)
e) Other, specify.....

14. What **are the major concerns** you have about honey?

1.
2.
3.
4.

5.
15. Give **suggestions** to address these concerns.
1.
2.
3.
4.
5.
16. What volume/quantity of honey do you buy?
17. How long does it take you to consume the honey you purchased?
18. How do you store your honey?
-
19. How long do you think honey can last before going bad?
- a) b) Don't know
20. How do you use honey?
- a) As a sweetener b) As a spread c) As an ingredient for food recipes
- d) As a beverage e) Other, specify.....

BENEFITS OF HONEY

21. Why do you eat honey? (You can tick/circle more than one) Please list the benefits.
- a) For nutritional purposes b) For medicinal purposes c) As a pleasure food
- d) Other, specify.....

Nutritional Benefits

Medicinal Benefits

.....	
.....	
.....	

.....

.....

PACKAGING OF HONEY

22. Is the honey that you buy packaged? a) Yes b) No
23. 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.....
24. What type of packaging materials do you want processors/vendors to use?
- a) Glass b) Plastic/Rubber c) Metal d) Other, specify.....

Thank you for your collaboration.

APPENDIX B: SURVEY RESULTS**Appendix B1: Skills and experience in honey production**

Parameters	Category	Frequency (n)*	Percentage (%)
How long have you been producing honey?	Less than a year	4	13.3
	1 – 5 years	19	63.3
	6 – 10 years	1	3.3
	Longer than 10 years	6	20.0
What is the source of your for production?	Through culturing of bees	15	50.0
	From the wild/forest	15	50.0
How did you acquire the skills of honey production?	From a relative who produces honey	20	66.7
	Through apprenticeship	1	3.3
	Others	9	30.0
Is honey production your main occupation?	Yes	1	3.3
	No	29	96.7
If no, what do you do in addition to the honey production	Carpentering	1	3.4
	Electrical wiring	1	3.4
	Farming	22	75.9
	Trading	3	10.3
	Training of beekeepers	1	3.4
	Civil servant (messenger)	1	3.4
(N = 30)	Others: Reading materials on beekeeping, attending of workshops		

Appendix B2: Quantity of honey obtained and honey combs used in a batch of honey production by artisanal producers surveyed

Parameters	Category	Frequency (n)*	Percentage (%)
Where you	40kg	17	56.7
use honey	60kg	1	3.3
combs, please	100kg	3	10.0
indicate the	120kg	6	20.0
proportion of	400kg	1	3.3
honey combs?	1000kg	1	3.3
	9000kg	1	3.3
What quantity	5.4431kg (per 40kg of honey combs)	17	56.7
of honey do	7.5000kg (per 60kg of honey combs)	1	3.3
you produce	13.1250kg (per 100kg of honey		
in a batch?	combs)	3	10.0
	81.0000kg (per 120kg of honey		
	combs)	6	20.0
	135.0000kg (per 400kg of honey		
	combs)	1	3.3
	880.0000kg (per 1000kg of honey		
	combs)	1	3.3
	1620.0000kg (per 9000kg of honey		
	combs)	1	3.3
(N = 30)			

Appendix B3: Grading system and packaging materials used by artisanal producers

Parameters	Category	Frequency (n)*	Percentage (%)
Are there any grading systems for produced honey?	Yes	16	53.3
	No	14	46.7
If yes, what criteria are used for grading?	Colour	16	100
How many grades are there and what are their characteristics?**	Two:		
	White honey;	15	93.8
	Do not know	14	93.3
	less thick	1	6.7
	Amber honey;	16	100.0
	Do not know	14	87.5
	Thick	2	12.5
	Dark amber honey;	1	6.3
	Very thick	1	6.3
What grade of product do you produce and why?	All grades; have hives in different locations with different floral pattern which determines the grade	16	100.0
Do you use any specialized form of packaging?	Yes	3	10.0
	No	27	90.0
What packaging materials do you use?	Glass	19	63.3
	Plastic/rubber	11	36.7
What is the nature of the packages that you use?	New packages	8	26.7
	Recycled packages	22	73.3
(N = 30) ** Answers include multiple responses			

Appendix B4: Skills and experiences of sellers in selling of honey

Parameters	Category	Frequency (n)*	Percentage (%)
How long have you been selling honey?	Less than a year	1	2.0
	1 – 5 years	14	28.0
	6 – 10 years	7	14.0
	Longer than 10 years	28	56.0
Where do you obtain your honey?	Direct from producers	19	38.0
	Distributors/retailers	31	62.0
	Region: Northern	10	20.0
	Brong Ahafo	4	8.0
	Ashanti	2	4.0
	Eastern	7	14.0
	Central	1	2.0
	Grater Accra	2	4.0
	Volta	19	38.0
	Others**	5	10.0
What volume do you buy at a time?	Less than 10 gallons	45	90.0
	11 – 20 gallons	3	6.0
	More than 40 gallon	2	4.0
Is selling of honey your primary business?	Yes	1	2.0
	No	49	98.0
If no, what do you do in addition?	Sells medicines/drugs	21	42.0
	Sells food stuffs	22	44.0
	Sells provisions	5	10.0
	Sews	2	4.0
How long does it take you to sell a batch of honey	Less than 6 months	13	76.0
	6 – 12 months	8	18.0
	13 – 18 months	1	2.0
	19 – 24 months	2	4.0
(N = 50)	A “Gallon” = 27 Litres	Others**: Benin, Togo	

Appendix B5: Handling and hygienic practices observed by honey sellers

Parameters	Category	Frequency (n)*	Percentage (%)
How do you handle your honey during the period of sale?	Packaged in aseptically sealed container	3	6.0
	Packaged in plastic or rubber bottle	2	4.0
	Packaged in glass bottle	1	2.0
	Packaged in recycled drinking water bottles	30	60.0
	Packaged in recycled alcoholic and/or soft drink bottles	14	28.0
	Yes	29	58.0
	No	21	42.0
Do you go in for fresh batch only when an old batch is completely sold out?	Unused container	4	8.0
	Used container	46	92.0
Do you reprocess your honey before selling?	Yes	16	32.0
	No	34	68.0
If yes, what do you do and give reason (s) for reprocessing?	Strain to remove dirt, dead bees, organic debris and particles	16	100.0
(N = 50)			

Appendix B5: Continuation

Parameters	Category	Frequency (n)*	Percentage (%)
Do you sometimes see the need to add something to honey to improve its quality before selling?	Yes	1	2.0
	No	49	98.0
If yes, what do you think can be added to honey to improve its quality and why?	Garlic to take out flavour and reduce sweetness for treating cough	1	100.0
Do you know of producers that add other ingredients to honey?	Yes	47	94.0
	No	3	6.0
If yes, please list ingredients and reasons for addition?**	Sugar; To increase volume for more profit	24	48.0
	Do not know	9	18.0
	Foam; To increase volume for more profit	8	16.0
	Do not know	15	30.0
	Water; To increase volume for more profit	3	6.0
	Do not know	1	2.0
Is adulteration of honey a known problem here?	Yes	25	50.0
	No	25	50.0
Do buyers/customers complain of adulteration?	Yes	48	96.0
	No	2	4.0

(N = 50)

** Answers include multiple responses

Appendix B6: Suggested methods of assuring honey quality by honey sellers

Parameters	Category	Frequency (n)*	Percentage (%)
How do you assure honey quality?	Check for presence of dead bees and particles in honey	1	2.0
	Check taste of honey	16	32.0
	Check thickness of honey	6	12.0
	Check ability of honey to diffuse in clean cold tumbler of water	10	20.0
	Check ability honey to soak up sand	3	6.0
	Check ability of a match stick dipped in honey to ignite fire	17	34.0
	Check for separation of honey left undisturbed for a period of time	1	2.0
	Check for smoothness of honey	1	2.0
	Check medicinal ability of honey	2	4.0
	Visual observation of viscosity	1	2.0
	Check flavour of honey	6	12.0
	Check solidification of honey under refrigeration condition	1	2.0
	Check colour of honey	2	4.0
	Do not monitor quality indices	3	6.0

