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DEPARTMENT OF NUTRITION AND FOOD SCIENCE

CHEMICAL COMPOSITION AND ANTIMICROBIAL EFFICACY OF ESSENTIAL OIL FROM *XYLOPIA AETHIOPICA* FRUIT PODS

BY

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DECLARATION

I declare that this thesis was carried out by me under supervision in the Department of Nutrition and Food Science, University of Ghana, Legon. I take full responsibility for whatever has been reported here. Related work by others, which served as a source of information has been duly acknowledged by reference to the authors.

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DEDICATION

This research work is dedicated to God Almighty, my dad Mr Benjamin Sefakor Cudjo Torku (of blessed memory), all my siblings, my mother Comfort Yevuyibor Stella Afua (Mrs. Torku), my bosom friend Mr Alexander Kwame Gavu and all who supported me financially during my study.

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ABSTRACT

Antimicrobial properties of essential oils (EOs) from plants have historically been explored and traditionally used as antiseptics and medicines. Recent studies on EOs of selected edible plants have shown great potential for inhibition of spoilage and pathogenic microorganisms that occur in foods. Food applications of essential oils have however been limited due to solubility issues, unaffordable extraction technologies, low yield and strong flavours etc. Indigenous spices are commonly used in traditional foods to add flavour. Limited studies have shown that spices such as *Xylopia aethiopica* may have antimicrobial properties. The objective of this study was the evaluate the chemical composition of *Xylopia aethiopica* fruit pods and assess its antimicrobial efficacy against prevalent foodborne pathogens in laboratory media and in food systems. The study assessed the chemical composition of the oil extracts using Gas Chromatograph-Mass Spectrometry (GC-MS) and determined the *in vitro* antimicrobial potential of the essential oil at 10%, 25%, 40%, 55%, 70% and 85% concentrations on *Escherichia coli, Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus* using the modified Kirby-Bauer disk diffusion method. Also, overall sensory differences between spiked (2 µl/ml essential oil) and unspiked samples of bissap extract and ice kenkey were assessed using the triangle test. The study further assessed the antimicrobial effects of essential oils in ice kenkey and bissap extract through a microbiological challenge test, using a cocktail of organisms comprised of *E. coli*, *S.* Typhimurium and *S. aureus*. The study revealed that *X. aethiopica* essential oil was composed of 105 different chemical compounds out of which 104 were identified. EO constituents worked synergistically to inhibit microorganisms, producing a clear zone referred to as the zone of inhibition. The highest EO concentrations to *S.* Typhimurium (17.00±1.00 mm), *S. aureus* (13.33+0.58 mm) and *E. coli* (11.67 ± 0.58 mm) were 70%, 85% and 85% EO respectively. The positive control, Gentamycin (200 µg) was inhibitory to all organisms, *E. coli* 20.33 ± 0.58 mm, *S.* Typhimurium 22.33 ± 1.15 mm and *S.*

aureus 23.67 ± 0.58 mm in ascending order, with zones of inhibition higher than those reported for the various EO concentrations. Essential oils of *X. aethiopica in vitro* could not inhibit any of the organisms at the least concentration (10%) tested on microorganisms. There was a significant sensory difference between EO spiked and unspiked samples of bissap extract and ice kenkey ($p<0.05$). Over 192hr of challenge studies, EO could not significantly influence pH in bissap and ice kenkey samples ($p>0.05$.) *S. aureus* and *E. coli* counts in ice kenkey spiked with EO under ambient conditions reduced for 323 (0hr) to 1 (192hr) and 41 (0hr) to 0 (192hr). In bissap samples kept under ambient and refrigerated conditions, growths of *S. aureus*, *E. coli* and S. Typhimurium were generally not recorded between the 0 hr to 192 hr challenge test. The antimicrobial efficacy of EO was generally higher in bissap extract than ice kenkey.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Ghana is a West African country located geographically between latitude 4° 44' N and 11° 11' N; longitude 3° 11' W and 1° 11' E, with a total land area of 238, 539 km^2 out of which 136,000 km^2 is considered as agricultural land (Oppong-Anane, 2006). Ghana is considered an agrarian country and therefore cultivates several crops including spices. Spices are either parts of the plant, leaves, stems, fruits, seeds, buds, flowers, roots; or whole plants used extensively in the food and beverage industry, pharmaceutical industry and cosmetics and aromatherapy industry to impart or enhance flavour, colour and aroma. It is also used as preservatives. Essentially, these spices function as seasonings in dishes rather than for nutritive purposes.

The spice wealth of the country include: aniseed ('nketekete', *Pimpinella anisum*), black pepper ('soro wisa', *Piper guineense*), ginger ('akakaduro', *Zingiber officinale*), Calabash nutmeg ('awedeaba', *Monodora myristica*), galbanum ('prekese', *Tetrapleura tetraptera*), Negro pepper ('hwintia', *Xylopia aethiopica*) and grains of paradise ('fam wisa', *Afromomum melegueta*) (Nkansah & Amoako, 2010; van Andel *et al.*, 2012). Spices, impart certain health benefits such as carminative, stomachic, analgesic, diuretic functions and sometimes aphrodisiac properties when consumed in food (Peter & Babu, 2012). Also, some spices have been proven to have cholesterol-lowering effects, antibacterial properties, antiparasitic properties and also serve as tonics and stimulants (Ahene *et al.*, 2011; Hyldgaard *et al.*, 2012; Lucchesi *et al*., 2004; Nkansah & Amoako, 2010; Perricone *et al.*, 2015; Pokhrel *et al.*, 2012; Tongnuanchan & Benjakul, 2014). Although, spices and their oils are known for their

beneficial uses, studies according to Pokhrel *et al*. (2012), surmises that some spices contain allergens, carcinogens, mutagens and abortifacients.

In Ghana, some of the spices and herbs that are used as medicines have been reported to cure asthma, intestinal problems, fever, malaria, sexually transmitted diseases (STD's) and are used as laxatives, baby care concoctions, for rituals and to improve women health especially during pregnancy and post-partum (van Andel *et al*., 2012).

Beyond the traditional fresh and minimally processed forms of spices such as dried and powdered forms which have found usage in food and herbal medicines, new forms such as their essential oil (EO) extracts have emerged. These oils are termed essential oils (EOs) because of their highly volatile nature, strong piquant aroma, tiny volumes and varying compositions (Tongnuanchan & Benjakul, 2014). These EOs are often extracted using some conventional methods such as steam distillation, hydrodistillation solvent extraction, and some modern technologies such as Microwave Assisted Extraction (MAE), Ultrasound Assisted Extraction (UAE) (Stratakos & Koidis, 2015).

Spice EOs and other extracts possess chemical components such as terpenes, terpenoids, and phenylpropanoids that confer functional properties such as antibacterial, antifungal, antihelminthic, anti-protozoal, antidiabetic, and anti-inflammatory (Bakkali *et al.,* 2008; Burt, 2004; Mohammed *et al.*, 2016; Raut & Karuppayil, 2014). The chemical components of these oils tend to work efficiently and synergistically rather than in isolation (Burt, 2004; Tajkarimi *et al.* 2010).

The use of EOs extracts in food applications has been piloted in several studies (Babarinde & Adegoke, 2015; Tajkarimi *et al*., 2010; Tongnuanchan & Benjakul, 2014). EOs are Generally Regarded As Safe (GRAS). Scientific data gathered by the US FDA, Codex Alimentarius

Commission and other independent research organisations support the use of essential oils from edible plants in food and beverages.

EOs have also found modern use in soaps, air refreshers, cosmetic products, mouthwash, shampoos, body lotions, kitchen sprays, antiseptics and general household cleaning detergents. EOs are also used to treat skin and oral infections, cold and flu, improper digestion and sometimes depression. The inherent medicinal properties of these spice essential oils cannot be underestimated in the search of potent natural antimicrobials which are necessary to combat multidrug-resistant strains of food spoilage and pathogenic organisms. The incorporation of EOs into the washing and cleaning regime of fruits and vegetables has also received wide attention because of its ability to inhibit microorganisms (Burt, 2004). The exploration of the antimicrobial food applications of the essential oil from *Xylopia aethiopica* could lead to novel applications that could extend the shelf-life of foods and beverages.

1.2 Problem statement and Justification

The exploration of essential oils for their antimicrobial properties as well as their food applications has been the subject of intense research (Abdel-Reheem & Oraby, 2015; Abolfazl, Frhad, Ahmadreza, & Hossein, 2013; Burt, 2004; Calo, Crandall, O'Bryan, & Ricke, 2015). The foundations for the use of essential oils as a natural antimicrobial is undergirded by the growing concerns against the incorporation of synthetic sources of antimicrobials in food (Burt, 2004). The need for novel, effective and efficient sources of natural antimicrobials has become indispensable hence the exploration of mostly plant and plant parts to obtain extracts and oils that will be potent alternatives to the synthetic antimicrobials (Burt, 2004). The application of spices and EOs as antimicrobials has been helpful in the control of many antibiotic resistant organisms. The success has been attributed to the multiplicity and complexity of the EO composition (Burt, 2004).

EO extracts have shown highest antimicrobial efficacy compared to water, ethanolic, methanolic extracts and pepper-soup extracts from edible plant parts. Nanasombat and Lohasupthawee (2005) observed that the inhibitory activity of spice essential oils was more pronounced than that of their own ethanolic extracts. However, amidst their antimicrobial potency, inherent challenges such as strong scent, poor solubility and sedimentation, in a liquid medium are of utmost concern in the food industry. Significant among the challenges include the strong, compelling sensory properties of essential oils when added to food. This phenomenon has posed problems for prospective consumers since very few consumers may be willing to compromise sensory pleasure for health benefits. The introduction and acceptance of these essential oils must be subjected to scrutiny as various factors account for their perceived antimicrobial activity. The beneficial antimicrobial effect of essential oils is dependent on the type of spices used, parts of the plant used, the age of the plant, quantity and duration of use (Bhattacharya, 2016; Elhassan & Ayoub, 2014).

Beverages are prone to spoilage and pathogenic organisms hence the need to explore other value addition options which will lead to an extension of their shelf-life. Ice kenkey, a maize-based fermented traditional beverage produced in Ghana is still at the artisanal stage. The production steps are not entirely free from possible microbial and fungal contamination. According to reports published by Feglo and Sakyi (2012), ice kenkey has a high risk of microbial contamination hence vulnerable to both spoilage and pathogenic organisms. *Hibiscus sabdariffa* 'bissap' extracts, although undergoes some form of heat processing, is often packaged cold and then kept under the cold chain. Poor handling practices of bissap extracts could also introduce microbial contaminants into the final product. Improper food handling, poor cleaning regimes, cross contamination, poor knowledge on food safety amongst others has been attributed to some of the foodborne illness episodes (Ababio & Lovatt, 2015).

In Ghana, most food poisoning episodes from both home and mass eatery sources are seldom diagnosed. Notwithstanding, reports on the microbiological safety of some street vended foods have indicated a high prevalence of the following pathogenic bacteria: *Escherichia coli, Pseudomonas aeruginosa. Staphylococcus aureus, Aeromonas pneumophila, Campylobacter jujeni, Enterobacter cloacae, Klebsiella pneumoniae, Clostridium perfringens,* and *Bacillus species* (Ababio & Lovatt, 2015; Feglo & Sakyi, 2012). There is, therefore, an urgent need to safeguard the integrity of such indigenous beverages as well as other foods through the use of natural antimicrobial ingredients to inhibit pathogenic and spoilage microorganisms that are commonly present to the ready-to-eat foods. The exploration of the antimicrobial efficacy of indigenous Ghanaian *Xylopia aethiopica* essential oils could also serve as evidence of potential applications in food to improve food quality and safety.

The foundational principles for the choice of the *X. aethiopica* fruits pods in the present study are undergirded by their unrestricted use as food spices in stews, soups beverages as well as herbal concoctions or drugs in major parts of the country.

1.3 General objectives

To evaluate the chemical composition of *Xylopia aethiopica* fruit pods and determine its antimicrobial efficacy against prevalent foodborne pathogens in laboratory media and in food systems.

1.4 Specific Objectives

i. To investigate the chemical composition of essential oil extracts from Negro pepper (hwintia or etso, *Xylopia aethiopica*) using Gas Chromatograph-Mass Spectrometry (GC-MS).

ii. To determine the antimicrobial potential of the essential oil on *Escherichia coli*, *Salmonella* Typhimurium and *Staphylococcus aureus* in laboratory media.

iii. To determine the perceived sensory difference between spiked and unspiked ice kenkey and bissap beverage treated with *X. aethiopica* essential oil extract at its minimum inhibition concentration.

iv. To investigate the inhibitory effects of the essential oil against a cocktail of *Escherichia coli*, *Salmonella* Typhimurium and *Staphylococcus aureus* in ice kenkey and bissap beverages.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of spices

The history of the emergence of spices on earth per historians are largely fragmented. Some accounts of history predate the Ancient Egyptian and Arabian beginnings (from about 2600 BC). Some historians suggest that the first authentic records of the use of spices date from the Pyramid Age in Egypt, around 2600 to 2100 BC. Other historians hold the view that labourers who toiled in the construction of the Great Pyramid of Cheops, were fed with onions and garlic for medicinal purposes thus to preserve their health (Rosengarten, 1970b, 1970a).

Spices in the days of old were used in food and embalming processes. Some of the essential components of the embalming concoctions include cassia, cinnamon, anise, cumin, marjoram. The embalming processes were meant to appease the gods of death, and preserve the bodies of important personages against decaying. Many historians believe that burning of the aromatic herbs, fragrant gums, hardened oozing's from resinous woods and shrubs led to the origin of perfumery. Myrrh, frankincense, balsam, bdellium and other famous spices from scriptural origin were not only used in foods but also to embalm, produce perfumes, anointing oil, incense offerings in fumigation, to appease the ancient gods and to expel evil spirits, serpents, household insects and pests (Rosengarten, 1970a).

2.2 *Xylopia aethiopica* (Negro pepper, Ethiopian pepper)

The word *Xylopia* is coined from the Greek word (xylon pikron) meaning "bitter wood". The latter part of the binomial name of the plant is *aethiopica*, refers to the Ethiopian origin of the tree. The proliferation of *Xylopia* species although predominant in West Africa, are also

common in the following parts of Africa; East Tropical Africa, Northeast Tropical Africa, South Tropical Africa, West-Central Tropical Africa. The major habitats of *Xylopia* plants are fringing forests, riverine communities and some arid savannah regions in tropical Africa. In Ghana, it is called "hwintia" in Twi, "etso" in Ewe and '*samaamdabile*' in Waala language spoken in the Upper West Region.

Xylopia aethiopica plant species is an evergreen, aromatic tree belonging to a family of flowering plants called Annonaceae that can grow up to 20 m high (Mohammed *et al*., 2016). The fruit or pod or hull shape is small and curvy, looking green when matured and dark-brown when fully dried and largely resembling a twisted bean pod (Elhassan & Ayoub, 2014; Ezekwesili *et al.*, 2010; Ogbonna *et al.*, 2015).

The most widely used part of the medicinal plant is the stem bark, root bark, and fruits. *X. aethiopica* fruits are predominantly used in food due to their ability to impart aroma and taste to food. The taste of the indigenous spices has in many ways been described as pungent and bitter. Dried fruits of *X. aethiopica* are also used as herbal medicine for curing various ailments and foods for the convalescing. Perceived medicinal benefits of *X. aethiopica* in drugs include the cure of rheumatism, bronchitis, gingivitis, stomach ache, constipation, infections, ulcers, dysentery, diabetes and infertility (Mohammed *et al*., 2016; Tatsadjieu *et al*, 2003; Yapi *et al.*, 2012).

X. aethiopica also serves as a herbal stimulant for some class of consumers. The indigenous pepper also possesses aromatic, carminative, insecticidal and antimicrobial properties (Habiba *et al*., 2010; Koné *et al.*,2012; Nguemtchouin *et al.,* 2010; Polatoğlu & Karakoç, 2016). The antibacterial property on *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Bacillus cereus*, antifungal property on *Aspergillus flavus,* insecticidal property on maize weevil *Sitophilus zeamais* (Motsch), anthelminthic property on *Echinostoma caproni,*

Heligmosomoides bakeri and *Schistosoma mansoni* exhibited by *X. aethiopica* has been reported by Bakkali *et al*. (2008); Koné *et al*. (2012); Nguemtchouin *et al*. (2010); Polatoğlu & Karakoç, (2016) and Tatsadjieu *et al*. (2003).

2.2.1 Scientific classification

Xylopia aethiopica has been identified and classified by various organisations, some of which are authoritative in the field of plant taxonomy. The Integrated Taxonomic Information Systems (ITIS) has been long known for providing authoritative taxonomic nomenclature and hierarchy for plants, animals, fungi and microbes hence the adaptation of their scientific classification of *Xylopia aethiopica*, which is also identified in the ITIS database as TSN 506195.

Kingdom: Plantae – plantes, Plantae, Vegetal, plants

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta – land plants

Superdivision: Embryophyta

Division: Tracheophyta – vascular plants, tracheophytes

Subdivision: Spermatophytina – spermatophytes, seed plants, phanérogames

Class: Magnoliopsida

Superorder: Magnolianae

Order: Magnoliales

Family: Annonaceae – custard apples

Genus: Xylopia L.

Species: *Xylopia aethiopica* (Dunal) A. Rich. – Ethiopian pepper

Plate 1: Dried fruits of *Xylopia aethiopica* sold at Nima Market, Accra, Ghana

2.2.2 Pre-and Post-harvest treatments and Usage of *X. aethiopica*

The fruits upon maturing are harvested and subjected to varying post-harvest treatments. In Ghana, smallholder farmers subject matured fruits to air or solar drying. The intensity of sun rays plays a critical role in the duration of drying of the fruits. The average drying time of the fruits has been speculated at around one week. Dried fruits from various smaller holder farms are often purchased and aggregated in sacks by middlemen and women. Problems such as spillage, breakages, bruising and leakage may occur during transportation. These problems are bound to occur when the integrity of packages are compromised. The presence of conditions such as rain, heat and humidity could trigger the growth of moulds. Birds and rodents are potent sources of concern during storage.

2.3 The need for novel and natural sources of antimicrobials

Ever since the golden age of microbiology begun and ended, there has been the consistent use of antimicrobials in chemotherapy. Notwithstanding, their long use has often led to the

resistance of various organisms to the antimicrobials. This has led to the reduction of their efficacy in the treatment of microbial infections hence the widespread public health concern over antimicrobial-resistant infectious microorganisms.

There has therefore been the need to explore other natural sources for these antimicrobials which would become the panacea to the emerging problems. Natural options for such antimicrobials include bacteria (prokaryotes), fungi (eukaryotic microorganisms), plants and animals. The recent and most successful sources of these antimicrobials are microbial and plant sources. The exploration of plant sources has been fuelled by the tendency for plants to yield crude extracts, essential oils and other secondary metabolites. Biotic and abiotic factors, however, influence the presence, volume, efficacy, quality, the composition of these antimicrobial extracts (Bakkali *et al*., 2008; Bhattacharya, 2016; Burt, 2004).

The need for natural antibiotics is further supported by the public concerns about the side effects of some synthetic antimicrobials (Abolfazl *et al*., 2013). Plant options have been exploited generally in folk medicine due to accepted reasons of lower cost, fewer side effects and their ability to inhibit a broad spectrum of diseases and microorganisms. The World Health Organization posits that about 25% of drugs prescribed worldwide come from plants sources and 252 are considered basic and essential (Abolfazl *et al.*, 2013).

Several studies have shown that these extracts are inhibitors of bacteria, fungi, yeast, mould, parasites and insects (Bevilacqua *et al.,* 2010; Burt, 2004; Burt & Reinders, 2003; Koné *et al*., 2012; Nguemtchouin *et al*., 2010; Omidbeygi *et. al* 2007; Prabuseenivasan *et al.,* 2006; Tatsadjieu *et al*., 2003; Xing *et al.*,2012). Pathogenic organisms from both human and veterinarian origin have also been inhibited by EOs. The inhibition of non-pathogenic and opportunistic organisms has been recorded (Chaieb *et al*., 2007). Foodborne pathogenic and

spoilage Gram positive and negative bacteria has also been shown to be appreciably inhibited by these plant extracts.