(N = 50)

*Answers include multiple responses

Appendix B7: Rank of honey quality characteristics in order of importance by honey sellers in markets in Accra Metropolis of Ghana

Parameters	Category	Frequency (n)*	Percentage (%)
Flavour	Most important	8	16.0
	Important	11	22.0
	Moderately important	12	24.0
	Least important	19	38.0
Taste	Most important	29	58.0
	Important	7	14.0
	Moderately important	13	26.0
	Least important	1	2.0
Appearance (colour)	Most important	4	8.0
	Important	12	24.0
	Moderately important	13	26.0
	Least important	21	42.0
Thickness	Most important	9	18.0
	Important	20	40.0
	Moderately important	12	24.0
	Least important	9	18.0
(N = 50)			

Appendix B8: Rank of quality characteristics in the order that informs consumer judgment of honey by honey sellers in markets in Accra Metropolis of Ghana

Parameters	Category	Frequency (n)*	Percentage (%)
Flavour	Most important	1	2.0
	Important	4	8.0
	Moderately important	14	28.0
	Least important	31	62.0
Taste	Most important	21	42.0
	Important	17	34.0
	Moderately important	7	14.0
	Least important	5	10.0
Appearance (colour)	Most important	5	10.0
	Important	14	28.0
	Moderately important	20	20.0
	Least important	11	22.0
Thickness	Most important	23	46.0
	Important	15	30.0
	Moderately important	9	18.0
	Least important	3	6.0

(N = 50)

Appendix B9: Quality characteristics consumers look out for in honey

Parameters	Category	Frequency (n)*	Percentage (%)
What are the quality characteristics you look for in honey you are going to buy?	Flavour	39	15.0
	Taste	113	43.5
	Thickness	73	28.1
	Appearance (colour)	35	13.5

(N = 260)

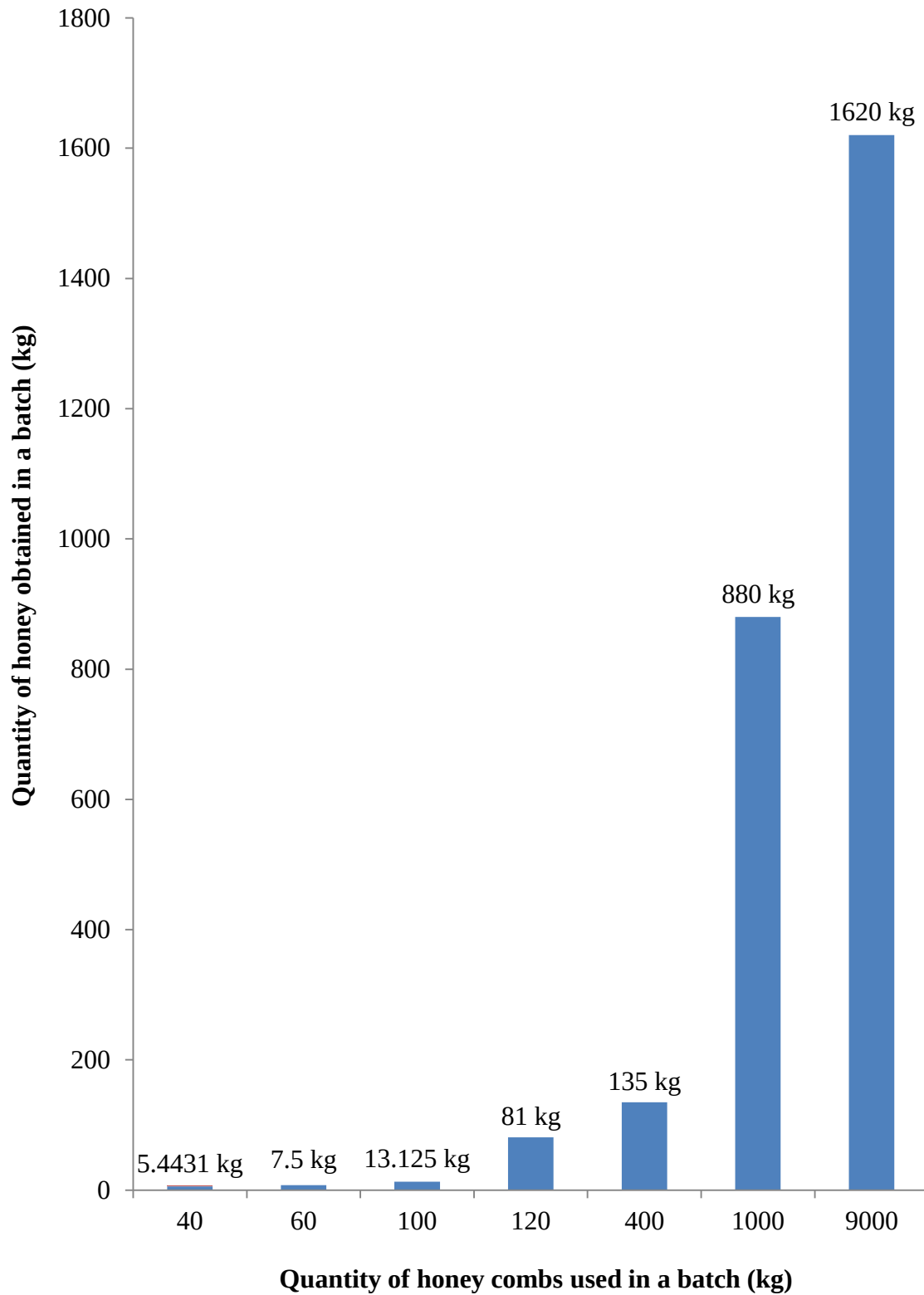
Appendix B10: Storage and packaging of honey

Parameters	Category	Frequency (n)*	Percentage (%)
What volume of honey do you buy?	Less than 1.0kg	157	60.4
	1.0 – 2.0kg	85	32.7
	2.1 – 3.0kg	3	1.2
	3.1 – 4.0kg	1	0.4
	More than 4.0kg	14	5.4
How do you store your honey?	Keep under room temperature	188	72.3
	Keep in fridge	72	27.7
How long do you think honey can last before going bad?	1 year and above	25	9.6
	Do not know	235	90.4
Is the honey you buy packaged?	Yes	183	70.4
	No	77	29.6
In what package do you buy your honey?	Glass bottle	42	16.2
	Plastic/rubber bottle	140	53.8
	Recycled plastic mineral water bottles	52	20.0
	Recycled alcoholic or soft drink bottle	25	9.6
	Recycled plastic cooking oil gallon	1	0.4
What type of packaging materials do you want producers/vendors to use?	Glass	95	36.5
	Plastic/rubber	161	61.9
	Metal	2	0.8
	Tetra pack	2	0.8

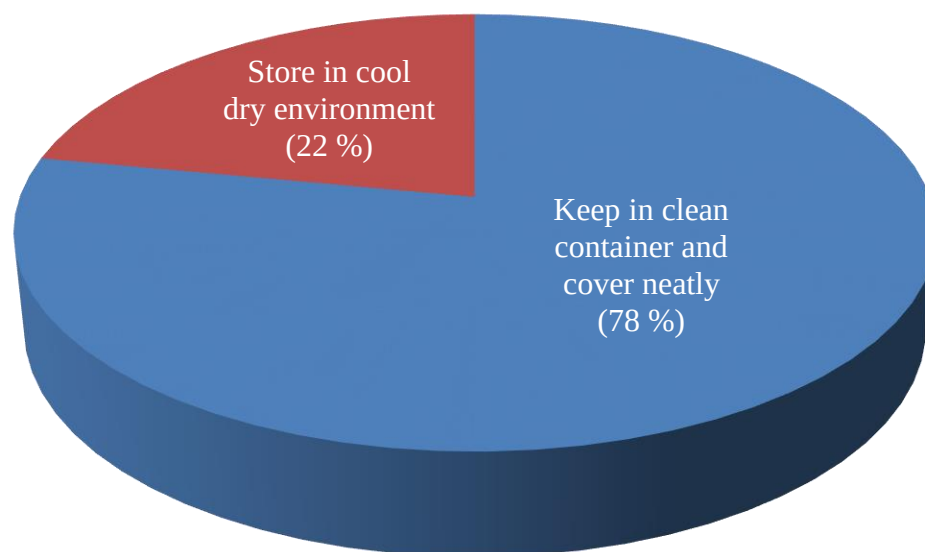
(N = 260)

APPENDIX C: SURVEY GRAPHS

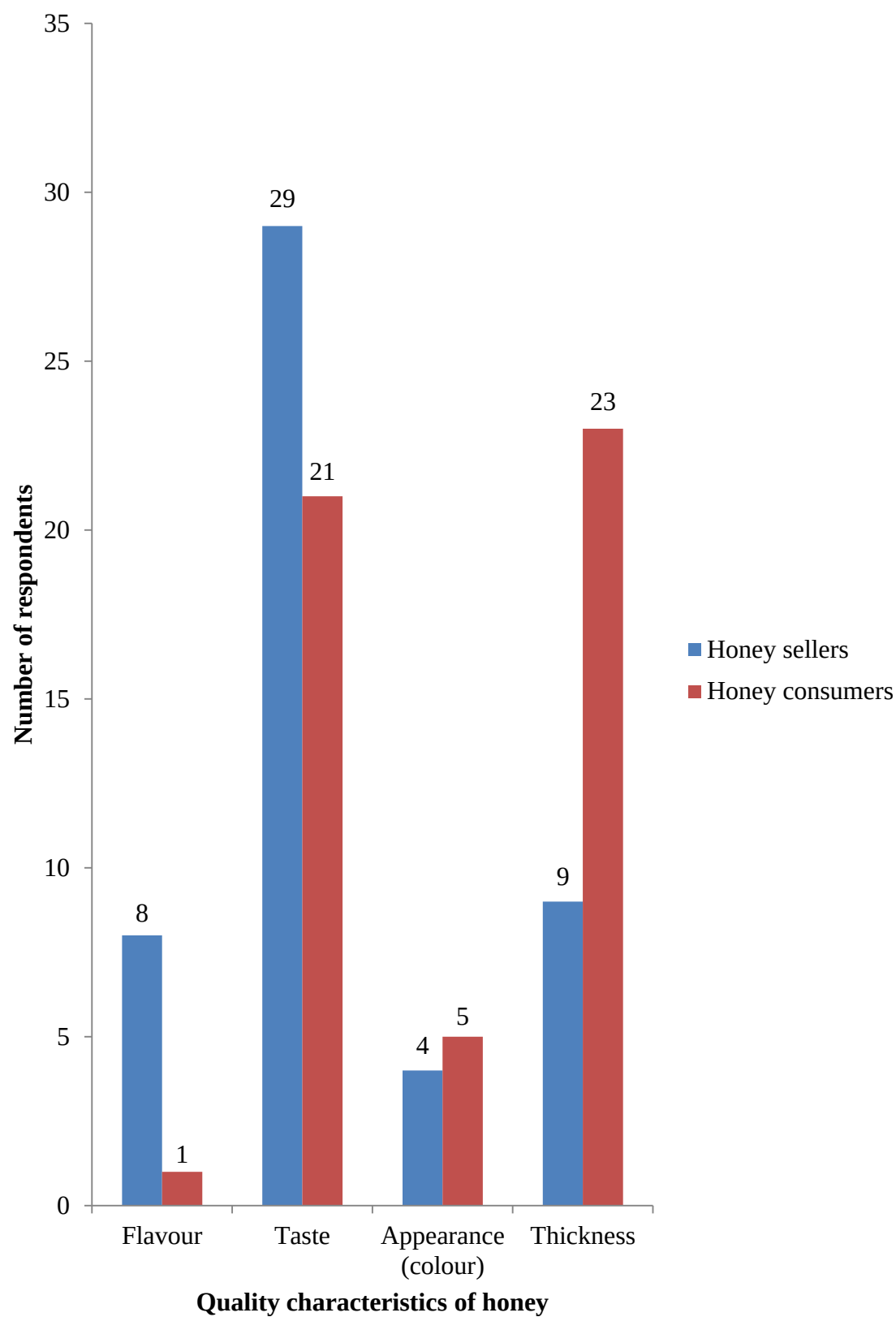
Appendix C1: Quantity of honey obtained from combs used in a batch production



**Appendix C2: Hygienic practices observed by sellers on the markets in the Accra
Metropolis of Ghana**



Appendix C3: Ranking of honey quality characteristics by honey sellers



APPENDIX D: STANDARD TABLES AND CHARTS**Appendix D1: Relationship of Water Content of Honey to Refractive Index**

Water Content,	Refractive Index	Water Content	Refractive Index
g/100 g	20°C	g/100 g	20°C
13.0	1.5044	19.0	1.4890
13.2	1.5038	19.2	1.4885
13.4	1.5033	19.4	1.4880
13.6	1.5028	19.6	1.4875
13.8	1.5023	19.8	1.4870
14.0	1.5018	20.0	1.4865
14.2	1.5012	20.2	1.4860
14.4	1.5007	20.4	1.4855
14.6	1.5002	20.6	1.4850
14.8	1.4997	20.8	1.4845
15.0	1.4992	21.0	1.4840
15.2	1.4987	21.2	1.4835
15.4	1.4982	21.4	1.4830
15.6	1.4976	21.6	1.4825
15.8	1.4971	21.8	1.4820
16.0	1.4966	22.0	1.4815
16.2	1.4961	22.2	1.4810
16.4	1.4956	22.4	1.4805
16.6	1.4951	22.6	1.4800
16.8	1.4946	22.8	1.4795
17.0	1.4940	23.0	1.4790
17.2	1.4935	23.2	1.4785
17.4	1.4930	23.4	1.4780
17.6	1.4925	23.6	1.4775
17.8	1.4920	23.8	1.4770
18.0	1.4915	24.0	1.4765
18.2	1.4910	24.2	1.4760
18.4	1.4905	24.4	1.4755
18.6	1.4900	24.6	1.4750
18.8	1.4895	24.8	1.4745
		25.0	1.4740

Source: International Honey Commission (2009)

Appendix D2: Honey colour standards according to the U.S. Department of Agriculture classifications measured in millimetres on Pfund scale

USDA honey colour standards	Pfund scale (mm)	Optical density
Water white	0 to 8	0.0945
Extra white	> 8 to 17	0.189
White	> 17 to 34	0.378
Extra light amber	> 34 to 50	0.595
Light amber	> 50 to 85	1.389
Amber	> 85 to 114	3.008
Dark amber	> 114	Not applicable

(Source: White, 1975c; Crane, 1980)

APPENDIX E: MEANS AND STANDARD DEVIATIONS OF PARAMETERS DETERMINED

Appendix E1: Microbial count and detection of *Botulinum* toxin of Ghanaian artisanal honey from different source