2.4 Essential oils (Eos), their nature and applications

EOs are odoriferous, aromatic and highly volatile liquids products that are extracted from flowers, roots, seeds, leaves, fruits, peels and whole plants. EOs have been revered for their importance even in distant history, however, Ríos (2016) suggests that their use has been lost with time. Essential oils, generally are composed of lipophilic and highly volatile secondary plant metabolites, largely terpenes (monoterpenes and sesquiterpenes) and allyl phenols. It also contains precursors of known derivatives like glycosides and sesquiterpene lactones (Ríos, 2016).

Essential oils are used in food applications to maintain or improve traditional flavour, aroma and taste of foods. Essential oils have also been used extensively in the agro-food industry where it has been chiefly used in the production of beverages and the flavouring of all kinds of foods; confectionery, soft drinks and distilled alcoholic beverages. Recent applications in food packaging materials (to extend shelf lives of food) is gaining ground (Adelakun *et al.*, 2016; Burt, 2004; Dini, 2016; Ríos, 2016; Tongnuanchan & Benjakul, 2014).

The names of oils are usually derived from the plant species or plant part but their uniqueness is usually detected by both smell and taste. According to Ríos (2016), essential oils, although in liquid form could sometimes be solid. The colour of these oils ranges from being colourless to slightly yellowish, especially when they have freshly distilled, being clean to touch and also providing an aromatic smell which can be easily absorbed by the skin. Essential oils are different from other oils because they are highly volatile, and the litmus test is that they quickly disappear when drops are placed on paper (Ríos, 2016)

In general, EOs are less dense than water, however, cinnamon, clove and sassafras oils are exceptionally denser than water (Ríos, 2016). EOs have varying properties with regards to solubility in solvents. They are soluble in organic solvents such as ethanol, diethyl ether and has been reported to mix well in vegetable oils, fats and waxes but conversely reported to be solubility poor (Ríos, 2016) According to Ríos (2016), the high refractive index and rotary power of essential oils could be instrumental in their identification and quality control.

2.4.1 Variations in essential oils

The variability of the quality essential oils poses challenges in terms of ensuring quality control measures. Essential oil types from different plant sources, described by several researchers are almost 3000 however, only 300 (10%) are used commercially for imparting flavours in the fragrance market (Burt, 2004). Bhattacharya (2016) also, posits that about 400 plant-based essential oils from 62 plant family species exist. Burt (2004) and Ríos (2016) share the same view that the difference in the chemical composition of these essential oils has the tendency to endanger the perfume manufacturing industries. Apart from the genetic factors which have been considered by many as the major cause of variations, recent a fortiori of science knowledge suggests that climate; temperature, rainfall, altitude, the length of the day, of the plant, play significant roles in the composition of essential oils (Ríos, 2016).According to Bhattacharya (2016) and Harris (2003), high temperatures enhance the formation of essential oils and high rainfall induces the loss of essential oils from leaves and roots by leaching. Also, long day and short day conditions have been shown to influence the major chemical components of some EOs such as the EO of peppermint leaves ((Bhattacharya, 2016; McKay & Blumberg, 2006). In that study, the long day weather conditions influenced the presence and concentrations of menthone, menthol and traces of menthofuran whiles the short day samples had only menthofuran as the major chemical component of the peppermint leaves. Other

environmental conditions such as the type of radiation; ozone depletion and altitude have been shown to influence the composition of essential oils. In other studies, cited by Bhattacharya (2016), the season of harvest, time of day of the harvest, the age of the plant and geographical location were shown to have also influenced yield and quality of the essential oils. Oils extracted from Greek oregano, harvested during the wet cold season, was higher in both inflorescence and leaves than those harvested in the dry and warm season.

2.4.2 Adulteration of essential oil

The complexity of the functional uses of essential oils makes it an indispensable component of the flavour and fragrance industry, medicinal or therapeutic industry and food industry. The adulteration of essential oils may limit their utmost functional use.

The common EO adulterants include; vegetable carrier oils, alcohol and synthetic oils which are often used as diluents. In other cases, cheaper oils of the same species but from different geographical ancestries are incorporated into essential oils. The incorporation of cheaper essential oils extracted from the other parts of the same or different plant part has also been reported (Ng *et al.,* 2016) . Also, essential oils obtained from similar or related species are sometimes used as adulterants. Synthetic adulterants such as phthalates and benzyl alcohol found in some EOs have been reported to be hazardous to health (Ng *et al*., 2016).

Several methods have been used in the identification of adulterated oils has been documented by Ng *et al*. (2016). Notable authentication methods include GC-MS, determination of enantiomeric composition, Supercritical Fluid Extraction GC-MS involving the use of multidimensional GC to resolve enantiomers, enantioselective capillary GC and online methods of Isotope Ratio Mass Spectrometry (IRMS). Also, other methods comprise of enantioselective capillary gas chromatography and IRMS coupled online with capillary gas

chromatography, Near Infrared (NIR) Spectroscopy, Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD) method.

2.5 Extraction of essential oils – conventional and modern methods and technologies

The extraction of essential oils from botanical species has become expedient due to the ever-increasing demands of these oils in diverse applications. The need, therefore, to unravel novel, efficient, effective, safe, cost-effective methods of extraction of these essences and oils must be duly explored. The choice of method for the extraction of essential oils is normally influenced by innumerable factors. Critical factors include the type of material or plant part, the state and form of plant material. These factors among others are major determinants of the quality of the essential oil. The method of extraction is crucial to the yield, composition of constituents, toxicity and other chemical properties including the antioxidant properties (Tongnuanchan & Benjakul, 2014).

The need for a suitable method of extraction cannot be underestimated because of the tendency of inappropriate methods leading to an overall chemical or pharmacological change in the essential oil. Inappropriate methods could also lead to damage, loss of bioactivity, discolouration, off-flavours/odours, increased viscosity (Tongnuanchan & Benjakul, 2014). On the other hand, a good choice of method for extraction will yield best quality aromatic substances that meet the demands of key players; flavourist, perfumers, aromatherapist, and research and development teams in the essential oil industry. An ideal method of extraction per the authors' views should be environmentally friendly and in accordance with all regulations and legislation governing the process of extraction.

The essential oil yield from plants are often found anywhere from 0.01 percent to 10 percent of the total (Kumar, 2010). Owing to the nature of the volatiles, there is the need to extract and

concentrate them into reasonable volumes for use. There are various methods of extraction of essential oils and their bioactive compounds in each spice. The methods revolve around conventional methods such as water distillation, water and steam distillation, steam distillation, maceration, cohobation and the enfleurage (Kumar, 2010; Stratakos & Koidis, 2015) The conventional methods according to Stratakos and Koidis (2015) have several drawbacks amongst which include high energy consumption, and less environmentally friendly because of high carbon dioxide (CO_2) emissions. On the other hand, modern or sophisticated methods (microwave assisted extraction, controlled pressure drop process and ultrasound assisted extraction), according to encourages sustainability, production of oils of same or higher quality, and cost-effectiveness (Stratakos and Koidis, 2015). Methods may also include the use of organic and inorganic solvents.

2.5.1 Solvent extraction methods

2.5.1.1 Extraction using organic solvents

The extraction of active components from the plant and plant parts, using solvents has been in existence for long. These methods include the use of polar and non-polar solvents. This method is often used for plant part or materials that are delicate or fragile. The parts may essentially include the flowers of some plants (Tongnuanchan & Benjakul, 2014). The solvents used in this process are myriad and may include petroleum ether, methanol, acetone, hexane, cooking oil, ethanol, isopropanol, and ethyl acetate (Hamed, 2006; Lee *et al.,* 2003; Ogunka-Nnoka & Igwe, 2013; Stratakos & Koidis, 2015; Thompson-Witrick *et al*., 2015; Thongson *et al.,* 2004; Tongnuanchan & Benjakul, 2014; Wilkes *et al*., 2000). The solvents often used in the extraction process are of analytical grade. The use of solvents for the extraction of volatile oils possess some advantages over other methods of extraction. Tongnuanchan and Benjakul (2014) posit that the general practice of the solvent extraction is to mix solvent with the respective

plant material and then the mixture is subjected to heating in order to extract the oil. The process is then followed by filtration, where the filtrate is concentrated through solvent evaporation. The same author records that, the concentrates that emanate from the filtration process usually contain a resin (resinoid), or alternatively described as concrete which is made up of a combination fragrance, wax and essential oils. Subsequently, the concentrate is then mixed with pure alcohol in order to extract the oil and distil at low temperatures. Tongnuanchan and Benjakul (2014) further posits that solvent extraction methods are sometimes advantageous than distillation, because of the lower uniform temperature that is maintained throughout the process. The low temperature tends to reduce the risk of chemical changes at high temperatures. According to Tongnuanchan and Benjakul (2014) during generic extraction, the absolute alcohol absorbs the fragrance and after the evaporation of the absolute alcohol, the essential oil is obtained.

Kumar (2010) explains that because of the nature of the waxy mass or concrete; the waxy concrete needs to be warmed and stirred with alcohol which then yields minute globules. The difference in solubility of the aroma molecules in alcohol and non-aromatic waxes and pigments leads to an efficient separation.

Solvent extraction methods are never devoid of merits and demerits. In a study conducted by Ozen *et al.* (2011), ethanol, methanol and water extracts of *Thymus praecox* subsp. s*korpilii* var. *skorpillii* (TPS), conferred significant free-radical scavenging activities. In a study conducted by Sarikurkcu *et al*. (2009), water extracts were found to exhibit higher antioxidant activity than hexane, ethyl acetate, methanol and dichloromethane. Generally, promoters and consumers of herbal extracts often express dissatisfaction about the use of some organic solvents in the extraction process. This obdurate notion is fuelled by perceived presence of residues of the organic solvents which could be unhealthy; causing allergies, the effect on the immune system and toxicity to end users (Kumar, 2010; Tongnuanchan & Benjakul, 2014).

Also, a major challenge for the patronage of essential oils that have been extracted using solvent is the higher cost of the solvents, unlike the distillation process. The long duration for extraction of oils as well as the large amounts of solvents required for the process has been cited as a major challenge. On the other hand, fanatics of the solvent extraction method posit that the process often confers a more natural odour that is incomparable to distilled oils (Kumar, 2010). Despite the claims of the organic solvent residues in oils, Stratakos and Koidis (2015) have hypothecated that, if alcohol is used in the extraction process, then it is deemed fit for consumption and considered as "food grade". Stratakos and Koidis (2015) surmised that solvent extraction is influenced by the rate of diffusion. The rate of extraction could be hastened using hot solvents because temperature also influences the rate of diffusion. The solvent extraction method is therefore revered in the perfume industry.

2.5.2 Other conventional methods of solvent extraction

The extraction of essential oils with varied quality has long been experimented by organic solvents such as isopropanol, methanol, ethanol, hexane and ethyl acetate. In an account of extraction of essential oils, Oke *et al*. (2009) used HPLC grade methanol to extract oils from *Satureja cuneifolia* Ten through the Soxhlet technique at 60 ℃ for 3 hours. With the aid of a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), the extract was filtered and concentrated under vacuum at 50 ℃. The waxy extract was then lyophilized and stored for at 4 ℃ for use.

2.5.2.1 Steam distillation

Distillation of essential oils from parts of aromatic plants and herbs has been most widely used (Kumar, 2010; Ríos, 2016; Stratakos & Koidis, 2015; Tajkarimi *et al*., 2010). The duration of distillation, according to Stratakos and Koidis (2015) can last for between 1 and 10 hours. The yield of essential oils is often influenced by the temperature, pressure, type of plant, the age of

the plant, geographical location and the length of distillation time among others. This special method which involves the use of steam to burst the oil repositories of plant materials to release considerable amount and quality of essential oils are collected in separate vessels. This method has proved to be very useful in distillation or separation of temperature sensitive materials like oils, resins and hydrocarbons from plant materials. The components are usually non-polar or insoluble in water and may undergo decomposition when boiling points are reached. The functional principle of steam distillation is to aid the release of compounds or their mixtures at temperatures equivalent to the boiling point of water (100 ℃). Although some of the compounds have boiling points around 200 ℃ or higher, they tend to be volatile in the presence of steam or temperature of boiling water (100 ℃) at atmospheric pressure. The distillation process, although seems simple, has inherent setbacks like the formation of artefacts due to the acidity of water or the high temperature. The presence of esters may also have serious implications on oil quality when hydrolysis of esters to alcohols and acids occur. The presence of undesirable impurities such as waxes, which impart unacceptable odours could be kerbed by a rectification which chiefly involves re-distillation of the oils (Stratakos & Koidis, 2015).

2.5.2.2 Supercritical carbon dioxide

Supercritical fluids have received broad usage in several applications. Pourmortazavi and Hajimirsadeghi (2007) records that more than 90% of the analytical grade of supercritical fluids is carbon dioxide. The ability to use supercritical fluids such as carbon dioxide (CO_2) for the extraction of essential oils is undergirded by some of the inherent deficiencies in other traditional or convention methods. According to several authors cited by Pourmortazavi & Hajimirsadeghi (2007), the method has been applied in metal cation extraction, polymer synthesis and particle nucleation. The choice of CO_2 is due to its relatively non-toxic, non-inflammable, relatively low cost, relatively high purity and easy removal of extracts.

The supercritical extraction according to Tongnuanchan and Benjakul (2014), offsets the low extraction efficiency, toxic solvent residue and the degradation of unsaturated compounds present in these oils. Based on the properties of CO_2 under high-pressure condition; safe and inert nature and its conversion to liquid, CO_2 is used to extract oils from the repositories of the botanical materials. In this process, it leaves no solvent residues in the final finished product. This according to Tongnuanchan and Benjakul (2014) is made possible because the liquid CO_2 reverts back into gas which is then evaporated under normal conditions of temperature and atmospheric pressure. Although it has posited that this method yields high solubility's of essential oils, another school of thoughts report the relatively slow extraction rates with pure CO_2 which is speculated to yield about 80% after 90 minutes. Another major drawback includes the lack of polarity needed for the extraction of polar analytes (Hawthorne *et al*., 1993; Pourmortazavi & Hajimirsadeghi, 2007; Tongnuanchan & Benjakul, 2014).

In the last two decades, other supercritical fluids that have seen their application in the extraction of essential oils includes N_2O, SF_6 and freons (Pourmortazavi & Hajimirsadeghi, 2007). The effects of the cogent extraction parameters, the effect of the matrix on the extraction process and the solubility and the mass transfer rate of plant oils in supercritical fluids has been described (Pourmortazavi & Hajimirsadeghi, 2007).

The SFE method according to Stratakos and Koidis (2015) "can be performed in batches, semi-batch and continuous modes". According to the same author, the solid botanical plant material is deposited in the "vessel in which the supercritical fluid is added under a specific flow rate until the appropriate extraction conditions are reached".

2.5.2.3 Cold expression or pressing

Stratakos and Koidis (2015) posits that the cold pressing method of extraction has been considered by many as the oldest method of extraction. This method of extraction has long

been used for the extraction of essential oils of the citrus fraternity. The evidence of the presence of such oils in citrus is best experienced during the manual zesting of the peels. The method utilises any physical process to exude oils from the glands in the peels and cuticles. Cold expression results in watery emulsions which are then concentrated or coalesced through a centrifugation process in order to obtain pure essential oils.

Stratakos and Koidis (2015) theorise that, until the twentieth century, large-scale industrial production of cold pressed citrus oil was artisanal or manual. Although, there has been a surge in industrial machinery; "sfumatric", "Pellatrici", bergamot oil extractors", "brown oil extractors" to extract oils through mechanical methods, the major setback is the thermal instability of the aldehydes present (Stratakos & Koidis, 2015). It is not a common practice to extract oils from non-citrus fruits.

2.5.3 New and emerging technologies for extraction of EOs

Some of the new methods that combine the conventional methods and microwave methods include; microwave-assisted solvent extraction, microwave hydrodistillation, compressed air microwave distillation, vacuum microwave hydrodistillation and microwave-accelerated steam distillation (Stratakos and Koidis, 2015). The development of microwave steam distillation (MSD) and microwave-assisted hydrodistillation has been reported by researchers and are cited by Périno-Issartier *et al.,* (2013); and Sahraoui *et al.,* (2008).

2.5.3.1 Solvent-free Microwave Extraction (SFME)

A relatively green and improved method of extraction of essential oil, Solvent-free microwave (SFME); entailing the application of microwave heating and a combination of dry distillation without the addition of water has been published by Lucchesi *et al*. (2004). The method although with some intrinsic disadvantages sought to improve the low extraction efficiency, long extraction time, degradation of ester compounds through hydrolytic effects or thermal

effects and the occurrence of toxic solvent residues. In addition to the above benefits, it is environmentally friendly, reduces waste and circumvents the usage of water and solvent. In this method, plant materials(aromatic herbs, dry seeds and spices) are deposited in microwave reactor without the addition of any solvent or water sample (Lucchesi *et al*., 2004; Tongnuanchan & Benjakul, 2014). The principle of microwave technology utilises the internal or *in situ* water in the plant material. The heating mechanisms lead to the distension and bursting of the glands and oleiferous receptacles of the plant materials in order to yield the oils. Subsequently, the oil is subjected to azeotropic distillation "a specific technique of adding another component to generate a new, lower boiling azeotrope that is heterogeneous" in which the in situ water is then evaporated (Lucchesi *et al*., 2004). The distillation process leads to the evaporation of vapour, which is collected through a condenser. The distillate is then collected in a fitted flask. EO's are then collected and dried without any addition of solvent. Additional reading on the SFME method has been documented by Lucchesi *et al*. (2004); and Tongnuanchan & Benjakul (2014).

2.5.3.2 Microwave- Assisted Extraction (MAE)

MAE has been categorised by Stratakos and Koidis (2015) as one of the novel "green" extraction methods that have become useful over time due to its unique heating mechanism that is based on friction. This method involves the use of non-polar or polar solvents. It has been surmised that it comes with a reasonable cost, higher selectivity, shorter extraction time, efficient performance under atmospheric conditions and reasonable yield as compared to the traditional or conventional methods of extraction (Stratakos & Koidis, 2015).

In Zhang *et al*. (2011) MAE was rated as higher than SFE in terms of operation cost and simplicity. In the event of environmental friendliness, SFE was rated higher than MAE due to the higher quantities of organic solvents that characterised the MAE process. The use of MAE

at the laboratory scale has been successful, however, the industrial scale of usage remains low (Leonelli & Mason, 2010; Stratakos & Koidis, 2015).

2.5.3.3 Ultrasound- Assisted extraction (UAE)

Ultrasonic- assisted extraction (UAE) has become a technology of utmost interest in the food processing fraternity due to its ability to facilitate the extraction of components such as oils, proteins and polysaccharides. The incorporation of the ultrasound technology professes minimum effects on the extractable compounds, reduction/ avoidance of organic solvents, reduction of extraction time (Stratakos & Koidis, 2015; Vilkhu *et al.*, 2008). The process of producing and breaking down of microscopic bubbles, called cavitation has played a vital role in the violent collapse of the bubbles. The mechanical force induced as a result of the violent closure of these bubbles leads to cell membrane damage hence high yields of essential oils and fast rate of extraction.

According to studies cited by Stratakos and Koidis (2015) UAE of *Jatropha curcas*, almond and apricot seeds have a higher yield and a reduced oil extraction time as compared to those obtained from enzymatic extraction. The UAE has high capital cost but produces a higher yield of EO in less time and this could serve as an alternative method of extraction to the conventional methods.

2.6 Key characteristics of essential oils (chemical, physicochemical, sensory, and biological stability of EO extracts)

The building blocks of the chemical compounds are hydrogen, carbon and oxygen. Some school of thoughts classify EOs into two major categories; the volatile fraction and the non-volatile residue. The volatiles fraction encompasses about 90 – 95% of the weight of the oil. The derivatives of oxygen and some esters, alcohols and aliphatic aldehydes also form part of

the volatile fraction of oils. On the other side, the non-volatile residue of the oils constitutes 1-10% of the oil. They are uniquely identified by the presence of sterols, carotenoids, fatty acids, waxes and flavonoids.