Honey	Average count in log ₁₀ (CFU/g) mean (range)			
sample	Total mesophilic bacteria	Total coliform	Faecal coliform	<i>Escherichia coli</i>
Honey A	4 ± 0	0	0	0
Honey B	2 ± 3	0	0	0
Honey C	2 ± 3	0	0	0
Honey D	3.8 ± 0.3	0	0	0
Honey E	4.2 ± 0.2	0	0	0
Honey F	4.78 ± 0.02	0	0	0
Honey G	2 ± 3	0	0	0
Honey H	4.0 ± 0.1	0	0	0
Honey I	3.8 ± 0.2	0	0	0
Honey J	0	0	0	0
Honey K	0	0	0	0
Honey L	2 ± 3	0	0	0
Honey M	3.8 ± 0.2	0	0	0
Honey N	3.8 ± 0.2	0	0	0
Honey O	2 ± 3	0	0	0
Honey P	4 ± 0	0	0	0
p-value	≤ 0.001			

Appendix E1: Continuation

Honey sample	Average count in log ₁₀ (CFU/g) mean (range)			
	Yeast and mould	<i>Salmonella</i>	Sulfite-reducing <i>Clostridium</i> spores	<i>Botulinum</i> toxin
Honey A	5.36 ± 0.5	0	0	
Honey B	0	0	0	0
Honey C	0	0	0	
Honey D	5.6 ± 0.3	0	0	0
Honey E	5.45 ± 0.3	5.7 ± 0.1	0	
Honey F	5.5 ± 0.2	5.7 ± 0.1	0	
Honey G	0	0	0	0
Honey H	5.1 ± 0.1	5 ± 0	0	
Honey I	0	0	0	
Honey J	3.8 ± 0.3	0	0	0
Honey K	0	0	0	
Honey L	0	0	0	
Honey M	2 ± 3	0	0	
Honey N	0	0	0	0
Honey O	0	0	0	
Honey P	0	0	0	
p-value	≤ 0.001	≤ 0.001		

Appendix E2: Antimicrobial activity MIC of honey against *Escherichia coli* and *Staphylococcus aureus*

Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
		MIC (%)	MIC (%)
Honey A	10	66.36 ± 0.09	53.54 ± 0.09
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	90.66 ± 0.09	91.11 ± 0.08
Honey B	10	97.9 ± 0.0	98.01 ± 0.09
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	98.9 ± 0.1	99.9 ± 0.0
Honey C	10	95.80 ± 0.04	98.2 ± 0.1
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	98.88 ± 0.06	99.53 ± 0.04
Honey D	10	76.84 ± 0.03	68.16 ± 0.07
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	89.07 ± 0.07	81.37 ± 0.04
Honey E	10	78.76 ± 0.09	57.97 ± 0.04
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	96.45 ± 0.07	90.08 ± 0.09
Honey F	10	93.00 ± 0.05	87.5 ± 0.1
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	96.8 ± 0.0	99.79 ± 0.07

Appendix E2: Continuation

Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
		MIC (%)	MIC (%)
Honey G	10	91.76 ± 0.05	85.2 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	96.9 ± 0.0	91.67 ± 0.04
Honey H	10	92.87 ± 0.08	89.4 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	93.7 ± 0.2	92.5 ± 0.0
Honey I	10	98.1 ± 0.0	95.3 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	100.0 ± 0.0	99.82 ± 0.08
Honey J	10	98.4 ± 0.0	98.8 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	99.4 ± 0.0	100.0 ± 0.0
Honey K	10	99.3 ± 0.0	97.0 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	100.0 ± 0.0	100.0 ± 0.0
Honey L	10	98.2 ± 0.0	97.3 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	100.0 ± 0.0	98.7 ± 0.0

Appendix E2: Continuation

Honey sample	Concentration (%)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
		MIC (%)	MIC (%)
Honey M	10	91.9 ± 0.8	90.4 ± 0.7
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	95.8 ± 0.0	93.8 ± 0.0
Honey N	10	98.0 ± 0.0	79.4 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	100.0 ± 0.0	86.3 ± 0.0
Honey O	10	98.4 ± 0.0	85.0 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	99.8 ± 0.0	93.9 ± 0.0
Honey P	10	98.5 ± 0.0	99.4 ± 0.0
	30	> 100	> 100
	50	> 100	> 100
	70	> 100	> 100
	100	100.0 ± 0.0	100.0 ± 0.0

Appendix E3: Physicochemical quality of Ghanaian artisanal honey from different sources

Honey sample	Refractive Index	Moisture Content (g/100 g)	Total Solids (%)
Honey A	1.48747 ± 0.00006	19.7 ± 0.1	80.3 ± 0.1
Honey B	1.4905 ± 0.00000	18.4 ± 0.0	81.6 ± 0.0
Honey C	1.4925 ± 0.00000	17.6 ± 0.0	82.4 ± 0.0
Honey D	1.48303 ± 0.00006	21.4 ± 0.0	78.6 ± 0.0
Honey E	1.4812 ± 0.00000	22.2 ± 0.0	77.8 ± 0.0
Honey F	1.4820 ± 0.00000	21.8 ± 0.0	78.2 ± 0.0
Honey G	1.486 ± 0.00600	21.4 ± 0.0	78.6 ± 0.0
Honey H	1.48857 ± 0.00006	19.2 ± 0.0	80.8 ± 0.0
Honey I	1.4971 ± 0.00010	15.8 ± 0.0	84.2 ± 0.0
Honey J	1.4938 ± 0.00000	17.0 ± 0.0	83.0 ± 0.0
Honey K	1.48413 ± 0.00006	17.0 ± 0.0	83.0 ± 0.0
Honey L	1.49413 ± 0.00006	17.0 ± 0.0	83.0 ± 0.0
Honey M	1.48207 ± 0.00006	21.8 ± 0.0	78.2 ± 0.0
Honey N	1.49077 ± 0.00006	18.2 ± 0.0	81.8 ± 0.0
Honey O	1.48953 ± 0.00006	18.8 ± 0.0	81.2 ± 0.0
Honey P	1.4909 ± 0.00000	18.2 ± 0.0	81.8 ± 0.0
p-value	< 0.001	< 0.001	< 0.001

Appendix E3: Continuation

Honey sample	Total Soluble Solids (%)	pH	Water Activity
Honey A	79.1 ± 0.00	4.317 ± 0.006	0.6443 ± 0.0006
Honey B	80.0 ± 0.20	4.58 ± 0.020	0.6293 ± 0.0006
Honey C	80.87 ± 0.06	4.417 ± 0.006	0.6200 ± 0.0000
Honey D	77.03 ± 0.06	4.303 ± 0.006	0.6773 ± 0.0006
Honey E	76.3 ± 0.00	4.12 ± 0.000	0.6803 ± 0.0006
Honey F	76.6 ± 0.00	3.793 ± 0.006	0.6823 ± 0.0006
Honey G	80.97 ± 0.06	4.757 ± 0.006	0.6170 ± 0.0000
Honey H	79.23 ± 0.06	4.337 ± 0.006	0.6450 ± 0.0000
Honey I	82.9 ± 0.00	5.39 ± 0.010	0.5910 ± 0.0000
Honey J	81.4 ± 0.00	5.187 ± 0.006	0.6480 ± 0.0000
Honey K	77.43 ± 0.06	3.813 ± 0.006	0.6650 ± 0.0000
Honey L	81.3 ± 0.10	5.013 ± 0.006	0.6100 ± 0.0000
Honey M	76.67 ± 0.06	4.103 ± 0.006	0.6900 ± 0.0000
Honey N	80.03 ± 0.06	4.53 ± 0.010	0.6230 ± 0.0000
Honey O	79.6 ± 0.10	3.98 ± 0.020	0.6150 ± 0.0000
Honey P	80.13 ± 0.06	4.71 ± 0.000	0.6240 ± 0.0000
p-value	< 0.001	< 0.001	< 0.001

Appendix E3: Continuation

Honey sample	Electrical Conductivity (mS/cm)	Ash (%)	Hydroxymethylfurfurals (mg/kg)
Honey A	0.5956 ± 0.0004	0.04180 ± 0.00004	12.17 ± 0.02
Honey B	0.3046 ± 0.0003	0.01764 ± 0.00003	27.1 ± 0.30
Honey C	0.3053 ± 0.0003	0.01770 ± 0.00002	16.90 ± 0.05
Honey D	0.4749 ± 0.0002	0.03178 ± 0.00001	8.89 ± 0.00
Honey E	0.5045 ± 0.0003	0.03424 ± 0.00003	9.72 ± 0.02
Honey F	0.5683 ± 0.0000	0.03953 ± 0.00000	18.94 ± 0.05
Honey G	0.3638 ± 0.0003	0.02256 ± 0.00003	15.69 ± 0.02
Honey H	0.6520 ± 0.0003	0.04648 ± 0.00003	22.5 ± 0.10
Honey I	0.2838 ± 0.0004	0.01592 ± 0.00004	14.4 ± 0.30
Honey J	0.1723 ± 0.0003	0.00666 ± 0.00002	14.4 ± 0.10
Honey K	0.1345 ± 0.0002	0.00352 ± 0.00001	22.8 ± 0.50
Honey L	0.1924 ± 0.0000	0.00834 ± 0.00000	28.5 ± 0.10
Honey M	0.8822 ± 0.0000	0.06559 ± 0.00000	6.96 ± 0.08
Honey N	0.2513 ± 0.0002	0.01322 ± 0.00001	22.00 ± 0.06
Honey O	0.3438 ± 0.0003	0.02090 ± 0.00002	14.67 ± 0.02
Honey P	0.3473 ± 0.0005	0.02119 ± 0.00004	19.9 ± 0.1
p-value	< 0.001	< 0.001	< 0.001

Appendix E3: Continuation

Honey sample	Free Acidity (milliequiv acid/kg)	Pfund (mm)
Honey A	56.3 ± 0.6	220.9 ± 1.0
Honey B	20.3 ± 0.6	332.3 ± 0.6
Honey C	25.3 ± 0.6	233.7 ± 0.6
Honey D	49.7 ± 0.6	200.2 ± 0.9
Honey E	53.0 ± 1.0	332.1 ± 1.0
Honey F	41.3 ± 0.6	863.8 ± 0.0
Honey G	30.0 ± 1.0	466.4 ± 1.0
Honey H	52.0 ± 1.0	556.3 ± 0.7
Honey I	19.7 ± 0.6	297.3 ± 2.0
Honey J	8.7 ± 0.6	158.1 ± 1.0
Honey K	21.3 ± 0.6	56.5 ± 0.9
Honey L	20.7 ± 0.6	113.9 ± 0.6
Honey M	50.3 ± 0.6	856.4 ± 0.0
Honey N	23.7 ± 0.6	178.6 ± 1.0
Honey O	37.0 ± 1.0	523.8 ± 1.0
Honey P	28.0 ± 1.0	493.4 ± 2.0
p-value	< 0.001	< 0.001

APPENDIX F: ANALYSIS OF VARIANCE (ANOVA) TABLES**Appendix F1: ANOVA Table for Total Mesophilic Bacteria by Honey sample**

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	62.6826	15	4.17884	1.68	0.1567
Within groups	39.7812	16	2.48632		
Total (Corr.)	102.464	31			

Appendix F2: ANOVA Table for Yeast and Mould by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	193.842	15	12.9228	26.28	0.0000
Within groups	7.86797	16	0.491748		
Total (Corr.)	201.71	31			

Appendix F3: ANOVA Table for *Salmonella* by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	140.521	15	9.36809	4833.88	0.0000
Within groups	0.0310081	16	0.00193801		
Total (Corr.)	140.552	31			

Appendix F4: ANOVA Table for Refractive Index by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00106892	15	0.0000712613	34.86	0.0000
Within groups	0.00006542	32	0.00000204438		
Total (Corr.)	0.00113434	47			

Appendix F5: ANOVA Table for Moisture Content by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	191.01	15	12.734	15280.80	0.0000
Within groups	0.0266667	32	0.000833333		
Total (Corr.)	191.037	47			

Appendix F6: ANOVA Table for Total Solids by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	191.01	15	12.734	15280.80	0.0000
Within groups	0.0266667	32	0.000833333		
Total (Corr.)	191.037	47			

Appendix F7: ANOVA Table for Total Soluble Solids by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	180.733	15	12.0489	2628.85	0.0000
Within groups	0.146667	32	0.00458333		
Total (Corr.)	180.88	47			

Appendix F8: ANOVA Table for pH by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	9.7937	15	0.652913	6964.41	0.0000
Within groups	0.003	32	0.00009375		
Total (Corr.)	9.7967	47			

Appendix F9: ANOVA Table for Water Activity by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0405896	15	0.00270598	25977.37	0.0000
Within groups	0.00000333333	32	1.04167E-7		
Total (Corr.)	0.040593	47			

Appendix F10: ANOVA Table for Electrical Conductivity by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.81642	15	0.121095	1334176.00	0.0000
Within groups	0.00000290444	32	9.07637E-8		
Total (Corr.)	1.81642	47			

Appendix F11: ANOVA Table for Ash by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0125133	15	0.000834222	1334175.07	0.0000
Within groups	2.00087E-8	32	6.25272E-10		
Total (Corr.)	0.0125133	47			

Appendix F12: ANOVA Table for Hydroxymethylfurfural by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1792.78	15	119.518	3871.52	0.0000
Within groups	0.987879	32	0.0308712		
Total (Corr.)	1793.77	47			

Appendix F13: ANOVA Table for Free Acidity by Honey sample

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	10086.3	15	672.422	1241.39	0.0000
Within groups	17.3333	32	0.541667		
Total (Corr.)	10103.7	47			

APPENDIX G: MULTIPLE RANGE TESTS TABLES**Appendix G1: Multiple Range Tests for Total Mesophilic Bacteria by Honey sample**

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups
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Honey K	2	0.0	a
Honey J	2	0.0	a
Honey C	2	1.95154	ab
Honey B	2	1.95154	ab
Honey G	2	1.95154	ab
Honey L	2	2.03959	ab
Honey O	2	2.03959	ab
Honey A	2	3.60206	b
Honey P	2	3.60206	b
Honey N	2	3.75257	b
Honey M	2	3.75257	b
Honey I	2	3.75257	b
Honey D	2	3.84062	b
Honey H	2	3.99114	b
Honey E	2	4.19011	b
Honey F	2	4.77791	b

Appendix G2: Multiple Range Tests for Yeast and Mould by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups
--------------	-------	------	--------------------

Honey L	2	0.0	a
Honey K	2	0.0	a
Honey N	2	0.0	a
Honey C	2	0.0	a
Honey O	2	0.0	a
Honey G	2	0.0	a
Honey B	2	0.0	a
Honey I	2	0.0	a
Honey P	2	0.0	a
Honey M	2	1.95154	b
Honey J	2	3.84062	c
Honey H	2	5.10206	cd
Honey A	2	5.36008	d
Honey F	2	5.451	d
Honey E	2	5.45424	d
Honey D	2	5.57918	d

Appendix G3: Multiple Range Tests for *Salmonella* by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups
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Honey O	2	0.0	a
Honey A	2	0.0	a
Honey P	2	0.0	a
Honey B	2	0.0	a
Honey C	2	0.0	a
Honey G	2	0.0	a
Honey D	2	0.0	a
Honey I	2	0.0	a
Honey J	2	0.0	a
Honey K	2	0.0	a
Honey L	2	0.0	a
Honey M	2	0.0	a
Honey N	2	0.0	a
Honey H	2	5.0	b
Honey E	2	5.38908	c
Honey F	2	5.69011	d