It is worth noting that, the chemical components of these oils are not fixed, however, they vary or change or get degraded based on how, when, where the plants were grown and subsequently harvested. Other schools of thoughts opine that the hydrocarbon components of the essential oils could be subdivided into 2 groups which are the terpenes and oxygenated compounds. The compounds include alcohols, esters, ketones, aldehydes, phenols and oxides (Kumar, 2010). Essential oils from different source have exhibited diverse chemical and physical properties. Their diversity spans across odours, refractive indices, optical and specific rotation properties (Mariod, 2016). Their often immiscible nature in water is a unique property. However, essential oils are generally known to be soluble in most organic, e.g. ether and alcohol. Essential oils are also not considered as fixed oils because they lack glyceryl esters of fatty acids and cannot undergo saponification reaction with alkalines, as in other oils (Mariod, 2016).

The basic descriptions of these compounds are expounded below.

2.6.1 Alcohols:

Alcohols are identified as the – OH component of the chemical structures of various compounds and know for the –ol usage in naming. An example of the components includes methanol, nerol, benzyl alcohol, citronellol in lemon, eucalyptus and rose. A common form of alcohol component in EO is their presence in lavender as linalool, geraniol in geranium and palmarosa (Kumar, 2010). The alcohol components are of GRAS status and either has a low or total absence of toxic compound which in turn becomes harmful to the human body. These compounds are either combined with terpenes or esters or in a free state. The usage of alcohol is undergirded by how it imparts an antibacterial, antiseptic and antiviral properties.

2.6.2 Aldehydes:

The aldehydes or alkanal components of EOs are uniquely identified by a chemical structure –CHO and –al usage in their naming. These group of compounds have been surmised to possess anti-fungal, antiseptic, bactericidal properties (Kumar, 2010). In some cases, they exhibit anti-inflammatory properties and also serve as sedatives and disinfectants. Their effect has been demonstrated in the treatment of candida infections and other fungal infections. Examples of aldehydes include citral in lemon, and citronellal in citrus eucalyptus, lemon balm and lemongrass (Kumar, 2010).

2.6.3 Esters:

Esters are compounds formed through a reaction of alcohols with acids. Esters are known for their soothing and balancing effects in essential oils. The alcohol components often impart antimicrobial agents (Kumar, 2010). The role of an ester of essential oils has been evident in the area of medicine. Esters have been used as antifungals and sedatives and also has the ability to balance the activities of the nervous system. Examples include linyl acetate in bergamot and lavender

2.6.4 Acids:

The acid components of essential oils are largely organic acids and are in free forms. The acids are often in very small or minute quantities. The acids in essential oils have been surmised to impart anti-inflammatory properties (Kumar, 2010). They also act as buffers systems to control acidity in food. Notable examples of such organic acids in EOs include citric acid, lactic acid and cinnamic acid.

2.6.5 Ketones

Ketones (alkenones) in general are inimitably identified structurally with their RC (=O) R' as a functional group. Their presence in essential oils has been very debatable due to their seemingly perceived toxic nature. Although research has it that, some natural forms are non-toxic, other schools of thoughts opine otherwise. The naturally toxic forms include thujone found in sage, wormwood oil and mustard oil, thuja and mugwort (Raut & Karuppayil, 2014). Pinocamphone in hyssops has also been reported as toxic. On the other hand, jasmine extracted from jasmine oil, menthone from peppermint oil, fenchone in fennel oil has been reported as non-toxic. Their beneficial use has been found in healing wounds and curing upper respiratory complaints. Their ability to aid the formation of scar tissue has been reported (Kumar, 2010).

2.6 Essential Oils applications in food: opportunities and challenges

Lime, juniper berry oil, fennel has been used extensively in the flavouring of drinks, spirits, and wines (Kumar, 2010). The application of lemongrass oil in the preservation of stored food crops has also been documented (Kumar, 2010). The application of various oils in food is undergirded by their antimicrobial properties, shelf-life extension, flavour and aroma addition, compelling taste and aphrodisiac properties etc. Their application of oils to raw and processed foods in order to combat pathogenic and spoilage microorganisms also impart some medicinal or natural therapeutic effects. The organoleptic enhancement of some of the cross-continental dishes served from the oils of lavandin, lavender, spike lavender, kernels of *Monodora myristica, Eugenia aromatica* and sweet basil, has widely been discussed in various literature (Mariod, 2016). Food applications of these oils are evident in frozen dairy, gelatin, puddings, non-alcoholic beverages, baked foods etc. The uniqueness or peculiarity of components such as thymol, cumene, thymine, stearoptene, often confer warm, aromatic, and pleasing flavours to sauces, snacks and sometimes vegetables (Malhotra, 2004; Mariod, 2016). To ascertain the

efficacy of oregano essential oils in preserving meat, Hulankova *et al.* (2013) proved that, samples of meat stayed up to the sixth day in storage as well as obtaining scores higher than both controls on the 5 scores neutral-point. In stored minced beef, oregano oil proved to have improved odour, colour and flavour but was slightly detectable after cooking (Hulankova *et al*., 2013).

The mode of post-harvest treatment of some spices has also left some traces of unique odour notes that greatly influence the organoleptic properties of the foods. In a study conducted by Calín-Sánchez *et al*. (2012), vacuum-microwave (VM) method and using convective (40 °C) drying methods yielded varying degrees of odour notes from fresh sweet basil. Odour notes intensely ranged from fresh, floral, herbaceous. The difference in odour notes for fresh samples that had not been subjected to any form of post-harvest processes yielded spicy, hay-like, earthy, sweet, woody and infusion. These odour notes together, play significant roles in imparting desirable or undesirable organoleptic properties in dishes or foods.

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The egregious odour and aftertaste left behind by thymol in tyndallized carrot broth often posed a minimal acceptance or liking degree when samples were evaluated (Valero & Giner, 2006). The use of cinnamaldehyde at low concentrations in carrot broth proved to have improved the taste without any adverse effect in the area of taste or smell (Valero & Giner, 2006).

The opportunity for further use of essential oils in the fruit and vegetable marketing industry has been studied. The urge to ensure availability and usage of cleaner and safer fruit and vegetables through means other than applying chlorinated water has been experimented. The move to incorporate essential oils in the cleaning regimes of fruit and vegetables has been heightened by the registration of carvacrol, carvone, citral, p-cymene, eugenol, limonene, menthol, thymol and cinnamaldehyde with the European Commission (Mariod, 2016).

In order to suggest tolerable limits for application of oregano essential oils, Gutierrez *et al*. (2009) conducted a study on the application of essential oils on lettuce and suggested the use of oregano essential oil at 250 ppm to impart an acceptable sensory pleasure. In another study, Wang *et al.* (2007) reported that the application of chemical components such as thymol, menthol and eugenol on strawberries caused a delay in the fruit senescence.

Wang *et al*. (2007) also posited that a better fruit quality was maintained with an increase in phenolic content, organic acids, sugars, anthocyanin's, flavonoids and oxygen radical absorbance capacity.

The opportunities for preserving cheese, on the other hand, has been reported after the application of essential oils of rosemary and oregano. The activity of essential oils improved the oxidative and fermentative stability of flavoured cheese prepared with a cream cheese base. The same author reported inhibition of the lipid oxidation as well as the development of rancid and fermented flavours (Mariod, 2016). Thyme oil applied at 500 ppm to tomato ketchup was considered acceptable after panellist evaluated the product. To summarise the purposeful uses of essential oil in food, Omidbeygi *et al*. (2007) opined that, it is best to incorporate the essential oil as flavour components rather than preservatives. The authors also suggested that major or active oil components could be used in isolation rather the whole oil. Also, they theorised that oils flavours could be used to mask foods with an already prevailing or conspicuous flavour. Alternative application methods to reduce the egregious smell of some essential has been suggested by Mariod, (2016). According to the author, the use of vapours from essential oil is essential to curb or combat the sensory effect of direct contact application of the oils.

In the quest to preserve button mushrooms (*Agaricus bisporus*), Gao *et al.* (2014), fumigated the mushrooms for 16 days with essential oils from cinnamaldehyde, clove and thyme, and

reported that the cinnamaldehyde fumigated mushroom although slightly brown, had commercial value as well as edibility.

The application of essential oils in sodas, “fizzy drink” and “minerals” has long existed, with exemplary use in the first popular soda, Coca-Cola or Coke at a stage in its history (Ameh & Obodozie-Ofoegbu, 2016; TCCC, 2017). The minor or major components of some of the other sodas are oils serving as flavourings. In the use of EOs as essential ingredients of these sodas, they are prepared and packaged as concentrates for distribution to smaller licenced bottling companies.

According to Ameh and Obodozie-Ofoegbu (2016), the primary motivation for the soda industry has been essential oils, as the combination of seven spices was crucial in popularizing the then John Pembroke’s cola formula rather than the psychoactive alkaloids incorporated from coca leaves. Also, in other brands of sodas, a combination of two or more essential oils are incorporated into the formulation of concentrates based on the supplier and bottlers brand and consumer choices.

According to Ameh and Obodozie-Ofoegbu (2016), the presence of EOs in soda concentrates aids interactions with other flavourings such as sweeteners and acids. Essential oils, often used in the soda industry spans between oils of coca leaf, cinnamon bark, coriander, lemon peel, lime peel, orange peel, neroli and vanilla (Ameh & Obodozie-Ofoegbu, 2016). These essential oils are added through suitable vehicles such as vegetable oils or polyhydric alcohols.

Furthermore, the application of essential oils in meat and fish has not long existed like pickling, salting, refrigeration, radiation and chemico-biopreservation methods. That notwithstanding, its adoption also contributes to the development of flavour, aroma, juiciness and texture, which cumulatively improve on the acceptability and taste. The application of essential oils further helps in the maintenance of the freshness through the impartation of antimicrobial,

hypoallergenic and antioxidant properties rather than the ancient pickling which existed for over 4000 years ago in some province of India (Chivandi *et al.* 2016).

The objection of synthetic preservatives often perceived to be extremely toxic and cancerous have also fuelled the incorporation various essential oils as preservatives in food and beverages. This trend has spearheaded the inclusion of rosemary, cinnamon, garlic, oregano, ginger and bay oils which have often shown positive results of but leaves much to be desired about the organoleptic properties as a result structural and chemical reactions or interactions (Chivandi *et al*., 2016). Also, pre-treating meat with oregano and sage EOs before storage has proved to reduce the rate of oxidative deterioration (Fasseas *et. al* 2008).

2.7 Selected foodborne microorganisms of food safety concern

2.7.1. Staphylococcus aureus

Staphylococcus spp. especially *S. aureus* has been considered by many as one of the most troublesome foodborne organisms (Azizkhani & Tooryan, 2015). These organisms are known for the menace they cause in the food and beverage industry for their ability to induce and produce enterotoxins. The strains of *Staphylococcus* are coagulase-positive. The emergence of symptoms in immunocompromised persons who have either consumed one or two of the Staphylococcal enterotoxins (SE's) barely last for 24 to 48h and a total day for health recuperation could span between 1 – 3 days (Azizkhani *et al.,* 2012).

The symptoms usually of any food poisoning episode include nausea, abdominal cramps, diarrhoea and vomiting. Vomiting during *S. aureus* poisoning is commonly placed hence perceived by many to result from stimulation of the enteric-vagus nerve reflex within the vomiting centres of the brains. (Arbuthnott *et al.,* 1990; Azizkhani *et al*., 2012). These heat-stable, often resistant toxins have caused many lives because of the lack of serologically

available drugs for endotoxins (Azizkhani *et al*., 2012). Essential oils of rosemary, lemongrass, oregano, clove, thyme, and tea bush (*Lippia spp*.) have been proved to inhibit the growth of *S. aureus in vitro* at different concentrations (Burt, 2004).

2.7.2. Escherichia coli

E. coli is one of the major bacterial groups implicated in food and waterborne illness. They broadly belong to the Gram-negative bacteria, facultative anaerobes, and are morphologically rod-shaped. *E. coli* strains are commonly found in the lower intestinal tract of warm-blooded organisms as well as foods and the environment. Although most strains remain harmless to humans, some strains have long been associated with diarrhoea, urinary tract infections and other illnesses. The *E. coli* O157:H7 strain has been reported to cause severe food poisoning episodes, haemolytic uremic syndrome cases, acute renal failure leading to death and anaemia (Rangel *et al.,* 2005)

E. coli contamination has been implicated in beef, pork, water, dairy products. Most of these foodborne illnesses are often acquired from mass eatery services in institutions and street vended foods but could also be domestically acquired. *E. coli* transmission to humans is largely through faecal-oral routes and could cause disease when the strain is pathogenic. The faecal-oral transmission indicts the strains as potential indicator organisms to test environmental samples and products for faecal contamination (Ishii & Sadowsky, 2008). In the event of the ingestion of viable strains of bacteria, symptoms such as abdominal cramps, bloody stools, loss of appetite, fatigue and fever begin within one to four days.

In the wake of replacing synthetic antimicrobials with novel and natural plant-based extracts, the essential oils of turmeric, tea bush (*Lippia spp*.), thyme, clove, sage, lemongrass and oregano have been cited to inhibit their growth at different concentrations (Burt, 2004).

2.7.3. *Salmonella* Typhimurium

Salmonella Typhimurium belongs to the Enterobacteriaceae family. The bacterium is Gram-negative and possesses a rod shape. The Centre for Disease Control and Prevention (CDC) reports that Salmonella has been implicated in over one million foodborne illnesses annually in the United States, with the death toll averaging 380 deaths. Generally, Salmonella infection is one of the possible causes of gastroenteritis, and in severe cases, causes Salmonellosis. Infections often emanate from the consumption of foods contaminated with animal faeces or humans carrying the bacteria. Salmonella infection lasting between 12 – 72 hours, poses symptoms such as loss of appetite, nausea, vomiting, headaches, stomach cramps, diarrhoea, and fever which could last for 4 to 7 days with or without treatment. The severity of infections bothers around factors such as the virulence of the pathogen, host factors and environmental factors among others (Thomas *et al*., 2013).

Salmonella Typhimurium is part of CDC's list of food germs and has caused some of the major foodborne illness outbreaks through the consumption of contaminated eggs, raw or undercooked meat, poultry, milk and other dairy products. Also, fruits, vegetables and water can also be contaminated by these bacteria. The essential oils or bioactive components of sage, thyme, clove, lemongrass and rosemary have been cited to inhibit the growth of *S.* Typhimurium (Burt, 2004). Also, active components such as α-terpineol, carvacrol, citral, eugenol, geraniol, perillaldehyde and thymol have also been posited to be inhibitory at different concentrations (Burt, 2004).

2.8 Brief background on *Hibiscus sabdariffa* (bissap) leaves and extracts

Hibiscus sabdariffa L. (Hs) is commonly known as roselle, Jamaica sorrel, 'bissap' (Ghana) and karkadeh (Arabic), a prominent shrub belonging to one of the many plants within the

Hibiscus genus. Its dominance has been documented in both tropical and subtropical regions and has long used in food and medicine in West Africa whiles its seeds are used for oil in China. (Da-Costa-Rocha *et al.,* 2014; Eslaminejad & Zakaria, 2011; Wang *et al.,* 2012)

The plant has traditional routes in food especially in herbal drinks which are either prepared hot or cold, fermented, served as wines, ice creams, used in chocolates, puddings and cakes. Their use as flavourings, colouring agents have been reported (Da-Costa-Rocha *et al*., 2014; Okoro, 2007).

Generally, all plant parts have found use in either medicine or food. The leaves and shoots have been cited as a colourant, sour-flavoured vegetable, condiment and also eaten raw or cooked. The seeds of roselle are also ground in meals or roasted.

Notwithstanding their use in food, their herbal preparations or infusions from leaves or calyces have often be attributed to their strong antioxidant, hepato-protective, diuretic, febrifugal antidiabetic, antihypertensive, some perceived antimicrobial and emollient properties as well as its ability to reduce the level of drunkenness (Da-Costa-Rocha *et al*., 2014; Morton, 1987).

In Ghana, bissap leaves have been processed into beverages (sobolo) through the extraction using hot or cold water and then the addition of various fruit and spice essences. Aqueous extracts of leaves have been associated to flavonoids such as catechin, ellagic acid, caffeic acid, gallocatechin gallate and protocatechuic acid (Lin *et al*., 2012; Yang *et al*., 2009). Despite the professed health benefits of bissap extract, gross concerns of safety exist as a result of improper hygienic practices, unsanitary conditions and post-processing handling and challenges. bissap

2.9 Ice kenkey, a maize-based beverage and its food safety concerns

Maize is a major staple in Ghana, and as such has been used in the preparation of foods and beverages including kenkey (Fante, Ga and *insiho*), *banku, abolo, korkli, koko, asana or liha*

etc. Ice kenkey is an indigenous beverage, made from kenkey, a stiff dumpling produced from fermented maize meal (Atter *et al.,* 2015).

The kenkey is mashed to yield a homogenous mixture and chilled, then sweetened with sugar or garnished with milk varieties or groundnut based on consumer preference. It is a satisfying delicacy for most commuters, city dwellers, the working class, and serves as a cost-cutter for low-income workers.

In the wake of food safety and quality concerns about street vended foods, ice kenkey has been implicated as a potential source for serious food poisoning episodes or outbreaks although it often has low pH, averaging about 3.7 (Atter *et al*., 2015). This implication emanates from factors such as poor packaging, poor quality of water used, poor manufacturing and hygienic practices, unsanitary production areas, utensils, milling machines. The breaking of kenkey balls with hand also has the tendency to initiate contamination and subsequent poisoning.

Escherichia coli, *Staphylococcus aureus* and coliforms, although not always present at the initial stages were found during production stages (reconstitution, packaging) or in the final product (ready to drink beverage). In ice kenkey sampled from major markets (Madina, Ashaiman, Mallam-Atta and Agbogbloshie) in Accra, Ghana, by Atter *et al*. (2015), total coliforms 10-1000 CFU/g, *E. coli* 0-90 CFU/g and *S. aureus* 30 – 140 CFU/g were reported. The authors reported that, although *S. aureus* CFU/g values were about the national standard (100 CFU/g), concentrations of *E. coli* deviated from the national standard. (Atter *et al*., 2015). Recommendations for curbing the high values of microbial counts generally revolved around pasteurization, use of safe or potable water, good handling and hygienic practices such as hand washing, cleaning of utensils or equipment's, the use of disposable gloves during packaging. None, however, recommended the use of essential oils of *X. aethiopica* as a potential remedy.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design:

To achieve the objectives of the study, qualitative and quantitative methods were adopted. Firstly, questionnaires were designed and administered to spice retailers, herbalist and food vendors in order to select spices having food and medicinal applications. Secondly, the selected spice was purchased from the Nima market, Accra and then supplied to Quin Organics for extraction of essential oils. Thirdly, the antimicrobial properties of the EO was tested on *E. coli, S. aureus* and *Salmonella* Typhimurium. Also, sensory test (triangle test) was done to determine consumer perceived differences between EO spiked and unspiked ice kenkey and bissap. Lastly, the antimicrobial efficacy of the EO was tested against three pathogens in the ice kenkey and bissap beverages.

3.2 Survey on spices sales and utilization

3.2.1 Selection of participants and choice of the study area

Thirty (30) structured questionnaires were administered amongst spice retailers, food vendors and traditional herbalist who consented to the study. The respondents had more than 3 years' experience in the use of spices for food or medicinal purposes. Questionnaires were administered at four major markets (Ashaiman, Nima, Makola, and Amasaman markets) in the Greater Accra Region. The choice of the area of study was based on prior knowledge obtained from van Andel *et al*. (2012), convenience and knowledge acquired from informal queries about places of bulk sale of spices in the capital.

3.3 Sampling methods and techniques

Respondents were identified using convenient sampling and snowball sampling. The concept of snowball sampling is based on referrals from the first identified respondent to other potential respondents who share a common interest or have attributes or knowledge about the subject of interest. The inclusion criteria were mainly the occupation of the prospective respondent; spice retailer, food vendor and traditional herbalist.