Appendix G4: Multiple Range Tests for Refractive Index by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey E	3	1.4812	a
Honey F	3	1.482	ab
Honey M	3	1.48207	ab
Honey D	3	1.48303	ab
Honey K	3	1.48413	bc
Honey G	3	1.4864	cd
Honey A	3	1.48747	de
Honey H	3	1.48857	def
Honey O	3	1.48953	ef
Honey B	3	1.4905	fg
Honey N	3	1.49077	fg
Honey P	3	1.4909	fg
Honey C	3	1.4925	gh
Honey J	3	1.4938	h
Honey L	3	1.49413	h
Honey I	3	1.49707	i

Appendix G5: Multiple Range Tests for Moisture Content by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey I	3	15.8	a
Honey J	3	17.0	b
Honey L	3	17.0	b
Honey K	3	17.0	b
Honey C	3	17.6	c
Honey P	3	18.2	d
Honey N	3	18.2	d
Honey B	3	18.4	e
Honey O	3	18.8	f
Honey H	3	19.2	g
Honey A	3	19.6667	h
Honey G	3	21.4	i
Honey D	3	21.4	i
Honey M	3	21.8	j
Honey F	3	21.8	j
Honey E	3	22.2	k

Appendix G6: Multiple Range Tests for Total Solids by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey E	3	77.8	a
Honey M	3	78.2	b
Honey F	3	78.2	b
Honey G	3	78.6	c
Honey D	3	78.6	c
Honey A	3	80.3333	d
Honey H	3	80.8	e
Honey O	3	81.2	f
Honey B	3	81.6	g
Honey N	3	81.8	h
Honey P	3	81.8	h
Honey C	3	82.4	i
Honey L	3	83.0	j
Honey K	3	83.0	j
Honey J	3	83.0	j
Honey I	3	84.2	k

Appendix G7: Multiple Range Tests for Total Soluble Solids by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey E	3	76.3	a
Honey F	3	76.6	b
Honey M	3	76.6667	b
Honey D	3	77.0333	c
Honey K	3	77.4333	d
Honey A	3	79.1	e
Honey H	3	79.2333	f
Honey O	3	79.6333	g
Honey B	3	79.9667	h
Honey N	3	80.0333	hi
Honey P	3	80.1333	i
Honey C	3	80.8667	j
Honey G	3	80.9667	j
Honey L	3	81.3	k
Honey J	3	81.4	k
Honey I	3	82.9	l

Appendix G8: Multiple Range Tests for pH by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups
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Honey F	3	3.79333	a
Honey K	3	3.81333	b
Honey O	3	3.98333	c
Honey M	3	4.10333	d
Honey E	3	4.12	e
Honey D	3	4.30333	f
Honey A	3	4.31667	f
Honey H	3	4.33667	g
Honey C	3	4.41667	h
Honey N	3	4.53	i
Honey B	3	4.58333	j
Honey P	3	4.71	k
Honey G	3	4.75667	l
Honey L	3	5.01333	m
Honey J	3	5.18667	n
Honey I	3	5.39	o

Appendix G9: Multiple Range Tests for Water Activity by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey I	3	0.591	a
Honey L	3	0.61	b
Honey O	3	0.615	c
Honey G	3	0.617	d
Honey C	3	0.62	e
Honey N	3	0.623	f
Honey P	3	0.624	g
Honey B	3	0.629333	h
Honey A	3	0.644333	i
Honey H	3	0.645	j
Honey J	3	0.648	k
Honey K	3	0.665	l
Honey D	3	0.677333	m
Honey E	3	0.680333	n
Honey F	3	0.682333	o
Honey M	3	0.69	p

Appendix G10: Multiple Range Tests for Electrical Conductivity by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey K	3	0.134466	a
Honey J	3	0.172267	b
Honey L	3	0.192434	c
Honey N	3	0.251279	d
Honey I	3	0.283819	e
Honey B	3	0.30457	f
Honey C	3	0.305252	g
Honey O	3	0.343832	h
Honey P	3	0.34734	i
Honey G	3	0.363804	j
Honey D	3	0.474869	k
Honey E	3	0.504486	l
Honey F	3	0.5683	m
Honey A	3	0.595579	n
Honey H	3	0.651988	o
Honey M	3	0.882203	p

Appendix G11: Multiple Range Tests for Ash by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey K	3	0.00352468	a
Honey J	3	0.00666215	b
Honey L	3	0.00833601	c
Honey N	3	0.0132201	d
Honey I	3	0.0159209	e
Honey B	3	0.0176433	f
Honey C	3	0.0176999	g
Honey O	3	0.0209021	h
Honey P	3	0.0211932	i
Honey G	3	0.0225598	j
Honey D	3	0.0317781	k
Honey E	3	0.0342363	l
Honey F	3	0.0395329	m
Honey A	3	0.041797	n
Honey H	3	0.046479	o
Honey M	3	0.0655868	p

Appendix G12: Multiple Range Tests for Hydroxymethylfurfural by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey M	3	6.96091	a
Honey D	3	8.89182	b
Honey E	3	9.71495	c
Honey A	3	12.1701	d
Honey J	3	14.3703	e
Honey I	3	14.4402	ef
Honey O	3	14.6694	f
Honey G	3	15.6879	g
Honey C	3	16.9001	h
Honey F	3	18.9363	i
Honey P	3	19.9243	j
Honey N	3	22.0041	k
Honey H	3	22.4546	l
Honey K	3	22.768	m
Honey B	3	27.1246	n
Honey L	3	28.4862	o

Appendix G13: Multiple Range Tests for Free Acidity by Honey sample

Method: 95.0 percent LSD

Honey sample	Count	Mean	Homogeneous Groups

Honey J	3	8.66667	a
Honey I	3	19.6667	b
Honey B	3	20.3333	bc
Honey L	3	20.6667	bc
Honey K	3	21.3333	c
Honey N	3	23.6667	d
Honey C	3	25.3333	e
Honey P	3	28.0	f
Honey G	3	30.0	g
Honey O	3	37.0	h
Honey F	3	41.3333	i
Honey D	3	49.6667	j
Honey M	3	50.3333	j
Honey H	3	52.0	k
Honey E	3	53.0	k
Honey A	3	56.3333	l

APPENDIX H: CORRELATIONS**Appendix H1: Correlation between RI, MC, TS, TSS, pH, a_w , EC, Ash, HMF and FA**

Parameter	RI	MC	TS	TSS	pH	a_w	EC	Ash	HMF
MC	-0.835								
	0.000								
TS	0.835	-1.000							
	0.000	*							
TSS	0.918	-0.744	0.744						
	0.000	0.000	0.000						
pH	0.784	-0.565	0.565	0.852					
	0.000	0.000	0.000	0.000					
a_w	-0.844	0.690	-0.690	-0.920	-0.666				
	0.000	0.000	0.000	0.000	0.000				
EC	-0.580	0.736	-0.736	-0.583	-0.443	0.579			
	0.000	0.000	0.000	0.000	0.002	0.000			
Ash	-0.580	0.736	-0.736	-0.583	-0.443	0.579	1.000		
	0.000	0.000	0.000	0.000	0.002	0.000	*		
HMF	0.415	-0.533	0.533	0.364	0.168	-0.443	-0.533	-0.533	
	0.003	0.000	0.000	0.011	0.252	0.002	0.000	0.000	
FA	-0.675	0.768	-0.768	-0.686	-0.592	0.572	0.857	0.857	-0.532
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Cell Contents: Pearson correlation

p-Value

RI – Refractive Index

MC – Moisture Content

TS – Total Solids

TSS – Total Soluble Solids

 a_w – Water Activity

EC – Electrical Conductivity

HMF – Hydroxymethylfurfural

FA – Free Acidity

APPENDIX I: PRINCIPAL COMPONENT ANALYSIS**Appendix I1a: Eigen analysis of the correlation matrix**

Parameter	Principal Component (PC)				
	1	2	3	4	5
Eigenvalue	7.0480	1.2910	0.6589	0.4612	0.3189
Proportion	0.705	0.129	0.066	0.046	0.032
Cumulative	0.705	0.834	0.900	0.946	0.978

Appendix I1b: Principal Component Analysis of RI, MC, TS, TSS, pH, a_w , EC, Ash, HMF and FA

Variable	Principal Component (PC)				
	1	2	3	4	5
RI	-0.339	-0.297	0.140	0.139	0.010
MC	0.345	-0.057	-0.119	-0.554	-0.019
TS	-0.345	0.057	0.119	0.554	0.019
TSS	-0.335	-0.362	0.030	-0.184	-0.099
pH	-0.275	-0.462	-0.248	-0.244	0.535
a_w	0.315	0.274	-0.229	0.292	0.602
EC	0.318	-0.373	0.337	0.152	0.216
Ash	0.318	-0.373	0.337	0.152	0.216
HMF	-0.220	0.404	0.732	-0.366	0.311
FA	0.330	-0.213	0.273	0.097	-0.389

RI – Refractive Index MC – Moisture Content TS – Total Solids

TSS – Total Soluble Solids a_w – Water Activity EC – Electrical Conductivity

HMF – Hydroxymethylfurfural FA – Free Acidity

Appendix I1a: Continuation

Parameter	Principal Component (PC)				
	6	7	8	9	10
Eigenvalue	0.1278	0.0738	0.0204	0.0000	-0.0000
Proportion	0.013	0.007	0.002	0.000	-0.000
Cumulative	0.991	0.998	1.000	1.000	1.000

Appendix I1b: Continuation

Variable	Principal Component (PC)				
	6	7	8	9	10
RI	-0.005	0.867	0.085	0.000	0.000
MC	-0.074	0.225	-0.017	-0.107	0.699
TS	0.074	-0.225	0.017	-0.107	0.699
TSS	-0.205	-0.151	-0.805	0.000	0.000
pH	0.455	-0.213	0.227	-0.000	-0.000
a_w	0.155	0.249	-0.490	-0.000	-0.000
EC	-0.253	-0.092	0.068	0.699	0.107
Ash	-0.253	-0.092	0.068	-0.699	-0.107
HMF	0.139	-0.005	-0.052	-0.000	-0.000
FA	0.754	0.025	-0.202	0.000	0.000