3.4 Identification and choice of spices:

Prior to the market survey, an exhaustive list of commonly sold and/or indigenous herbs and spices was collated from various literature. Subsequently, a photo album detailing locally available spices was also created in addition to some 'herbarium' samples of the spices.

Spices were identified using their indigenous Ghanaian name, common and scientific or botanical names through the help of technicians at the University of Ghana Herbarium. Respondents were made to identify spices either in the photo album and/or through the physical examination of the 'herbarium' samples. Spices perceived by respondents as having medicinal properties, used in food, indigenously Ghanaian, available on the market were considered to have met the criteria and therefore were shortlisted. Shortlisted spices were then compared to relevant literature (in scholarly databases; AGORA, TEEAL, ScienceDirect) on their demonstrated ability to yield essential oils, usage in food, medicinal property and nativity to Africa but more especially Ghana.

X. aethiopica was selected based on previously proven antimicrobial efficacy as reported by Bakkali *et al*. (2008); Kiin-Kabari *et al.,* (2011); Tatsadjieu *et al*. (2003) and Wouatsa *et al.,* (2014).

3.5 Extraction of essential oils

Dried fruit pods of *X. aethiopica* spices, were procured from El-Shamma Ventures (a major dealer in natural spices) at the Nima market, Accra for the study. The spice trader aggregates spices from multiple locations in Ghana and retails them at the traditional Nima market in Accra. *X. aethiopica* purchased from El-Shamma Ventures was outsourced to Quin organics, (Cape Coast, Ghana) a local manufacturer and exporter of spice and citrus essential oils for extraction of essential oils. The EO was extracted by the conventional steam distillation method as described by Boutekedjiret *et al.* (2003).

3.6 Characterization of the essential oil extracts from spices

The EO of *X. aethiopica* was analysed for its chemical constituents at the Pesticide Residue Laboratory of the Ghana Standards Authority. The essential oil was analysed using a Varian GC/MS - 3800 GC/2200 Saturn MS. The column oven temperature was set at 40 ºC and was held for 2 min and then increased to 250 ºC at a rate of 10 ºC min^{-1} and held for 7 min. The injection temperature was kept 250 ºC. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. Mass Spectrometry was held under the following conditions; Trap temperature; 200 ºC, Manifold Temperature; 80 ºC, Transferline temperature; 250 ºC. Overall mass ranges were between (30 - 650) m/z. The volume of each essential oil injected into the gas chromatograph was 2 µl.

Chemical constituents of the essential oil were compared with Wiley 275 GC-MS library, Mass Spectrometry data bank (NIST) at Ghana Standards Authority.

3.7 Bacterial cultures used for the efficacy study

Bacterial cultures of *E. coli* (gram-negative), *Salmonella* Typhimurium (gram-negative), and *Staphylococcus aureus (*gram-positive) were obtained from the Centre for Scientific and Industrial Research (CSIR) – Food Research Institute (FRI) for the study.

Stock cultures were inoculated in Brain Heart Infusion broth supplemented with glycerol and stored in an (Arctiko Freezer) at -80 ºC. Test cultures; isolated from the stock cultures were temporally stored in 16mm deep Nutrient Agar (NA CM0003; Oxoid Basingstoke, England, UK) slants under cold storage of 4 ± 2 ºC. Standard microbiological procedures were used during the preparation of agar slants.

3.8 Dilution of essential oils into various concentrations for testing of minimum inhibitory concentration (MIC)

Essential oils were diluted in dimethyl sulfoxide (DMSO) (VWR Chemicals, France) of analytical grade. Working concentrations of 10, 25, 40, 55, 70 and 85 % (v/v) were used in this study. These concentrations were calculated as follows:

From the basic conversion of *1 ml = 1000 µl or 1 µl = 0.001 ml*, the various concentrations were calculated as presented in (Table 1). Desired test volumes were obtained either through scaling up or down.

Table 1: *Xylopia aethiopica* essential oil concentrations used for the study

Essential oil volume (µl)	DMSO volume (µl)	Final volume (1000ul = 1ml)	Final Working Concentration in %
100	900	1000	10
250	750	1000	25
400	600	1000	40
550	450	1000	55
700	300	1000	70
850	150	1000	85

3.9 Determination of minimum inhibitory concentration (MIC) of the essential oils against *E. coli, S. aureus* and *S.* Typhimurium

To determine the antimicrobial property of the *X. aethiopica* essential oils and their tendency to inhibit or prevent the microbial growth of *Escherichia coli, Staphylococcus aureus* and *Salmonella* Typhimurium, the MIC of the essential oil was examined at different concentration levels. The MIC of the essential oil was determined using a modified (Kirby-Bauer) agar disc diffusion method as described by Balouiri, Sadiki and Ibnsouda (2016) but also in consultation with American Society for Microbiology (2005); Franklin and Cockerill III (2011) test protocols. The area of clearing (zone of inhibition) around each disc, imparted by the essential oil on the microorganisms was measured and recorded in (mm) for their respective level of concentration.

3.10 Antimicrobial sensitivity testing

A sterile swab was dipped into each broth culture of *Escherichia coli, Staphylococcus aureus* and *Salmonella* Typhimurium and then gently pressed against the walls to remove excess

liquid. Bacterial cultures were streaked uniformly to yield growth patterns that represent bacterial lawns. Plates were left to dry for approximately 5 minutes, then with the aid of an Antibiotic Disc Dispenser, 200 µg Gentamycin (Oxoid), was added to plates to serve as positive controls. Essential oils of *X. aethiopica* was added to empty sterile 6mm disc papers at different concentrations and then allowed to diffuse into the agar medium for a period of 2 hours at 4 °C. The assumption that diffusion occurs in a uniform and multidirectional fashion largely undergirds the principles of the disc diffusion method. Flame-sterilized forceps were then used to press each antibiotic disc onto the agar, to ensure firm attachment to agar. Plates were then incubated for a period of 18 – 24 hours at an incubation temperature of 37 °C. The antimicrobial sensitivity testing procedure was adapted from (American Society for Microbiology, 2005; Balouiri *et al*., 2016; Cockerill, 2011).

3.11 Mutual Antagonism of *Escherichia coli, Staphylococcus aureus* and *Salmonella* Typhimurium

A mutual antagonism test for a cocktail of *E. coli, S. aureus* and *Salmonella* Typhimurium was conducted to evaluate, the presence (if any) of inherent antagonism that exists between organisms. All organisms were cultured on Nutrient Agar (Oxoid), a suitable agar capable of sustaining the growth of all organisms. Plates were incubated at 37 °C for 24 hour and results of possible antagonism were assessed through microscopy of Gram stained slides. The quest was to limit the possibility of obtaining erroneous results when the cocktail is used in the challenge test.

3.12 Microbiological Challenge testing; Organism – essential oils challenge test on ice kenkey and bissap extracts

The microbiological challenge test was performed as described in ("Microbiological Challenge Testing," 2003). Microorganisms in their natural habitat do not often grow in isolation, hence, a cocktail of *Escherichia coli, Salmonella* Typhimurium and *Staphylococcus aureus* was prepared using 1 ml each of live cultures (Bacterial cultures were set to 0.5 McFarland standard prior to the formulation of the cocktail). The microbial cocktail was then homogenised using a vortex (Stuart vortex mixer, ISA7). The choice of organisms was influenced by their association with contamination of high-risk foods and beverages such as ice kenkey and *Hibiscus sabdariffa* 'bissap' extract. Two microliters (2 µl) of *X. aethiopica* essential oil was added to One (1) ml of each food sample (bissap extract and ice kenkey). The challenge test was conducted at 5 data point collection periods, with each data collection point having a 48-hrs interval thus 0hr to 192hrs. At each samples collection point, both control (food substrate) and test samples (food substrate, essential oil and cocktail of microorganism) were plated (in duplicates) and counts duly enumerated using Stuart Colony Counter, $SC6^{+}$ (Staffordshire UK). Although organisms were inoculated as a cocktail, they were cultured on specific selective media, Baird-Parker Agar (*Staphylococcus aureus),* MacConkey Agar (*Escherichia coli)* and Xylose Lysine Deoxycholate (XLD) (*Salmonella* Typhimurium*)* using strict microbiological protocols and agar preparations according to manufacturer's instructions (Franklin & Cockerill III, 2011; "Microbiological Challenge Testing," 2003). To ensure that only the cocktail of *Staphylococcus aureus, Escherichia coli* and *Salmonella* Typhimurium, the food substrates (ice kenkey and bissap extract) were pasteurised before the cocktail of organisms were inoculated and the essential oils added. The challenge samples were kept under both abuse refrigerated conditions (14 ± 2°C) and ambient room temperature conditions (35 ± 2°C).

3.12.1 Determination of pH during the challenge test

The pH of control samples (food substrate) and test samples (a mixture of food substrate, essential oils and cocktail of organisms) were measured using a pH meter (Mettler-Toledo GmbH, Switzerland). The aim was to assess possible essential oil influence on pH of food samples.

3.12.2 Colour determination of test and control samples during the challenge test

Colour of both control (food substrate) and test samples (a mixture of food substrate, essential oils and cocktail of organisms) was measured using colourimeter (Chroma meter, Konica Minolta, Japan). The purpose was to determine if essential oils could impart or induce or influence colour changes in the food products during the challenge test.

3.13 Sensory Analysis

3.13.1 Triangle test

To achieve the test objective, a triangle test was adopted for the study, from (Meilgaard, Carr, & Civille, 2011). The extent for like or dislike for perceptibility or non-perceptibility of the essential oil was not researched in the present study.

Twenty-eight screened panellists with a speciality in detecting overall differences in taste between samples were randomly recruited from a pool of screened panellists already working with the Sensory Laboratory, Department of Nutrition and Food Science at the University of Ghana.

The triangle test was carried out on two samples: Bissap beverage and Ice kenkey. In the bissap test, bissap extract with *X. aethiopica* EO (2µl/ml) and bissap extract without EO was used. Sample volumes of 20ml each were presented to each panellist in a disposal plastic cup at (14

± 2) °C. Samples were labelled with random three-digit codes and presented to each panellist. In each presentation, two of the samples were identical and one was different (odd). Each panellist was asked to examine bissap extract samples by tasting samples from left to right and identify the odd sample.

Panellists were asked to comment on what they thought was different about the sample they chose as the odd sample.

In the ice kenkey test, ice kenkey with *X. aethiopica* EO (2µl/ml) and ice kenkey without EO was used. Sample volumes of 20ml each were presented to each panellist in a disposal plastic cup at (14 ± 2) °C. Samples were labelled with random three-digit codes and presented to each panellist. In each presentation, two of the samples were identical and one was different. Each panellist was asked to examine ice kenkey samples by tasting samples from left to right and identify the odd sample. Panellists were asked to comment on what they thought was different about the sample they chose as the odd sample.

A complete block balanced randomised presentation order design found in Compusense cloud software (Compusense, Guelph, Ontario, Canada) was used for all assessments. The three samples were presented at the same time to panellists for evaluation. The correct selected odd sample is counted and analysed based on the proportion of distinguishers model. All assessments and analysis were completed using Compusensecloud software (Compusense, Guelph, Ontario, Canada).

3.14 Microbiological analyses

To maintain a sterile working environment, 70% of Ethanol (Product; 20821.321, France) was used to disinfect workbenches. Glassware and microbiological media were autoclaved to ensure utmost sterility. Aseptic techniques were used throughout the microbiological study to prevent the possibility of contamination.

Muller-Hinton agar (MHA; Oxoid, Basingstoke, England) was used for the antimicrobial sensitivity test for *Staphylococcus aureus, Escherichia coli and Salmonella* Typhimurium. The cocktail of organisms was plated using the spread plate method. Organisms were incubated at 37°C for 24 hrs prior to enumeration. Muller-Hinton Broth (MHB CM0001, Oxoid, Basingstoke, England) was used for sustaining the growth of *Staphylococcus aureus, Escherichia coli and Salmonella* Typhimurium 37 °C for 2 hours prior to plating on Muller Hinton agar.

Nutrient Agar (NA CM0003; Oxoid Basingstoke, England, UK) was used for culturing the growth of *Staphylococcus aureus, Escherichia coli* and *Salmonella* Typhimurium by spread plating. Inoculated plates were incubated at 37 °C for 24 hour.

Brain Heart Infusion (BHI: CM1135; Oxoid, Basingstoke, England, UK) supplemented with glycerol was used for long term storage of stock cultures of *Staphylococcus aureus, Escherichia coli* and *Salmonella* Typhimurium at -80 °C (Arctiko Freezer) throughout the study. The broth was prepared strictly according to the manufacturer's instructions.

MacConkey Agar (MAC CM0007: Oxoid Basingstoke, England, UK) was used for the isolation and enumeration of *Escherichia coli* at 37 °C for 24 hours. Red, non-mucoid colonies were enumerated.

Baird-Parker (BPA: CM0275; Oxoid, Basingstoke, England, UK) was used for the isolation and enumeration of *Staphylococcus aureus.* Inverted dishes were incubated at 35 °C for 24 hours. Grey-black shiny typical colonies of *Staphylococcus aureus* were examined and enumerated after the 24 hours. Negative cultures were re-incubated for a further 24 hours.

Xylose Lysine Deoxycholate (X.L.D.) CM0469, Basingstoke, England, UK) was used for the isolation and enumeration of *Salmonella* Typhimurium strains at 35 ± 2°C for 18-24 hours.

Violet Red Bile Glucose (VRBG; CM1082, Oxoid, Basingstoke, England) was used to detect the presence of coliform bacteria in ice kenkey and bissap samples prior to the sensory evaluation of the products. Samples were incubated at 37 °C for 24 ± 2 hours. Enumeration of the total number of characteristic (purple/pink) colonies were subsequently made.

Plate Count Agar (PCA CM0325; Oxoid, Basingstoke, England) was used to enumerate total viable counts of *E. coil, S. aureus, Salmonella* Typhimurium in ice kenkey and bissap samples prior to the sensory evaluation of the products. Plates were incubated at 35 °C for 48 hours.

3.17 Statistical analysis

SPSS software (SPSS v22.0, SPSS Inc., Chicago, Illinois) and conventional statistical methods were used for calculating the mean and standard deviation of zones of inhibition obtained from the minimum inhibitory concentration.

One way-ANOVA was applied to data obtained from the zone of inhibition in order to determine differences between the various concentrations; EOs, 200 µg Gentamycin and DMSO ($P< 0.05$), using SPSS v. 22.

The unpaired t-test was applied to mean pH measurements of EO spiked and un-spiked bissap extract and ice kenkey to determine if EO influenced pH ($P< 0.05$), using Minitab 14

Triangle test results were analysed using the proportion of distinguishers' model in Compusense Cloud (SAAS) software.

Data on colour, pH and microbial counts were analysed, graphed and charted using SPSS software (SPSS v22.0, SPSS Inc., Chicago, Illinois) and Microsoft Excel 2013 (Microsoft, Seattle, WA, USA).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Spice market survey

From table 2, the occupations of the respondents were spice retailers (70.0%), herbalist (3.3%) and food vendors (26.7%). Also, the majority (80%) of the respondents had a form of formal education (basic, second cycle and tertiary), whilst only 20% received no formal education. Considering the 21 spice retailers, 28.6% had no form of formal education whilst 71.4% had basic, secondary/technical/vocation (second cycle), and tertiary education.

Table 2: Crosstabs between occupation and educational level of respondents

		Educational Levels				Total
		None	Basic	Second Cycle	Tertiary	
Occupation	Spice retailer	6	9	5	1	21
	Herbalist	0	1	0	0	1
	Food Vendor	0	3	4	1	8
Total		6	13	9	2	30

Data from table 3 suggests that majority, 40% of the total respondents (30) had been practicing their occupation above 8 years, followed by 0-2 years (16.67%), 6-8 years (16.67%) and 2-4 years (13.33%), 4-6 years (13.33%). The experiences gathered within the years of plying trade culminated in their abilities to readily describe spices and expound on the various use.

Table 3: Crosstabs between occupation and number of years of trade

		No of years of trade					Total
		0-2 years	2-4 years	4-6 years	6-8 years	Above 8 years	
Occupation	Spice retailer	4	1	2	4	10	21
	Herbalist	0	1	0	0	0	1
	Food Vendor	1	2	2	1	2	8
Total		5	4	4	5	12	30

In the quest to obtain data on spices used in the food and for herbal preparation, which hitherto was currently extinct (table 4), 73.3% of the respondents indicated that they had no knowledge on such spices. On the other hand, 26.7% of respondents posited that they had knowledge of some extinct spices but could not recall their names. It is worth noting that 75% of those who had knowledge of the extinct spices but could not recall their names were spice retailers.

Table 4: Crosstabs between occupation and knowledge about extinct spices

		Extinct Spices		Total
		Yes	No	
Occupation	Spice retailer	6	15	21
	Herbalist	1	0	1
	Food Vendor	1	7	8
Total		8	22	30

From table 5, data shows the respondents were evenly distributed between the two major religions in Ghana, Christianity 50%), and Islam (50%). However, Muslims were marginally more involved in the retail of spices.

Table 5: Crosstabs between occupation and religion practised by respondents.

		Religion		Total
		Christian	Muslim	
Occupation	Spice retailer	10	11	21
	Herbalist	1	0	1
	Food Vendor	4	4	8
Total		15	15	30

Spice cultivation in Ghana is largely limited to small settler communities along the forest belt, cocoa growing areas and some mountainous communities. The scale of production of these spices are undoubtedly small to medium, often labour intensive and largely for subsistence

purposes and low-scale commercial purposes. The mix cropping system of farming practised in the country enables farmers to grow spices together with other crops. The country, although known to be an importer of some varieties of spices, can boast of a wealth of other indigenously cultivated spices some of which are listed in (Table 6) below.

Table 6: List of common indigenous Ghanaian spices

Name of spice	Botanical name	Common local name
Aniseed	*Pimpenella anisum*	Nketekete, Ahaliwe
Black pepper	*Piper guineesis*	Soro wisa
Calabash nutmeg	*Monodora myristica*	Awedeaba
Galbanum	*Tertrapleura tetraptera*	Prekese
Ginger	*Zingiber officenalis*	Akakaduro
Negro pepper	*Xylopia aethiopia*	Hwintia, Etso
White pepper	*Piper nigrum*	Famu wisa
Clove	*Eugenia caryophylata*	Pepre
Chili pepper	*Capsicum frutescens*	Mako
Nutmeg	*Myristica fragans*	
	Afromomum hanburyi	Adowa wisa
	Afromomum geocarpum	Nsensam
	Costus afer	Semanini

Although there are no formalised map-outs of areas where spices are cultivated in bulk, the market survey, according to spice retailers, intermediaries and exporters revealed that some notable or major locations include Nkwanta, Kpeve, Kpeze, Kpoeta, Kadjebi all in the Volta region. In addition, Tarkwa, Bogoso and Prestea in the Central Region, villages surrounding Obuasi in the Ashanti Region and Bawku in the Upper East region were also cited as places for growing spices. The communities in the Volta Region were surmised to be major suppliers of pepper, ginger, Negro pepper and clove. The forest areas of the central region were also

cited as a place of bulk galbanum, Calabash nutmeg, Negro pepper, clove, and pepper cultivation.

According to 96.7% (food vendors and spice retailers) of the respondents, Galbanum, *Tetrapleura tetraptera* (prekese), is a highly traded spice in Ghana, because of its medicinal properties and extensive piquant properties it imparts when used in the preparation of indigenous dishes such as palm nut soup and peanut soup. *T. tepraptera* is however not cultivated, or deliberately planted or farmed but rather obtained from the wilds' and in isolated places around Bawgyiase and Prestea and many other farmer settler communities., Also, according to 96.7% of respondents (spice retailers and food vendors), ginger is also a well-traded spice, known for the unique taste and aroma it imparts to meat and poultry during steaming or roasting. Bulk spice retailers recounted that the vast majority of their suppliers were from Kadjebi in the Volta Region and Bawku in the Upper East Region of Ghana.