RI – Refractive Index

MC – Moisture Content

TS – Total Solids

TSS – Total Soluble Solids

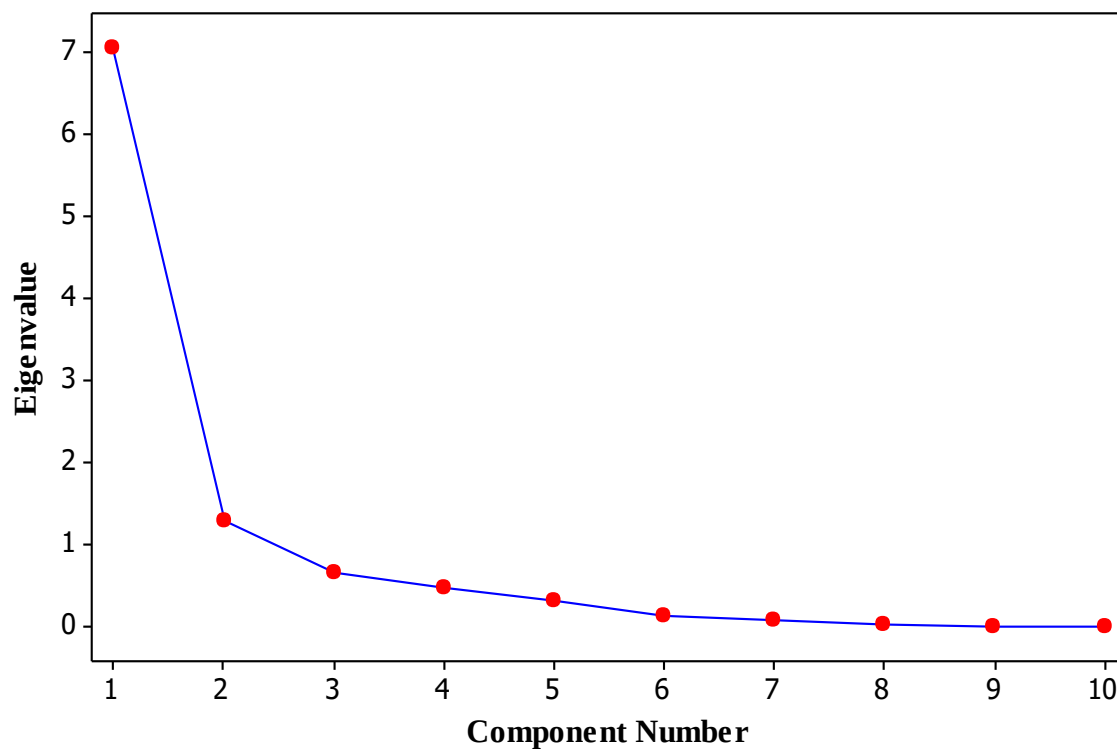
 a_w – Water Activity

EC – Electrical Conductivity

HMF – Hydroxymethylfurfural

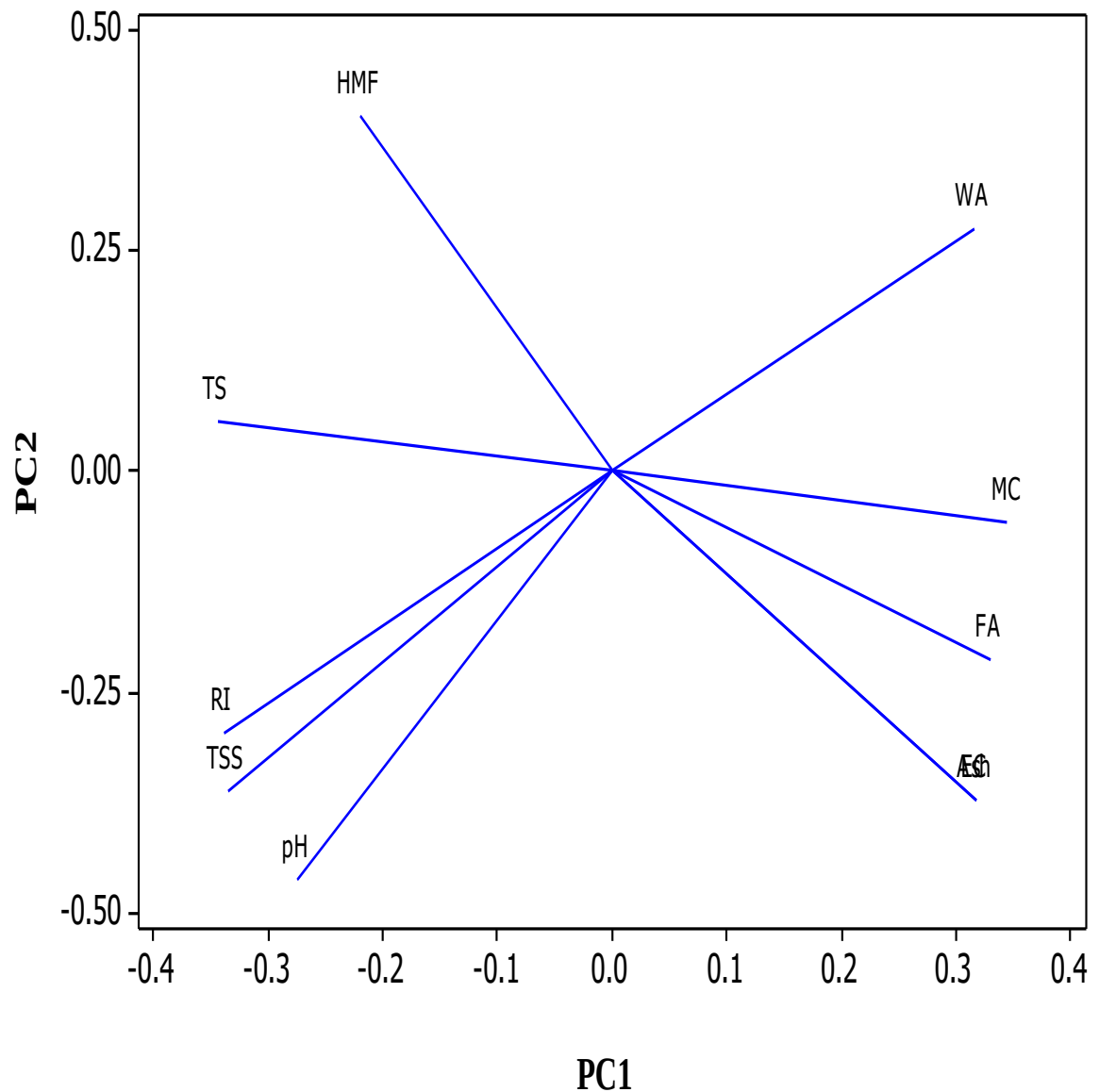
FA – Free Acidity

Appendix I2: Scree plot of Refractive Index (FI), Moisture Content (MC), Total Solids (TS), Total Soluble Solids (TSS), pH, Water Activity (a_w), Electrical Conductivity (EC), Ash, Hydroxymethylfurfural (HMF) and Free Acidity (FA)



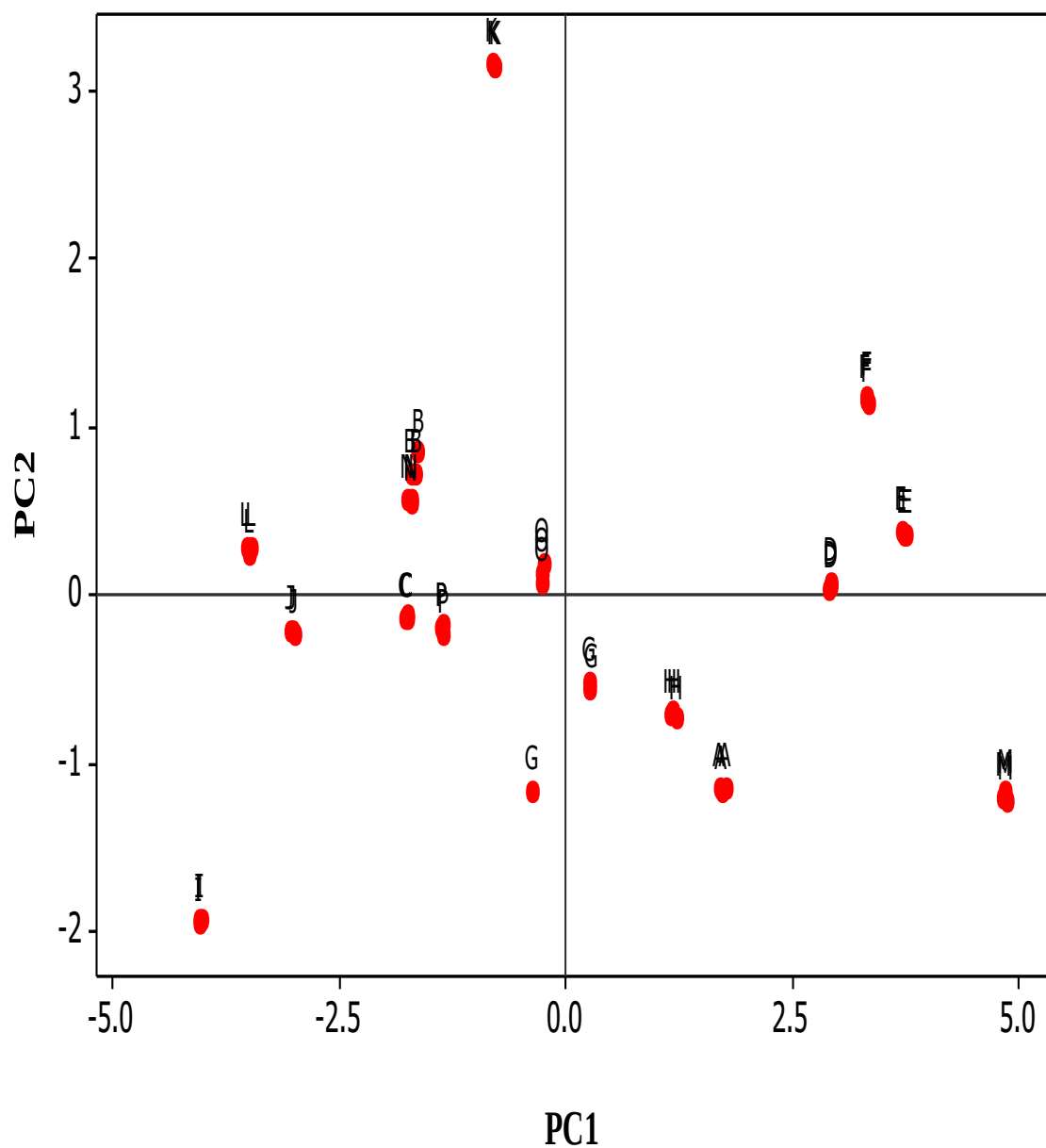
Appendix I3: PCA loading plot of Refractive Index (RI), Moisture Content (MC), Total Solids (TS), Total Soluble Solids (TSS), pH, Water Activity (WA), Electrical Conductivity (EC), Ash, Hydroxymethylfurfural (HMF) and Free Acidity (FA)

Goodness of fit: PC1 = 70.5 %, PC2 = 12.9 %, Total = 83.4 %



Appendix I4: PCA score plot of Refractive Index (FI), Moisture Content (MC), Total Solids (TS), Total Soluble Solids (TSS), pH, Water Activity (a_w), Electrical Conductivity (EC), Ash, Hydroxymethylfurfural (HMF) and Free Acidity (FA)

Goodness of fit: PC1 = 70.5 %, PC2 = 12.9 %, Total = 83.4 %



APPENDIX J: Honey samples

Appendix J1: Honey **A:** Apiary honey from Nkwanta (North Volta); Honey **B:** Apiary honey from Ho (West Volta); Honey **C:** Apiary honey from Nkwanta (North Volta)



Appendix J2: Honey **D**: Forest honey from Dzolokpuita (West Volta); Honey **E**: Forest honey from Dzolokpuita (West Volta); Honey **F**: Forest honey from Somanya (Eastern Region)



Appendix J3: Honey **G**: Honey from Mallam Atta market; Honey **H**: Honey from Achimota market; Honey **I**: Honey from Dome market; Honey **J**: Honey from Nima market (All in Greater Accra Region)



Appendix J4: Honey **K**: Honey from Agbogbloshie market; Honey **L**: Honey from Kaneshie market; Honey **M**: Honey from Makola market; Honey **N**: Honey from Timber market (All in Greater Accra Region)



Appendix J5: Honey **O**: Honey from Ashaiman market; Honey **P**: Honey from Madina market (All in Greater Accra Region)