4.2 Market survey results on *X. aethiopica*

The market survey conducted during the study revealed that all (100%) of respondents were able to identify and correctly name the *X. aethiopica*, by their common name. Probes on the origin of the spice revealed that bulk of the spice was procured from the surrounding villages of Kpeve, Kpedze in the Volta Region. On the concept of post-harvest handling treatments, respondents (100%) revealed that fruits from the plant are often dried in the open air or solar dried before they are bagged for onward haulage. Generally, post-harvest challenges such as bruising, spillage, breakage, insect infestation could lead to loss of volatiles, which has the tendency to influence the method of extraction of oils, the rate of yield, quality and quantity of the essential oils. All respondents (herbalist, food vendors and spice retailers), posited that *X. aethiopica* possesses stomachic properties, and is a useful inclusion in herbal and medicinal concoctions, herbal blends for the convalescing and masking of off-flavours of rancid foods.

Reports from the survey revealed that the spice often forms part of other drugs used in curing cough, ulcers of the stomach, diarrhoea and nausea.

The findings that all morphological part of the *X. aethiopica* plant possess some medicinal property hence its use in curing ailments such as a cough, candidiasis, cough, dysentery and fever also influenced the choice of the spice (Hassan, Almagboul, & Kabbashi, 2016).

4.3 The chemical composition of indigenous Ghanaian *X. aethiopica* essential oil

The EO obtained from the steam distillation was subjected to Gas Chromatograph – Mass Spectroscopy (GC-MS) analysis. The broad-spectrum chromatograph (Appendix 4) was also generated after the analysis. The chromatograph revealed that the scan range of the chromatograph falls within 1 to 3082 counts whiles the time range spans between 0.00 to 29.98 minutes. The report on peak names showed that one hundred and five (105) compounds were present, out of which one hundred and four (104) individual chemical compounds were identified whiles one (1) remained unidentified. The complete list of identified chemical compounds from *X. aethiopica* has been presented in the Appendix. However, presented in (Table 7 below is the list of twenty (20) dominant chemical compounds based on the magnitude of the retention time and the area covered by peaks. The various chemical constituents have been grouped into their functional groups (Table 8).

The composition of these oils revealed that these dominant compounds may not singly induce antimicrobial property; however, the synergistic effects of all components yields optimal antimicrobial effects.

In the present study, two isomers of (E,Z,Z)-2,4,7-Tridecatrienal, were identified at the retention index of 7.463 and 7.515, covering a percentage area of 4.31 and 5.54 respectively. This active component has also been identified and attributed to antifungal properties in clove.

Although the compound was not tested in isolation, it was among the dominant compounds that proved inhibitory against *Aspergillus flavus*, *Penicillium citrinum* and *Rhizopus nigicans* in vitro and in wounded fruits (Xing *et al*., 2012).

Studies conducted by Asekun and Adeniyi (2004), tested the oils of *X. aethiopica*, indigenous to Nigeria on selected species of gram-negative, gram-positive bacteria and some fungi and further assessed the cytotoxic activities of the oils. The study revealed that, the volatile composition of the oil, comprised mainly of (15.15%) of monoterpenoids and 1,8-cineole while terpinen-4-ol was 6.6% but were not attributed to any previously observed antimicrobial and cytotoxic effect.

In a similar study on the composition of essential oil extracts of *Xylopia aethiopica* from Cameroon, 68 constituents were identified and were mainly oxygenated monoterpenes (48.83%) trailed by monoterpene hydrocarbons (25.08%). Some sesquiterpenes were also identified but constituted only about 10% of the composition (Wouatsa *et al*., 2014). In effect, the total number of chemical constituents reported in the present study exceeds those reported by Wouatsa *et al*. 2014.

Reports on another chemical composition of "Ethiopian pepper" in Cameroon suggests that more than 100 volatile compounds have been identified amongst which β-pinene (18%), terpinen-4-ol (8.9%), sabinene (7.2%), alpha-terpineol (4.1%), 1,8-cineole (2.5%) and kaurane derivatives (4.2%) are considered predominant (Hassan *et al*., 2016; Jirovetz, Buchbauer, & Ngassoum, 1997). Although 1,8-cineole and some kaurene derivatives were reported in the present study, their quantities have not been reported. In another study conducted in the Republic of Benin, Ayedoun *et al*. (1996) reported on (50 – 60) chemical components of *X. aethiopica* fruits which were predominantly mono- and sesquiterpenoid such as β-pinene, myrcene, p-cymene, limonene, linalool and 1,8-cineole. On the account of sesquiterpenes, the

authors reported on the presence of guaiol and elemol. The terpene contents included p-mentha3, 8-diene and p-menthat-3,8-triene (Ayedoun *et al*., 1996). In contrast, these terpenes were not found in the present study.

Chiefly amongst the contents of native Sudanese *X. aethiopica*, were the monoterpene fractions of the oil such as alphainene (11.36%), alpha-phellandrene (10.50%) (El-Kamali & Adam, 2009). The oxygenated monoterpenes composition of the same essential oil were 4-isopropylbenzyl alcohol (16.67%), $C_{10}H_{16}O$ (8.12%) and 1,8-cineole (5.28%). Sesquiterpene hydrocarbons such as gamma-cadinene (11.11%) and copaene (0.95%) were also identified in the native Sudanese *X. aethiopica* fruit essential oils. In total, El-Kamali and Adam (2009) reported on forty-five (45) chemical compounds in Sudanese *X. aethiopica*.

In a similar study conducted in Sudan, Hassan *et al*. (2016) reported that seventy-two (72) compounds were identified whiles ten 10 were unidentified. Major constituents included β-pinene (15.06%), 3Cylohexen-1-ol,4methyl-1-1 (13.22%), β-phellandrene (8.24%), α-pinene (7.85%).

The components of essential oils of *X. aethiopica* from neighbouring Togo, according to Koba *et al*. (2008) suggests that α-pinene (23.6%), β-pinene (11%), sabinene (9.8%) and 1,8- cineole (8.2%) were the predominant constituents of the oil. In a study conducted by Keita *et al*. (2003), thirty (30) compounds were detected from *X. aethiopica* oils in Mali and the major constituents reported were β-pinene (19.1%), γ-terpinene (14.7%), pinocaveol (8.6%) and p-cymene (7.3%) as major constituents of the oil.

Studies conducted in Ivory Coast by Konan *et al*. (2009) reported β-pinene concentration in the range of (16.606 – 20.56%). Germacrene D (25.1%), β-pinene (20.6%), α-pinene (8%), 1,8, cineole (7.4%) and a trace amount (0.1%) of kaur-16-ene, a diterpene were amongst the thirty-

nine (39) compounds reported in indigenous Ghanaian *Xylopia aethiopica* oil (Karioti, Hadjipavlou-Litina, Mensah, Fleischer, & Skaltsa, 2004).

In the present study, kaur-16-ene was also identified at a retention time of 20.881 and covering an area percentage of 0.01%. In a similar faction, Olonisakin, Oladimeji and Lajide (2007) reported on twenty-three (23) chemical compounds in *X. aethiopica* of Nigerian origin, and further cited β-pinene (13.78%), β-phellandrene (12.36%), γ-terpinene (7.66%), eucalyptol (6.9%) and α-pinene (5.56%) as the predominant compounds in the oil.

The difference in the quantities of chemical constituents could be accounted for by; difference in geographical location, the age of the plant, the method of extraction, genetic factors, climatic conditions, and soil and cultivation techniques, seasonal and maturity variation, growth stages, part of plant and post-harvest treatment; fresh, dried, boiled and dried or smoked and dried fruits (Elhassan & Ayoub, 2014; Keita *et al*., 2003). The difference in chemical composition could ignite a heuristic approach to exploring the cause of varietal difference and possibilities of difference in antimicrobial efficacy.

Reporting on the association between antimicrobial efficacy and the chemical composition of essential oils, Devi *et al*. (2010); Nissen *et al*. (2010) theorised that the antimicrobial activity could result when the composition of oxygenated monoterpenes which are noted for their antimicrobial prowess is around ≥ 50%.

Habiba *et al*. (2010), posited that a combination of α-pinine, β-pinine ≥ 3carene and terpinene-4-ol could induce insecticidal properties leading to about 96% of mortality of maize weevil, *Sithophylus zeamais Motsch*.

Table 7: List of twenty (20) dominant chemical compounds from GC-MS profile of *X. aethiopica* essential oil

No.	# on output in Appendix	RT	Peak Name	Res Type	Quan Ions	Area	Area %	Amount/RF
1	**8**	6.677	Borazine, 2,4,6-triethyl	Id.	81	6.797E+06	10.03	6797000 Counts
2	**11**	7.463	(E,Z,Z)-2,4,7-Tridecatrienal	Id.	81	2.921E+06	4.31	2921000 Counts
3	**12**	7.515	(E,Z,Z)-2,4,7-Tridecatrienal	Id.	80.7	3.753E+06	5.54	3753000 Counts
4	**13**	7.639	(4-Dimethylaminomethyl-1H-pyrrol-3-ylmet	Id.	136.1	3.418E+06	5.04	3418000 Counts
5	**14**	7.684	3-Cyclohexene-1-methanol,.alph., alpha	Id.	137.1	905211	1.34	905211 Counts
6	**17**	7.989	Decahydronaphtho[2,3-b]furan-2-one, 3-[	Id.	136	1.407E+06	2.08	1407000 Counts
7	**18**	8.306	4-Hydroxy-3-methoxybenzyl alcohol	Id.	137.3	7.571E+06	11.17	7571000 Counts
8	**20**	8.681	3-Benzylsulfonyl-2,6,6-trimethylbicyclo	Id.	136	1.437E+06	2.12	1437000 Counts
9	**25**	9.337	Cyclohexane, 1-methyl-3-(1-methylethenyl	Id.	81.2	969013	1.43	969013 Counts
10	**26**	9.337	Fenchyl acetate	Id.	81.2	969013	1.43	969013 Counts
11	**31**	10.049	Bicyclo[4.4.0]dec-5-ene-1-acetic acid	Id.	135	1.931E+06	2.85	1931000 Counts
12	**32**	10.123	8a-Methyl-5-methylene-3--([(pyridin-2-ylm	Id.	93.2	1.923E+06	2.84	1923000 Counts
13	**33**	10.358	Benzene, 1-[(2-bromophenoxy)methyl]-3,4	Id.	150.9	2.582E+06	3.81	2582000 Counts
14	**35**	10.626	1-(2,4-Dihydroxybenzoyl)-3-ethyl-5-trifl	Id.	136.9	4.417E+06	6.52	4417000 Counts
15	**37**	10.907	2,4,6-Tris(1-(2-methoxycarbonylpyrrolidi	Id.	135.1	2.413E+06	3.56	2413000 Counts
16	**49**	12.825	Cyclohexane, 1-ethenyl-1-methyl-2-(1-met	Id.	121.2	960745	1.42	960745 Counts
17	**54**	13.73	Ethanone, 1-[5-[(5-methy-2-furanyl)meth	Id.	204	2.543E+06	3.75	2543000 Counts
18	**61**	14.802	.gamma.-Himachalene	Id.	161.3	3.844E+06	5.67	3844000 Counts
19	**62**	14.802	1,4-Methanoazulene, decahydro-4,8,8-trim	Id.	161.1	3.378E+06	4.98	3378000 Counts
20	**74**	16.526	1H,4H-Pyrazolo[3,4-b]pyran-5-carbonitril	Id.	203.1	933010	1.38	933010 Counts

Table 8: Grouped components of the essential oil of *Xylopia aethiopica* from Ghana

Alkanes/ aromatic hydrocarbons	Alkenes/Olefins	Carbonyls	Alcohols/ Enols	Esters/terpenes	Carboxylic acids	Ethers	Others
Cyclohexane, (1-methylethylidene)	3,5,5-Tetramethylcyclopentene	Cyclopentanone, 2,2-dimethyl	Methanol, (1,4-dihydrophenyl)	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl	Cyclopentanecarboxylic acid, 2,4-dimethy	Decahydronaphtho[2,3-b]furan-2-one, 3-[	Pentalene, octahydro-2-methyl
7-Propylidene-bicyclo[4.1.0]heptane	Pentalene, octahydro-2-methyl	(E,Z,Z)-2,4,7-Tridecatrienal	3-Cyclohexene-1-methanol,.alph., alpha	(E,Z,Z)-2,4,7-Tridecatrienal	Bicyclo[4.4.0]dec-5-ene-1-acetic acid		(4-Dimethylaminomethyl-1H-pyrrol-3-ylmet
Cyclohexane, 1-methyl-3-(1-methylethenyl	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1	6-Isopropenyl-3-methoxymethoxy-3-methyl	Decahydronaphtho[2,3-b]furan-2-one, 3-[	2-Cyclohexen-1-ol, 1-methyl-4-(1-methyle	1,2-Benzenedicarboxylic acid, butyl octy		1-Oxaspiro[4.5]deca-3,6-diene2,6,10,1
Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-me	1,4-Cyclohexadiene, 1-methyl-4-(1-methyl	Benzaldehyde, 4-(1-methylethyl)	4-Hydroxy-3-methoxybenzyl alcohol	Fenchyl acetate	5,8,11,14,17 -Eicosapentaenoic acid, meth		Cyclohexanone, 2-(1-methylethylidene
2-(3,4-Dibromo-4-methylcyclohexyl) propane		Ethanone, 1-[5-[(5-methy-2-furanyl)meth	2-Propanol, 1-[(1-ethynycyclohexyl) oxy]	(2R,4R)-p-Mentha-6,8-diene, 2-hydroperox	Docosahexaenoic acid, 1,2,3-propanetriyl		Cyclohexanone, 2-mythyl-5-(1-methylethen

Cyclohexane, 1-ethenyl-1-methyl-2-(1-met	Longipinocarvone	3,7-Dimethyl-2,6-nonadien-1-ol	O-Trifluoroacetyl-isopulegol	5-Benzofuranacetic acid, 6-ethenyl-2,4,5	Benzene, 1-[(2-bromophenoxy)methyl]-3,4
Napthalene, 1,2,3,4,4a,5,6,8a-octahydro	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-me	Benzenemethanol, 4-(1-methylethyl)	cis-p-Mentha-2,8-dien-1-ol	1,2-Benzenedicarboxylic acid, diisooctyl	Benzenaminium, 3-hydroxy-N,N,N-trimethyl
1,4-Methanoazulene, decahydro-4,8,8-trim	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl	1-Cyclohexene-1-methanol, 4-(1-methleth	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2		2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-oct
Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-me		Cyclohexanemethanol, 4-ethenyl-,alpha	Cyclohexanone, 2-(2-butynyl)		1,4-Cyclohexadiene, 1-methanol, 4-(1-meth
2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahyd		Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-me	alpha.-Cubebene		1H-Cycloprop[e]azulene, decahydro-1,1,7
2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a		Ledene alcohol	Copaene		Diepicedrene-1-oxide

1,3,6,10-Cyclotetradecatetraene, 3,7,11

1H-Naphtho[2,1-b]pyran, 3-ethenyldodecah

2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a

2-Naphthalenemethanol, 1,2,3,4,4a,8a-hex

5,6-Azulenedimethanol, 1,2,3,3a8,8a-hex

gamma.-Himachalene

Methyl (Z)-5,11,14,17-eicosate

Kaur-16-ene

2-[4-methyl-6-(2,6,6-trimethylcyclohex-1

Isoaromadendrene epoxide

3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bic

Isoaromadendrene epoxide

1H,4H-Pyrazolo[3,4-b]pyran-5-carbonitril

Columbin

Longipinocarveol, trans-

Longipinocarvone

9-Isopropyl-1-methyl-2-methylene-5-oxatr

4.4 Assessment of the inhibitory effect of *X. aethiopica* essential oil on bacterial pathogens

Essential oils of different concentration (10%, 25%, 40%, 55%, 70% and 85%) were tested for their inhibitory effect on a cocktail of *Salmonella* Typhimurium, *Escherichia coli*, and *Staphylococcus aureus* using the Mueller-Hinton agar base and the modified Kirby-Bauer disc diffusion method. In order to obtain benchmarks for measuring the performance of the indigenous Ghanaian *X. aethiopica* oils, 200 µg Gentamycin and dimethyl sulfoxide (DMSO) (100%) were used as positive and negative controls respectively. DMSO was used as the surfactant in achieving the various concentration of the essential oils.

The results of the study showed that the essential oils were inhibitory to all test organisms (*Salmonella* Typhimurium, *Escherichia coli*, and *Staphylococcus aureus*) and imparted zones of inhibitions (mm) but at different concentrations of the essential oils. The inhibitory nature of the essential was more pronounced in *Salmonella* Typhimurium, 17.00±1.00 mm at 70% EO concentration, followed by *S. aureus* 13.33±0.58 mm at 85% EO concentration and *E. coli* 11.67 ± 0.58mm 85% EO concentration. These findings are contrary to most scientific findings on the antimicrobial susceptibility of the gram positives and gram-negative bacteria. In this study, *Salmonella* Typhimurium was more susceptible to the essential oil than *S. aureus*.

Table 9: (MIC) Zone of inhibition imparted by *X. aethiopica* essential oils on *S. Typhimurium, S. aureus* and *E. coli*

Zones of inhibition (mm)								
Essential oil concentration (v/v) in %								
Bacterial Pathogens	**10**	**25**	**40**	**55**	**70**	**85**	**Gentamycin**	**Negative control-DMSO**
S. Typhimurium	$6 \pm 0.00^{a*}$	$12.33 \pm 0.58^{a*}$	$13.67 \pm 1.15^{a*}$	$14.33 \pm 0.58^{a*}$	$17 \pm 1.00^{a*}$	$14.67 \pm 0.58^{a*}$	$22.33 \pm 1.15^{b*}$	$6 \pm 0.00^{a*}$
S. aureus	$6 \pm 0.00^{a*}$	$6 \pm 0.00^{a*}$	$6 \pm 0.00^{a*}$	$7.67 \pm 0.58^{a*}$	$10.67 \pm 0.58^{a*}$	$13.33 \pm 0.58^{a*}$	$23.67 \pm 0.58^{b*}$	$6 \pm 0.00^{a*}$
E. coli	$6 \pm 0.00^{a*}$	$7.67 \pm 0.58^{a*}$	$8.67 \pm 0.58^{a*}$	$9.67 \pm 0.58^{a*}$	$9.67 \pm 0.58^{a*}$	$11.67 \pm 0.58^{a*}$	$20.33 \pm 0.58^{b*}$	$6 \pm 0.00^{a*}$

Legend

[a] = Rows with the same alphabet signify that there was no statistically significant difference (CI=95%; $p>0.05$) between the various concentrations

b= There was a statistically significant difference between the concentrations (CI = 95%; $p<0.05$)

*= There was no statistically significant difference between how the various organisms were inhibited by the same concentrations (CI=95%; $p>0.05$)

The results on the minimum concentrations at which organisms were inhibited as well as the zones of inhibition imparted on each organism are presented in (Table 9). The results from (Table 9) revealed that EO concentration of 10%, could not induce an inhibitory effect on all organisms (*Salmonella* Typhimurium, *Escherichia coli, and Staphylococcus aureus*). However, at 25% concentration of EO, the zone of inhibition conferred on *S.* Typhimurium and *E. coli* was 12.33 ± 0.58 (mm) and 7.67 ± 0.58 (mm) respectively. *Staphylococcus aureus,* on the other hand, was still insensitive to the essential oil at both 25% and 40% concentration of essential oil. The range of mean zone of inhibition of *S.* Typhimurium was between 12.33 ± 0.58 (mm) at 25% of EO and 17.00 ± 1.00 (mm) at 70%. According to (Table 9) the zone of inhibition imparted on *S.* Typhimurium at 70% concentration of EO was higher than at 85% concentration of EO. This result suggests that there is the possibility of a non-uniform diffusion of the bioactive components of the essential oils throughout that portion of the plate where 85% concentration of EO was applied. Also, an actively growing bacterial load also has the tendency to suppress the thorough efficacy and inhibition of the essential oil prior to the total diffusion of the oil. In summary, the zone of inhibition was highest at 85% concentration of essential oil hence imparted 13.33± 0.58 on *S. aureus* (mm) and *E. coli* 11.67 ± 0.58 (mm) respectively.

From (Table 9), the closest zone of inhibition to 200 µg Gentamycin was 70% of EO on *S.* Typhimurium. Also, 200 µg Gentamycin tend to impart a higher zone of inhibition (23.67 ± 0.58 mm) on *S. aureus* than on *S.* Typhimurium (22.33 ± 1.15) mm and *E. coli* (20.33 ± 0.58) mm.

Although Asekun and Adeniyi (2004) reported that essential oil of *X. aethiopica* was active against *Stellocapella maydis, Aspergillus flavus, A. ocheraceus, Fusarium oxysporum,* the essential oils did not express any antibacterial properties on *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213.

The antimicrobial prowess of most essential oils has been attributed to the numerous and varying chemical compositions. Brenes and Roura (2010); Burt (2004) posited that this phenomenon could be the panacea to bacterial resistance, due to the complex nature of the components juxtaposed with the synthetic drugs. Brenes and Roura (2010) further surmised that in addition to the antimicrobial property EOs impart, they enhance the production of digestive secretions, stimulate blood circulation, exert antioxidant properties, and enhance immune status in poultry birds.

The antibacterial activity of indigenous Ghanaian essential oil of *X. aethiopica* on the *S.* Typhimurium was not very different from findings of Wouatsa *et al*. (2014) who also reported that *S.* Typhimurium MTCC1251 were most susceptible to *X. aethiopica* oils from Cameroon. The harmony in both results could emanate from some identical components within each essential oils. In the present study, as well as Wouatsa *et al*. (2014) S. Typhimurium cells were the most sensitive to *X. aethiopica* essential oils.

The findings of this study contrast, the general concept of Gram-negative bacteria proving to be less sensitive or susceptible to antibacterial agents as compared to Gram-positive bacteria. The thick or extramembranous layer of lipopolysaccharide has often been cited as a potent barrier to these antimicrobials. The mechanism of inhibition for indigenous Ghanaian spices should, therefore, warrants further investigation.

Generally, there was a statistically significant difference between concentrations of EOs inhibited the *S.* Typhimurium, *S. aureus* and *E. coli (CI=95%; p=0.000), Appendix 3c.* Also, there was no statistically significant difference between how the various organisms were inhibited by the same concentrations of the essential oil *(CI=95%; p=0.381), Appendix 3b*.

4.5 Sensory analysis

4.5.1 Overall difference test between EO spiked bissap extract and unspiked bissap extract

The triangle test was conducted to determine if screened panellist would be able to determine an overall difference between unspiked bissap and bissap spiked with 2 µl/ml of *X. aethiopica* EO. The result presented in (Table 10) is based on the proportion of distinguisher's model. The underlying assumption prior to the overall difference test was that the addition of essential oil at levels they retain their antimicrobial potential does not induce a sensory difference because of the differences in nature of food matrices. The outcome of the study reveals the need to establish rejection thresholds for which EO spike bissap extract will be rejected despite the EO conferring antimicrobial properties, thus inhibiting spoilage and other organisms.

Table 10: Response of overall difference test between essential oil spiked bissap and unspiked bissap extract

Difference Test							
Sample 1 - Sample 2	Chance	N (Panellist)	Correct	Incorrect	d'	p-value	Significant at 0.05
Triad 1	1 in 3	28	28	0	Inf	0.00	YES

From Table 10 there was a statistically significant difference between both spiked and unspiked samples ($p<0.00$). The magnitude of difference based on the d' value signified that there was an infinite difference between both samples, implying a very large difference between the samples.

Some comments from panellists on how the samples differed are outlined below:

- *"there is no mint in sample 622"* ***(P 22)***
- *"There is no spice in the sample as compared to the other two samples"* ***(P 11)***

On the other hand, 14 panellists provided descriptors such as minty aroma, spicy taste, earthy flavour, sharp taste, strong bitter flavouring and strong pungent flavour for the *X. aethiopica* spiked bissap extracts. In addition, two (2) respondents perceived the spiked bissap extract as those from the citrus origin, hence described it as lemon flavoured and citrus taste respectively. Four (4) panellist also reported on the oily appearance on the surface of the bissap extract.

Although the use of EO in food as antimicrobials looks promising, there is a need for food product developers to experiment on novel processing conditions of limiting the differences between traditional food samples and food samples containing EOs.

4.5.2 Overall, difference test between EO spiked ice kenkey and unspiked ice kenkey

The triangle test was conducted to determine if screened panellist would be able to determine an overall difference between unspiked ice kenkey and ice kenkey spiked with 2 µl/ml of *X. aethiopica* EO. The result presented in (Table 11) is based on the proportion of distinguisher's model. The underlying assumption prior to the overall difference test was that the addition of essential oil at levels they retain their antimicrobial potential would not induce a sensory difference because of the differences in nature of food matrices. The outcome of the study reveals the need to establish rejection thresholds for which EO spike ice kenkey will be rejected despite the EO conferring antimicrobial properties, thus inhibiting spoilage and other organisms.

Table 11: Response on overall difference test between essential oil spiked ice kenkey and unspiked ice kenkey

Difference Test							
Sample 1 - Sample 2	Chance	N (Panellist)	Correct	Incorrect	d′	p-value	Significant at 0.05
Triad 1	1 in 3	28	27	1	5.14	0.00	YES

From Table 11 there was a statistically significant difference between both spiked and unspiked ice kenkey samples ($p<0.00$). The magnitude of difference based on the d' value of 5.14 signifies that there was an overwhelming difference between both samples, implying a very large difference between the samples.

Some comments from panellists on how the samples differed are outlined below:

- *"there is a minty taste in the sample"* ***(P 10)***
- *"it [ice key with X. aethiopica essential oils] has sharp irritating mouth feel after taste"* ***(P 6)***

The unspiked ice kenkey was described as possessing glossy surface, bland taste, sour and without a hint of spice or mint whiles the ice kenkey spiked with *X. aethiopica* essential oil was described as possessing minty taste, spicy tastes, hot sensation and lingering ointment-like taste. Knowledge about the tolerable consumer limits of the EO in the high starch diet will aid in product value addition processes.

4.6 Microbiological Challenge test

4.6.1 pH trends of bissap extract during microbiological challenge test.

Two (2) bissap extract samples each, were stored under both ambient and refrigerated conditions were monitored during the microbiological challenge test (0 hr – 192hrs). The controls depict bissap extract without *X. aethiopia* essential oils and a cocktail of three organisms, *S.* Typhimurium, *S. aureus* and *E.coli* and under both ambient (35 ± 2) °C and refrigerated conditions (14 ± 2) °C . The two (2) treatment samples were also bissap samples spiked with *X. aethiopica* essential oils together with a cocktail of three organisms, *S.* Typhimurium, *S. aureus* and *E. coli* and under both ambient (35 ± 2) °C and refrigerated

conditions (14 ± 2) °C. The pH for all samples was measured using a pH Meter (Mettler-Toledo GmbH, Switzerland).

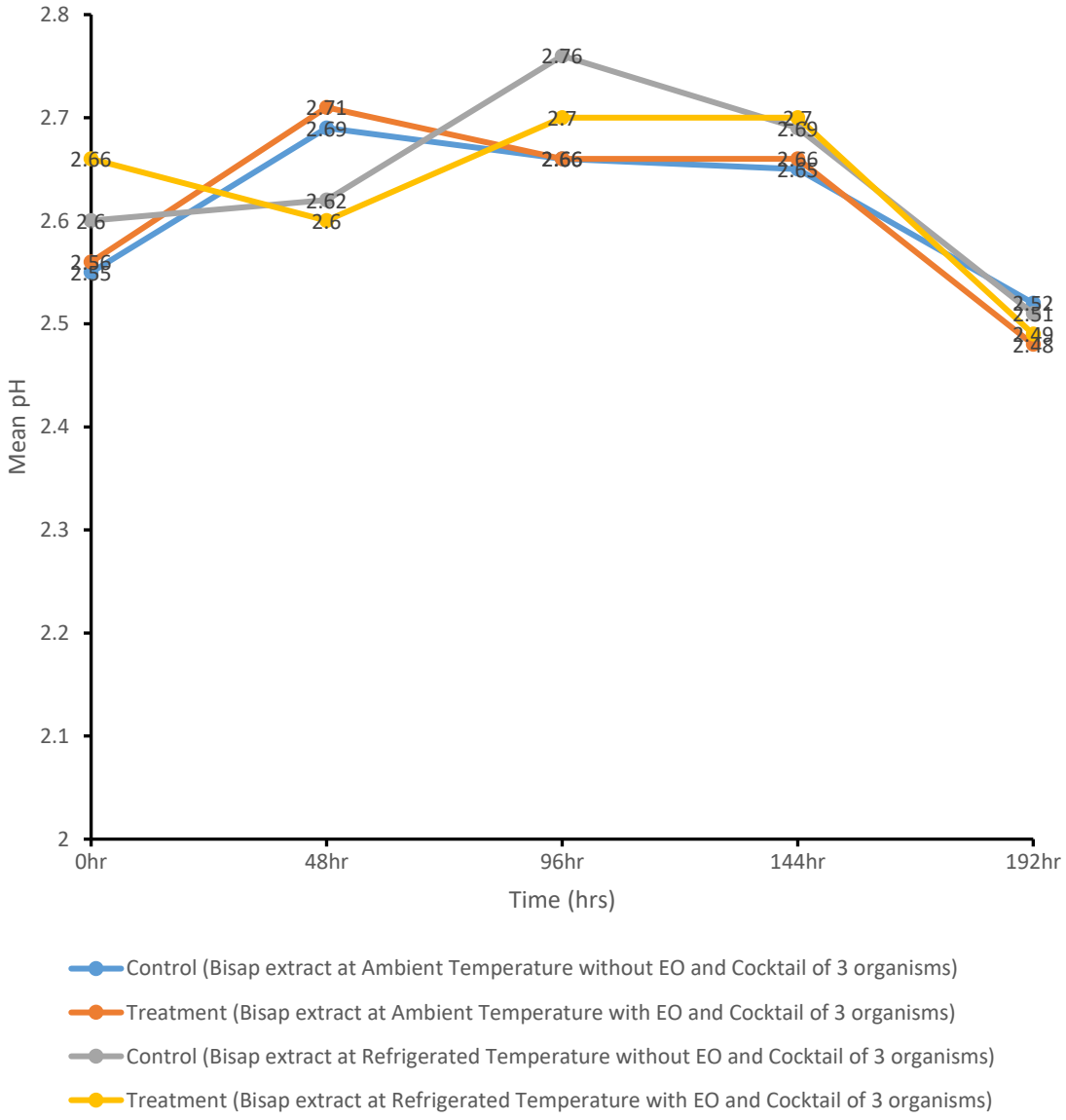

Figure 1: Effect of essential oils on pH of bissap extracts under different temperature conditions.

From (Figure 1), the pH for control sample under ambient temperature ranged from 2.55 to 2.52 and it is indicative of high acidity in bissap. The pH of the same sample increased between the first 48hr, 2.55 to 2.69 and could be indicative of the breaking down of acids.

The treatment samples under ambient temperature, on the other hand, followed a similar pattern, thus pH at 2.56 (0hr) and rising to 2.71 after 48hrs, and a decline from 2.66 (144hr) to

2.48 (192hr). Statistical analysis of mean pH from an unpaired mean t-test at 95% confidence interval reported at a p-value of 1.00, which depicts the EO had no significant influence on the pH of the samples. The non-difference in pH could be useful to food product developers, as composition or nature of food might not significantly change due to the addition of the essential oil. Possible factors that could account for the undulating pH in both control and treatment could be possible room temperature fluctuations or microbial activity.

Generally, from (Figure 1) the pH for control sample under refrigerated temperature ranged from 2.6 (0hr) to 2.51 (192hr), further observing a rise between the 2.62 (48hr) and 2.76 (96hr) and then decreases from 2.69 (144hr) to 2.51 (192hr). The rise in pH values signifies a decrease in the acidity of the bissap beverage, whiles the gradual decrease in pH values, signifies a rather increasing acidity on the pH scale.

On the other hand, the pH values of bissap beverage spiked with essential oil ranged between 2.66 (0hr) to 2.49 (192hrs) in the study. The treatment sample observed an increase in acidity between 2.66 (0hr) to 2.60 (48hr). The pH values suggest that it is unfavourable for the proliferation of some foodborne organisms.

Analysis of the means of the values of pH for the control and treatment under refrigerated condition samples using the unpaired t-test at 95% confidence interval revealed that there was no statistically significant difference between samples (p-value, 0.920). Bissap extracts proved to be acidic in nature under the refrigerated condition and the presence of essential oils could not induce a significant change in pH.

Analysis of the mean values of pH of EO spiked bissap sample stored under ambient condition and EO spiked bissap sample under refrigerated condition using the unpaired t-test at 95% confidence interval revealed that there was no statistically significant difference. The p-value

was 0.884. This finding suggests that temperature conditions did not necessarily influence changes in pH.

4.6.1.3 pH trends of ice kenkey during the microbiological challenge test

In (Figure 2), unspiked ice kenkey and treatment samples were kept at ambient temperature over a-192 hr period and pH of the samples monitored. Ice kenkey, like other cereal-based products that undergo fermentation, often records an increasing acidity (decreasing pH values) as fermentation time increases. pH values of the control sample span between 4.21 (0hr) to 3.23 (192hr). On the other hand, the treatment (ice kenkey containing spiked with *X. aethiopica* and a cocktail of three organisms) followed a steady increase in acidity (decreasing pH values) between the 192hrs of storage under ambient temperatures. The pH values ranged from 4.21 (0hr) to 3.23 (192hr). Comparatively, the presence of essential oils, as well as cocktail of organisms, did not yield a statistically significant difference in pH at a 95% confidence interval as an unpaired t-test revealed a p-value of 0.712. This signifies that the presence of EO in ice kenkey would not necessarily alter the pH. Understanding and application of this concept are useful in ice kenkey value addition trials which will be useful to product developers whose product specificity and product attribute include pH.

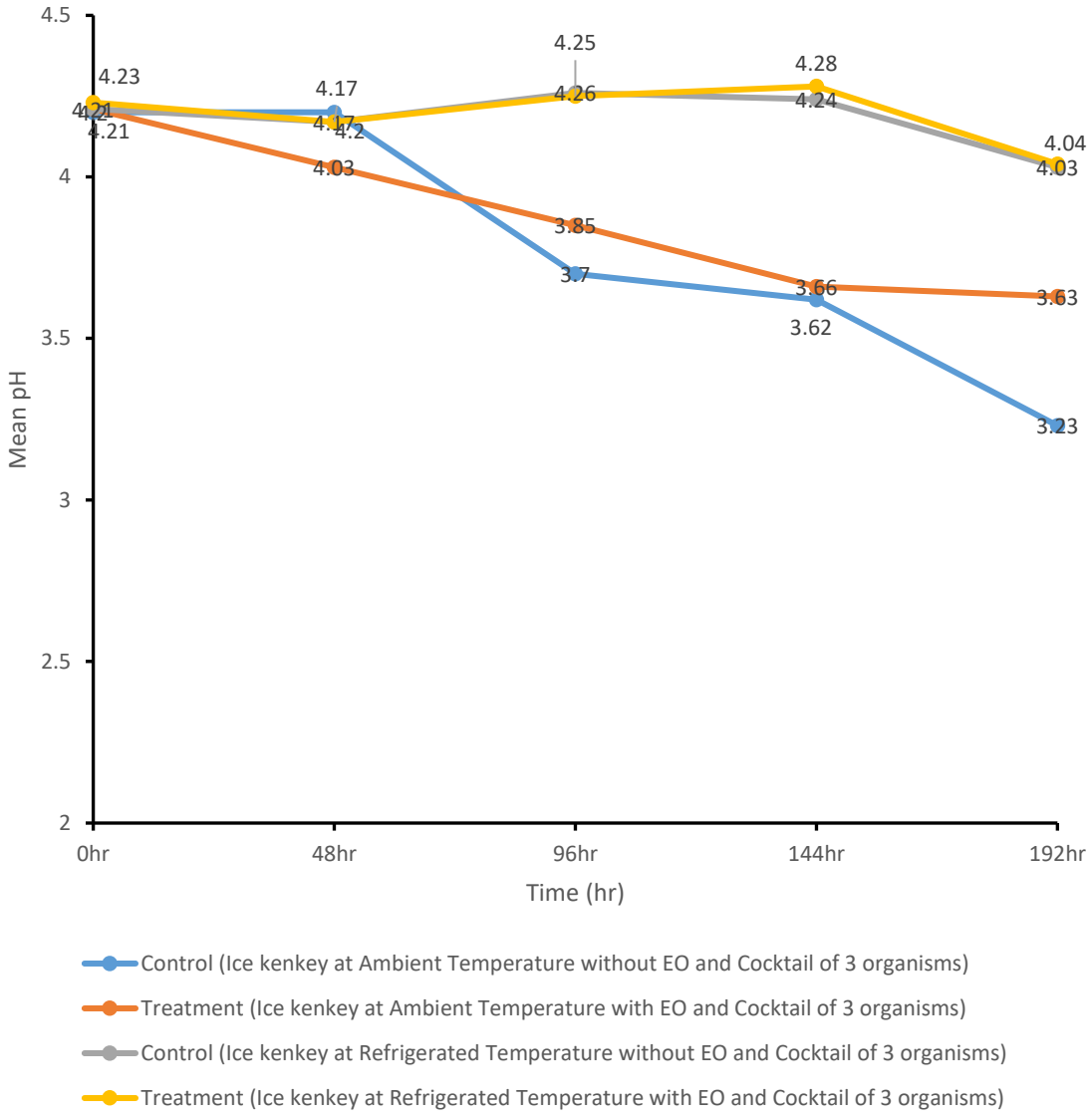

Figure 2: Effect of essential oils on pH of ice kenkey under different temperature conditions

The findings on the pH changes in ice kenkey and ice kenkey spiked with essential oil and cocktail of microorganisms all under refrigerated conditions suggest a similar trend. (Figure2) The pH of control samples ranged from 4.21 (0hr) to 4.03 (192hr) whiles the treatment sample (ice kenkey with essential oil and cocktail of microorganisms) ranged from 4.23 (0hr) to 4.04 (192hr). Comparatively, the essential oils could not impart any statistically significant difference in the pH (*CI=95%; p=0.845*). Analysis (unpaired t-test) of the mean values of pH of EO spiked ice kenkey sample stored at ambient condition and EO spiked ice kenkey sample under refrigerated condition indicates there was statistical significance (*CI=95%; p=0.027*).

4.7 Food (Bissap and Ice kenkey) Colour analysis during microbiological challenge test using Hunter L,a,b System

In the quest to assess possible colour changes in bissap extract and ice kenkey over the 192 hr challenge test, the colour of both control and treatment samples under ambient and refrigerated temperature were monitored for the various colour codes on the Hunter Lab colour system. Generally, colour plays a quintessential role in food acceptability, therefore serving as a litmus test for the determination of the initiation, presence or occurrence of chemical changes. The L-value which depicts lightness on the Hunter L,a,b colour system was measured for both treatment and control of bissap extract and ice kenkey. The L values generally span between 0 and 100 where the former and latter depicts dark/ black and whiteness respectively. Some authors hold the thoughts that, (0-50) depict black whiles (51-100) depicts light. However, because of this intellectual divide, all analysis and discussions are made in relation 100 which depicts light or lightness of bissap or ice kenkey. The ‘a-value’ on the same colour scale or system also depicts redness or greenness of a given sample. The colour values have no numeric thresholds; however, positive values depict redness whiles negative value suggests greenness. Furthermore, the ‘b-value’ on the Hunter colour system depicts yellowness or blueness of the sample. In the same vain as ‘a-value’, the b-colour value scale has no numeric threshold, but positive values suggest yellowness whiles negative values imply blueness.

4.7.1 Analysis of (L- value) colour of bissap extract under both ambient and refrigerated temperature.

The data from colour (white/dark) changes in bissap extract obtained under different storage temperature conditions has been graphically presented.

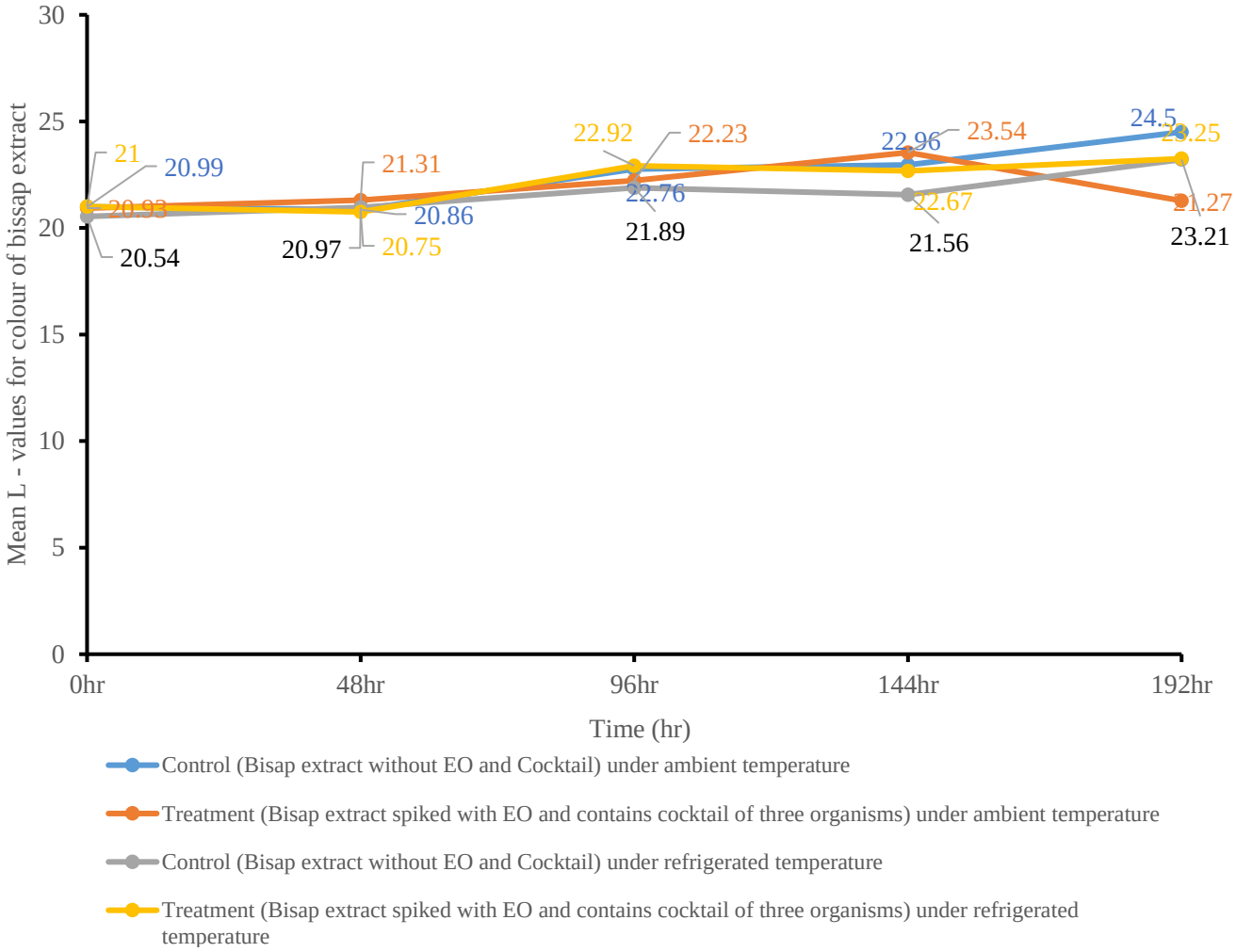

Figure 3: Changes in L-values (white/dark) of the colour of bissap extract under different storage temperature conditions

The L-value of control bissap sample under ambient temperature according to (Figure 3) above spans from 20.99 (0hr) to 24.5 (192hr), with a decline in the lightness or whiteness of the bissap during 48hr (20.86). A rather gradual increase in the whiteness (increase L-values) 22.76, 22.96 and 24.5 of the bissap extract occurred at (96hr), (144hr), (192hr) respectively. Comparatively, bissap extracts containing a cocktail of (*S. aureus, E. coli, and S.* Typhimurium) and also spiked with *X. aethiopica* essential oil under ambient conditions had colour values spanning from 20.93 (0hr) and 21.27 (192hr). Per Figure 3, L-value of the treatment increased from 0hr to 144hr (23.54) and then sharply decline to 21.27 (192hr). The presence of essential oils could not maintain the rise in the lightness of bissap extract under ambient temperature conditions as

expected. Assessment of the L-value of the same control and treatment samples under refrigerated conditions revealed similar trends like the control sample under ambient temperature conditions.

For control sample under refrigerated conditions, L-value ranged through 20.54 (0hr) to 23.21 (192hr), with a gradual rise in lightness or whiteness from 0hr (20.54), 48hr (20.97) to 96hr (21.89).

4.7.2 Analysis of colour (L- value) of control and test samples of ice kenkey under both ambient and refrigerated temperature

The comparative assessment of the L colour value of ice kenkey (control) and essential oil spiked ice kenkey, which contains a cocktail of microorganisms under ambient and refrigerated conditions, was conducted over a 192 hr period. Figure 4 shows that all samples followed a similar trend, thus a gradually increasing L-value over the period of study. Ice kenkey vended on Ghanaian streets and in supermarkets' are generally whitish - creamy. This was corroborated as L-values of all samples were above 50. The general scale of L-value falls between zero (0) and hundred (100) where the former and latter depict dark and white respectively. Control sample stored under ambient temperature condition observed L-values spanning from 56.58 (0hr) to 66.69 (192hr) with an occasional decline at 144hr. Treatment sample under ambient temperature also recorded L-values between 54.94 (0hr) to 69.63 (192hr). On the other hand, samples stored under refrigerated conditions could not significantly induce a change on the L-values of the ice kenkey, as similar pathway was observed. Ice kenkey under refrigerated conditions had L-values spanning across 55.35 (0hr) and 64.83 (192hr). L-values of treatment samples of the ice kenkey under refrigerated condition gradually increased between 58.0 (0hr) and 69.43 (192hr). In summary, treatment samples under refrigerated conditions recorded the highest L-value after the 192 hr experimentation. It is therefore suggested that, because the

colour (whiteness) is a crucial indicator for the acceptability of ice kenkey, temperature conditions and essential oil interactions should further be researched.

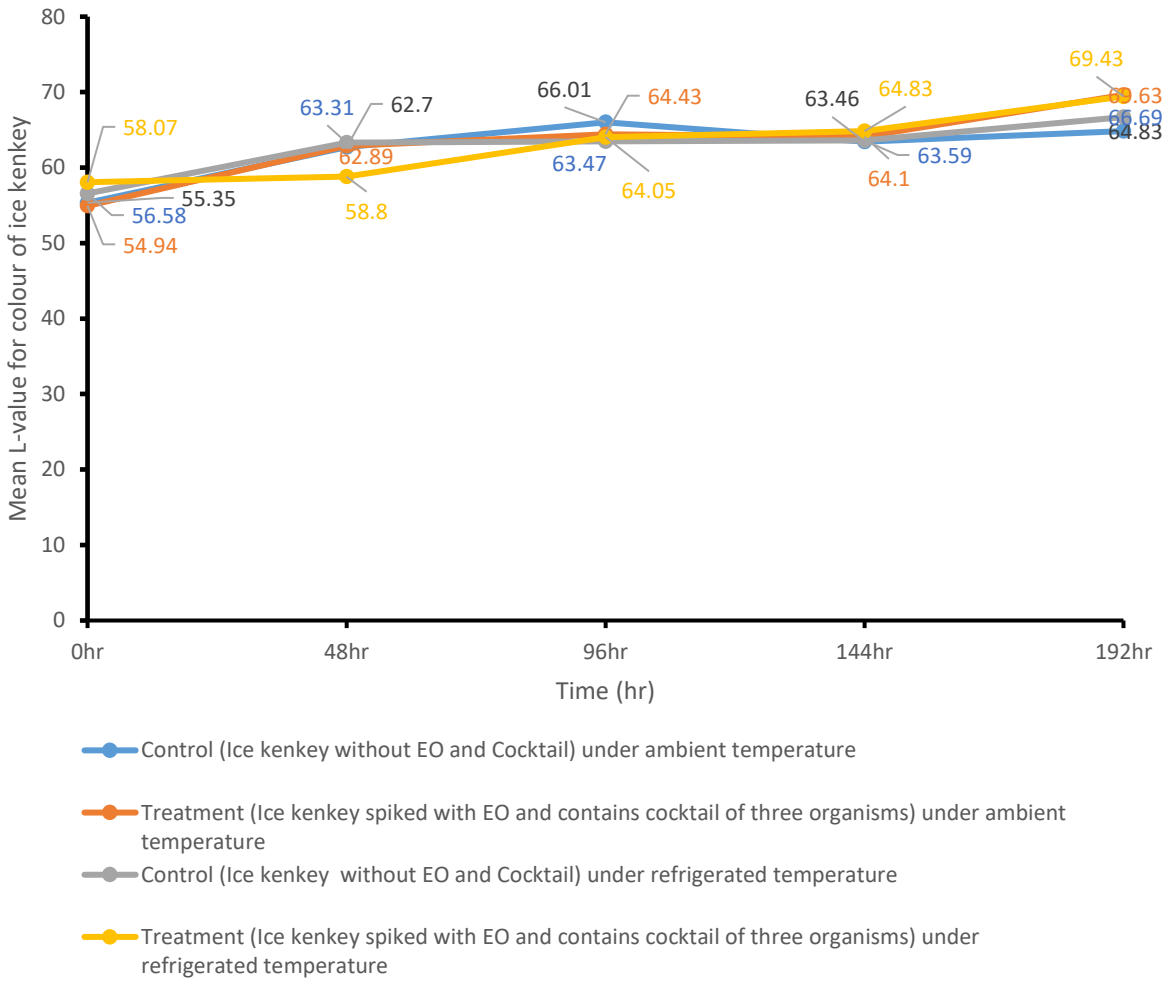

Figure 4: Changes in colour, L-values (white/dark) of ice kenkey under different storage temperature conditions

4.7.3 Analysis of colour (a- value) of essential oil spiked and raw (control) bissap extract under both ambient and refrigerated temperature

Control bissap extract sample under ambient temperature conditions had a-values ranging between 8.09 (0hr) to 29.96 (192hr). The a-values (redness because of the positives values) increased as the duration of the study increased. The treatment bissap sample also recorded a-

values between 8.39 (0hr), a relatively higher a-value at 19.5 (144hr) and then a sharp decline to 9.99 (192hr). In the case of bissap extract control and treatment under the refrigerated condition, a-values of the control span between 7.09 (0hr) which was the lowest amongst all samples to 21.12 (192hr). In the case of bissap extract spiked with essential oil under refrigerated conditions, a-values were within a range of 7.94 (0hr), and 21.27 (192hr) which was far above bissap extract spiked with essential oil and under ambient temperature 9.99 (192hr). It was therefore indicative that the refrigerated conditions help in maintaining a steady redness of bissap extract. Comparing a-values at the end of the 192 hr period, control samples under ambient temperature recorded the highest a-value. Generally, all samples were within the red region of the a-value scale.

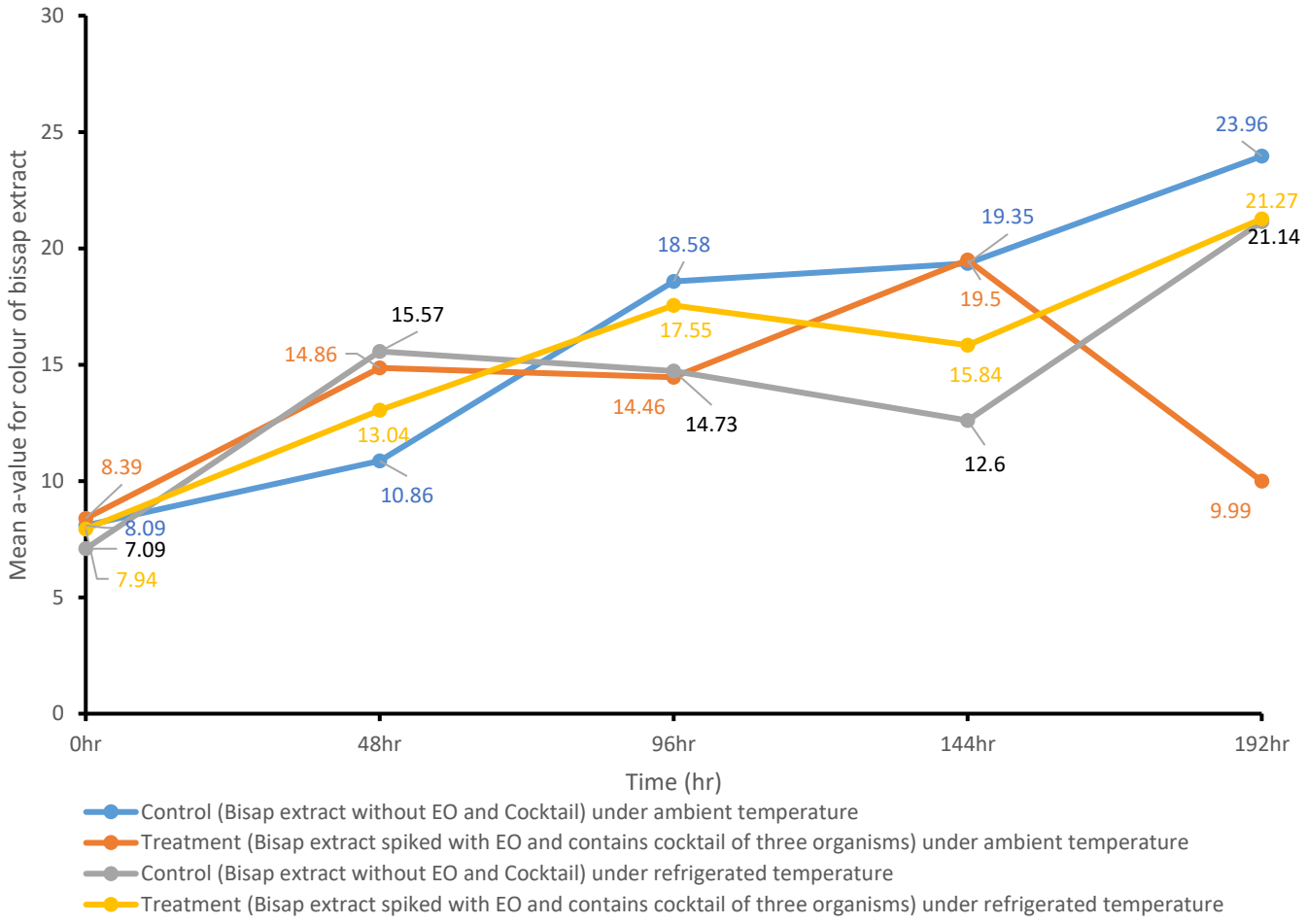

Figure 5: Changes in colour, a-values (red/green) of bissap extracts under different storage temperature conditions

4.7.4 Analysis of colour, (a- value) essential oil spiked and control ice kenkey under both ambient and refrigerated temperature

From Figure 6 mean of a-values of the control ice kenkey samples under ambient temperature conditions ranged from a relatively high 2.87 (0hr) to 1.84 (192hr). Ice kenkey samples containing a cocktail of organisms as well as essential oils of *X. aethiopica* under ambient temperature conditions recorded a-values between 2.74 (hr), then gradually decline to 1.22 (192hr). Similar gradually declining trends were observed in control and treatment samples under refrigerated conditions. The a-values of colour for control samples were recorded at 2.36

(0hr) to 1.46 (192hr), which was lower than 1.4 (192hr) of similar control sample under ambient temperature. The a-value of the treatment sample under refrigerated condition recorded the lowest a-value colour at both 0hr (2.22) and 192hr (1.16). Generally, control sample under ambient temperature recorded the highest a-values (redness)), 2.87 (0hr) and 1.84 (192hr).

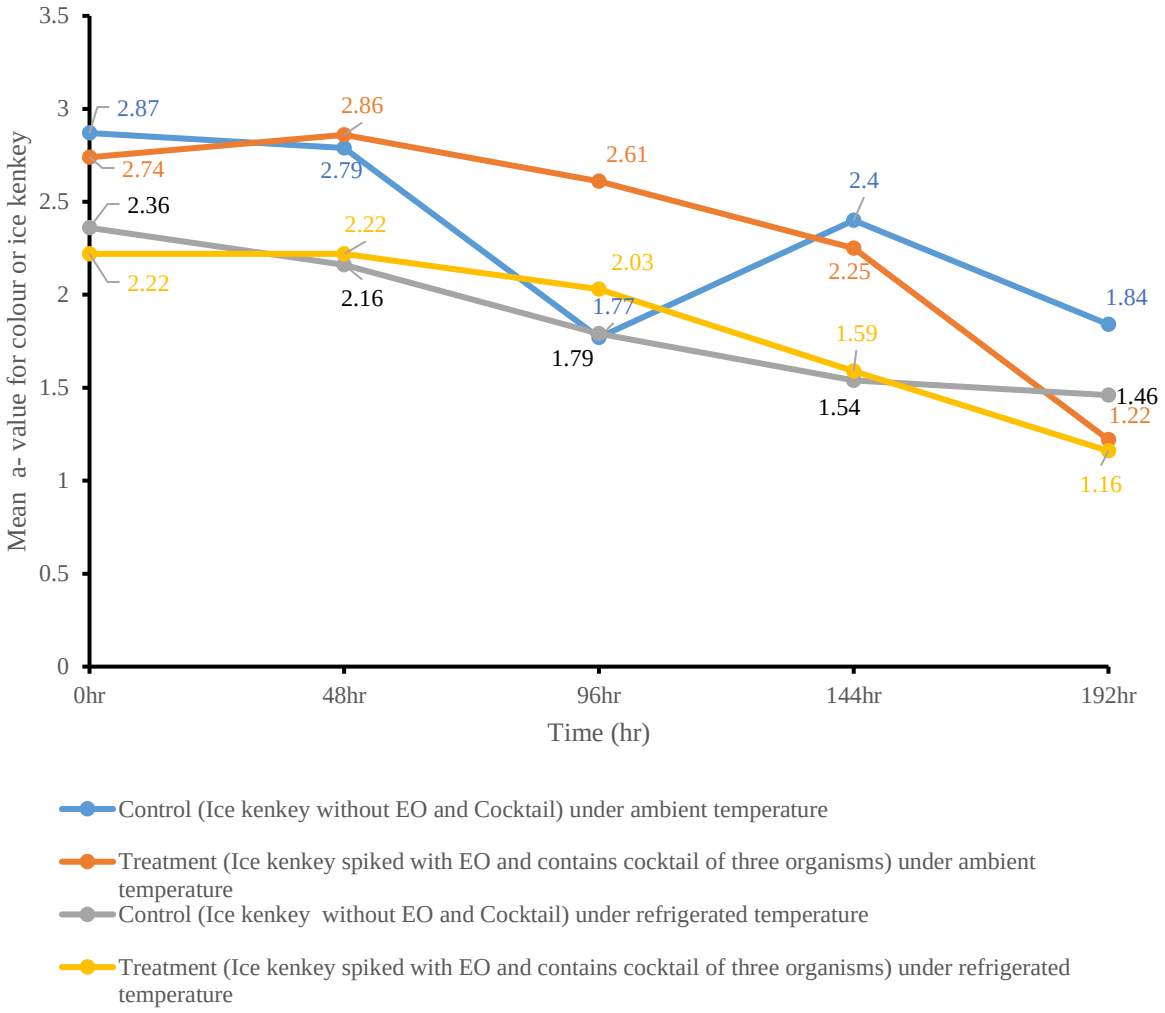

Figure 6: Changes in colour, a-values (red/green) of essential oil spiked and raw (control) ice kenkey under different storage temperature conditions

4.7.5 Analysis of (b- value) colour of control and test samples of bissap beverage under both ambient and refrigerated temperature

The measurement of the extent of yellowness or blueness of control and treatment samples of bissap was conducted through the use of b-value on the Hunter L,a,b colour system. The general, non-numeric scale surmise all positive values as depicting yellowness whiles the negative values depict blueness. All values recorded for b-values of bissap extracts under both ambient and refrigerated temperature, per figure 7, were positive values, indicating taints or traces of yellowness in samples. For control bissap samples under ambient temperature, b-values increased from 1.86 (0hr) to 5.89 (192hr), which was higher than all the other samples measured. For treatment sample under ambient temperature, the highest b-value was recorded at 0hr (2.00) and increased steadily in yellowness to 4.95 (144hr) before declining sharply to 1.57 (192hr) which was the lowest compared to all other samples assessed. The assessment of b-values of bissap extract stored under refrigerated condition showed that samples recorded the lowest b-value (yellowness due to positive b-values) at 0hr (1.44) but increased gradually to 4.65 (192hr). It is however uncertain, why there are fluctuations in the b-value during the per 0hr to 192hr time period. According to Figure 7 above, the treatment sample under refrigerated condition increased from 1.63 (0hr) to 4.5 (192hr).

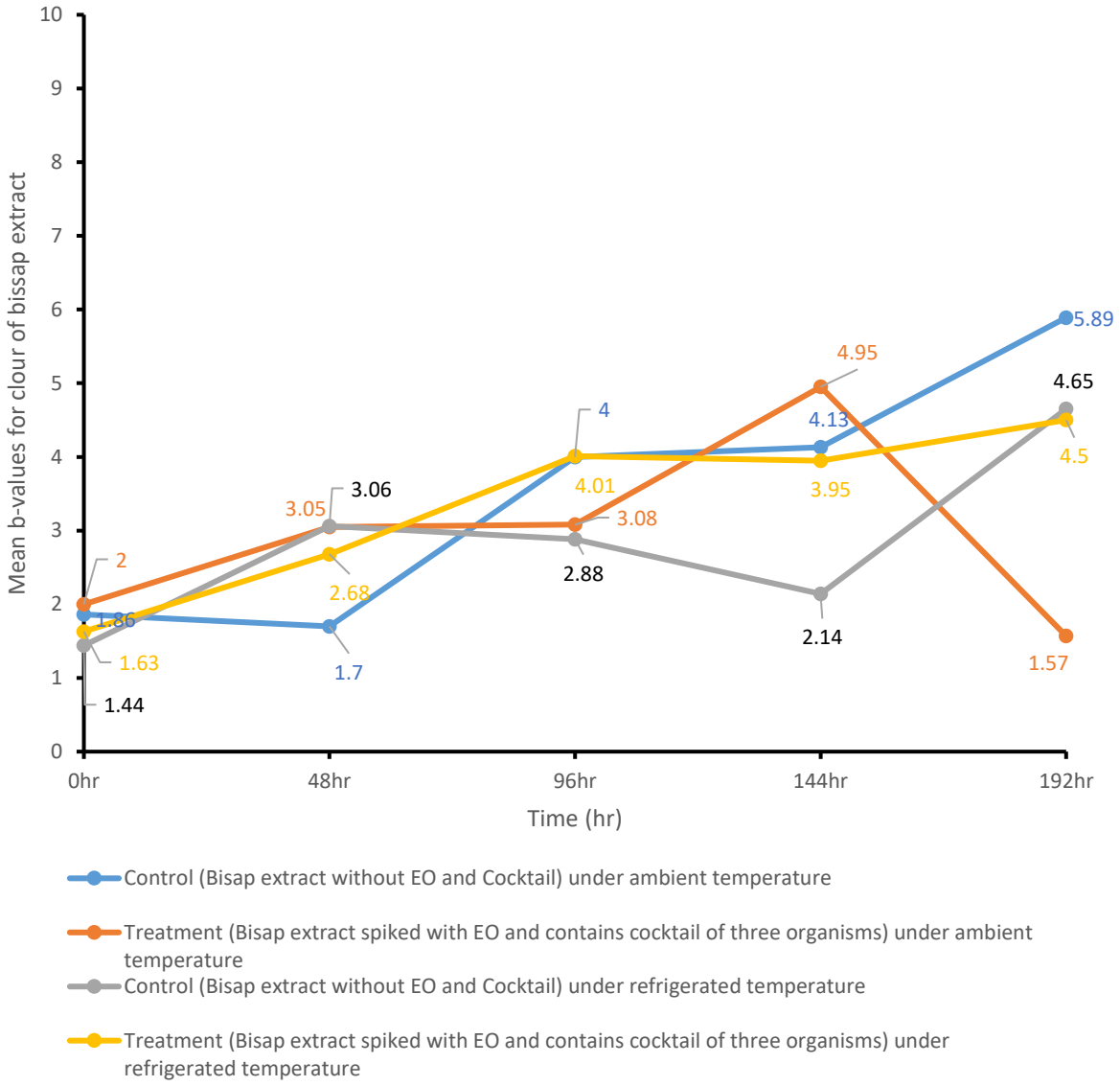

Figure 7: Changes in colour, b-values (yellow/blue) of essential oil spiked and raw (control) ice kenkey under different storage temperature conditions

4.7.6 Analysis of (b- value) colour of control and test samples of ice kenkey under both ambient and refrigerated temperature

From figure 8, the mean b-values (yellowness/blueness) of ice kenkey samples (control and treatment) under both ambient and refrigerated temperature was plotted against the 192hr duration of the study. Generally, b-values of all samples increased sharply during the first 48hr and then resumed a rather gradual increase over the rest of the study period. Based on the scale

of the non-numeric threshold, where positive denotes yellowness and negative denotes blueness, all samples were suggestive of taints or traces or shades of yellow.

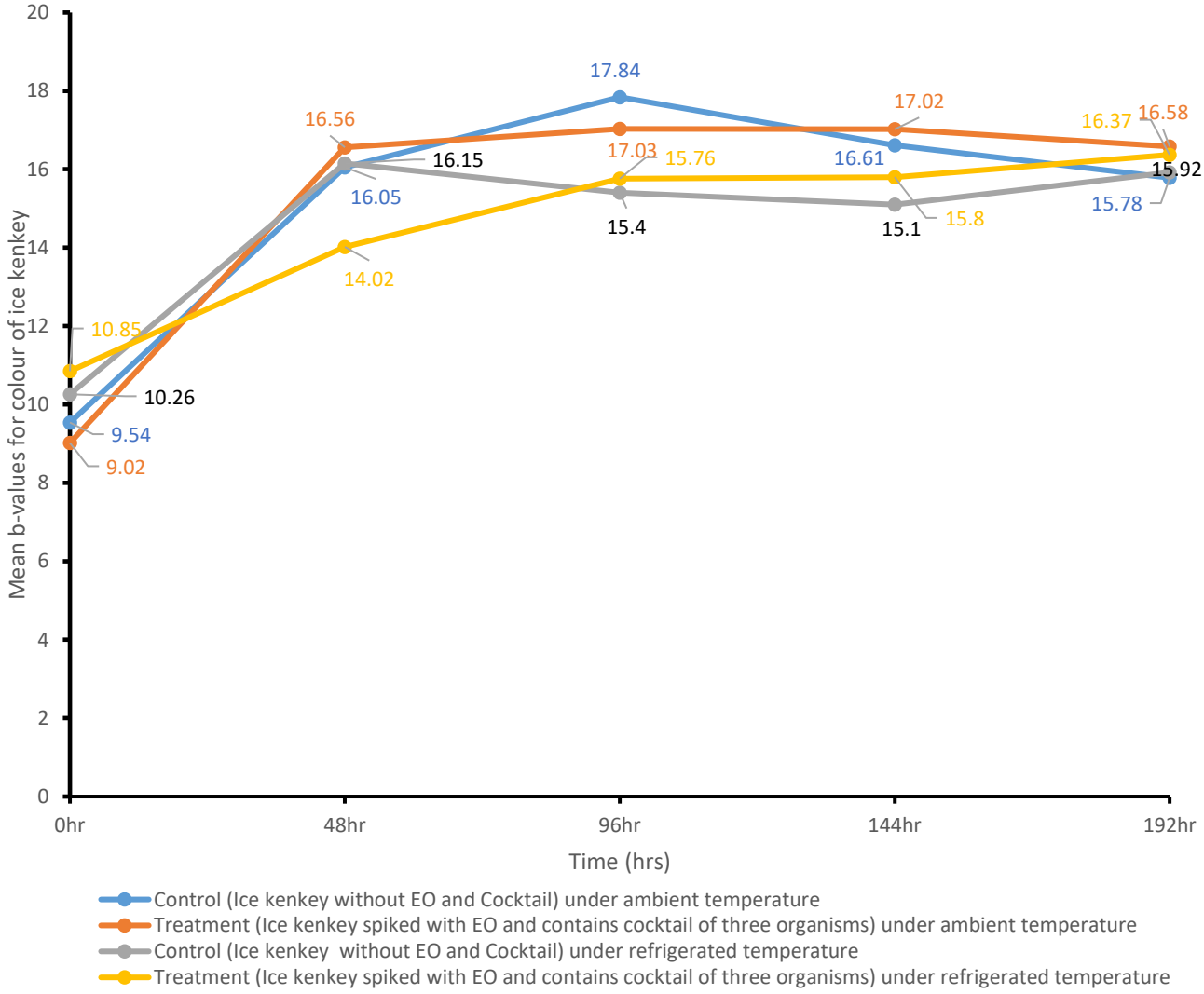

Figure 8: Changes in colour, b-values (yellow/blue) of essential oil spiked and raw (control) ice kenkey under different storage temperature conditions

Control samples of ice kenkey under ambient temperature rose from 9.54 (0hr) to 16.05 (48hr) then to apex b-value of 17.84 (96hr) and later decline to 15.78 (192hr). The treatment sample recorded the lowest b-value, 9.02 (0hr), but increased sharply and highest at 48hr (16.56) before resuming a steady b-value through to 16.58 (192hr), which also is the highest amongst all

samples under both ambient and refrigerated temperatures. The control sample under refrigerated condition also followed the same trend as the other samples, where there was a sharp rise from 10.26 (0hr) to 16.15 (48hr).

4.8 Food based antimicrobial properties of *X. aethiopica* essential oils

4.8.1 Microbial counts and analysis of Bissap beverage containing a cocktail of microorganisms during food-based antimicrobial application of essential oils

Results from Table 12 showed that *S. aureus* and *S.* Typhimurium did not grow or thrive in control samples (without the cocktail of organisms and essential oils) under both ambient and refrigerated temperature conditions during the 192hr of the study. The finding indicates that when good hygienic conditions are implemented during processing, pasteurization, bottling and refrigeration (only refrigerated sample) of the bissap, contamination can be prevented. Despite the hygienic conditions implemented, the control bissap extract at 0hr (initial time) recorded a growth of 4 cfu/ml of *E. coli.* The 4 cfu/ml was, however, lower than the national standard of 10 cfu/ml (Ghana Standards Authority). Also, observed on control samples of bissap extract after 144hr of storage under ambient temperature were visible growths of sixty (60) distinct colonies of moulds The results indicate that bissap extract kept under ambient temperature for 144hr could lead to mould growth, hence may compromise consumer safety and product quality. On the other hand, treatment samples (the cocktail of 3 organisms and the essential oil) under ambient condition at both 144hr and 192hr neither supported the growth of organisms nor moulds. The results, therefore, suggest that the essential oil has both antibacterial and antifungal properties.

Similarly, EO bissap samples stored at ambient temperature for 192 hr did not support mould growth. However, similar unspiked sample showed 25 fluffy white spreading colonies on

Baird-Parker plates originally meant for isolating *S. aureus*. In cases where any of the organisms survived, they were lower than the national limits.

Generally, essential oils inhibited the growth of *S. aureus, E. coli, and S.* Typhimurium under ambient and refrigerated conditions. Understanding the mechanism of action or inhibition of the EO will provide a sound basis for their continuous use in other food matrices.

The surmised causes of the non-survival and growth of test organism are not very different from those posited by Burt (2004) that, essential oils tend to work effectively under physical conditions such as low pH and low temperature. Although post processing handling could indict bissap extract in food contamination, their surmised antibacterial properties against methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii* has been reported by Da-Costa-Rocha *et al*., (2014); Liu *et al*.(2005). Also, the bissap has been associated with protocatechuic acid, which is considered to have some level of antibacterial properties.

Table 12: Colony counts from food applications of essential oils in bissap extract

Mean						
Food Matrix	**Storage condition (°C)**	**Sampling time (hrs)**	**Sample Code**	***Staphylococcus aureus* (CFU/ml)**	***Escherichia coli* (CFU/ml)**	***Salmonella* Typhimurium (CFU/ml)**
Bissap extract	AT 30±2°C	0	C0hrAT	0	4	0
		48	C48hrAT	0	0	0
		96	C96hrAT	0	0	0
		144	C144hrAT	0	0	0
		192	C192hrAT	0	0	0
		0	T0hrAT	0	0	0
		48	T48hrAT	0	1	0
		96	T96hrAT	0	0	0
		144	T144hrAT	0	0	0
		192	T192hrAT	0	0	1
	RT 14+2°C	0	C0hrRT	0	5	0
		48	C48hrRT	0	0	0
		96	C96hrRT	0	0	0

144	C144hrRT	0	0	0
192	C192hrRT	0	0	0
0	T0hrRT	0	3	0
48	T48hrRT	0	0	0
96	T96hrRT	2	0	0
144	T144hrRT	0	0	0
192	T192hrRT	0	0	0

Legend

C = Control sample T = Treatment sample (bissap extract + X. aethiopica essential oil + cocktail of S. Typhimurium, *S. aureus* and *E. coli*; hrs = hours

AT= Ambient Temperature RT= Refrigerated Temperature 0= No growth

4.8.2 Antimicrobial food applications of essential oils in ice kenkey

Ice kenkey, according to Feglo and Sakyi (2012) is a high-risk food or beverage prone to contamination and poisoning hence it was suggestive that possible antimicrobial applications of *X. aethiopica* EOs on the indigenous beverage should be investigated.

From Table 13 below, *S.* Typhimurium was inhibited in the ice kenkey matrix containing essential oil under both atmospheric and refrigerated temperature storage. This was not surprising, as, the same organisms was the most inhibited in vitro. *Staphylococcus aureus* and *Escherichia coli* growth trends generally declined in ice kenkey samples containing the cocktail of organisms and essential oil as the duration of the study increased. Also, from table 13, ice kenkey samples (containing both essential oils and cocktail of organisms) stored under refrigerated conditions, showed that, *S. aureus* was present throughout the 192hr period.

Physical examination of control (ice kenkey) and treatment samples kept under ambient temperature for 144hr, prior to the inoculation on various agar revealed that all samples showed visible growths of moulds. Morphologically, they appeared white coloured. On the other hand, corresponding samples (control and treatment, 144hr, refrigerated temperature) revealed no signs of mould growth. The examination of the anti-mould efficacy of essential oils was however beyond the scope of the present study. That notwithstanding, control samples kept for

144hr at ambient temperature recorded an average of 56 “pale to dark” coloured colonies of mould on Baird-Parker agar. For the same samples treated with essential oils at ambient temperature, an average of 13 mould colonies, morphologically white and centred with pale-dark brown colour was observed.

Results on moulds growth were also recorded at 192hr for both control and treatment samples under ambient conditions. Mould growths were not spotted in refrigerated samples. r.

Table 13: Colony counts from the application of essential oils in ice kenkey

				Mean		
Food Matrix	**Storage condition (°C)**	**Sampling time (hrs)**	**Sample Code**	***Staphylococcus aureus***	**Escherichia coli**	**Salmonella Typhimurium**
Ice Kenkey	AT 30±2°C	0	C0hrAT	0	0	0
		48	C48hrAT	36	3	0
		96	C96hrAT	0	0	0
		144	C144hrAT	0	0	0
		192	C192hrAT	1	0	3
		0	T0hrAT	323	41	0
		48	T48hrAT	214	22	0
		96	T96hrAT	1	0	0
		144	T144hrAT	0	0	0
		192	T192hrAT	1	0	0
	RT 14+2°C	0	C0hrRT	2	3	0
		48	C48hrRT	0	3	0
		96	C96hrRT	4	2	2
		144	C144hrRT	0	0	0
		192	C192hrRT	0	0	0
		0	T0hrRT	197	TNTC	0
		48	T48hrRT	390	103	0
		96	T96hrRT	358	+2	0
		144	T144hrRT	130	0	0
		192	T192hrRT	72	0	0

Legend

C = Control sample T = Treatment sample (bissap extract + X. aethiopica essential oil + cocktail of S. Typhimurium, *S. aureus* and *E. coli*; hrs = hours

AT= Ambient Temperature RT= Refrigerated Temperature 0= No growth

NG/0= No growth TNTC= Too Numerous to Count 2+ = S. Typhimurium was found on MacConkey agar

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In the present study, a preliminary market survey across four major markets in the national capital supported findings that indigenous Ghanaian *X. aethiopica* both medicinal and food applications.

The essential oil composition of *X. aethiopica* was largely classified into their various functional groups. In all, 104 compounds were identified out of 105 compounds profiled by GC-MS. Similarities existed between the chemical constituents of indigenous Ghanaian *X. aethiopica* and those of other neighbouring countries. The dominant chemical constituents include the cyclohexane, ethenone, 1,4methanoazulene, beneze, 4-hydroxy-3-methoxybenzyl alcohol, kaur-4-ene. Quantitatively chemical compounds reported in the present study exceeds the others reported from neighbour West African countries. This does not, however, suggest a comparative advantage in terms of yield, quality and potency, as this was beyond the scope of this study.

X. aethiopica showed antimicrobial effects on *Escherichia coli, Salmonella* Typhimurium, and *Staphylococcus aureus* at different concentrations of the essential oil. Essential oils proved to be strongly inhibitory to *S. Typhimurium* and weakly inhibitory to *E. coli* and *S. aureus* at different concentrations.

X. aethiopica essential oils were easily detected by taste in bissap extract and ice kenkey at levels they still conferred antimicrobial properties. The study revealed statistically significant differences between essential oil spiked and unspiked bissap extract and ice kenkey samples.

Also significant amongst findings is that EO's did not significantly impact or influence pH and colour of both bissap and ice kenkey beverages.

In the wake of numerous unreported and underreported mild and worst cases of food poisoning episodes of some foodborne organisms such as *S. aureus, E. coli* and *S.* Typhimurium, the use of *X. aethiopica* essential oil proved to be a useful natural alternative for synthetic antimicrobials, Gentamycin.

5.2 Recommendation

There existed some limitations which when researched or addressed will contribute to knowledge in the area to antimicrobial properties and other applications of essential oils to foods and beverages. The noteworthy challenges include the under listed;

- The study was bedevilled with challenges in obtaining enough yields of essential oils. The extraction method used in the extraction of essential oils was still at the remotely artisanal stage. The low yield of essential oils from *Xylopia aethiopica* greatly interrupted work flow.
- Another major challenge in the study was the difficulty in retaining the antimicrobial properties of *X. aethiopica* essentials oils as well as conferring a pleasurable sensory appeal to the trained discriminative panellist. The strong, piquant and compelling aroma of the essential oils could hardly be circumvented.

Based on the above limitations and other findings of the study, I recommend the following;

- There is the need to report findings of the chemical composition of the essential oils in common names and not IUPAC names, as the present GC-MS output of the chemical constituents makes it difficult to classify the chemical constituents.

- There is the need to study the mechanism of inhibition with which essential oils were able to inhibit the various bacterial strains *in vitro*.
- There is the need to map out *X. aethiopia* growing areas in Ghana. This is important because geographical distribution has been cited severally to influence the chemical composition, yield and antimicrobial efficacy of essential oils. Therefore, knowledge of the growing area and their respective chemical position will serve as a foundation for knowing areas within which the most potent essential oils can be obtained.
- There is also the need to conduct a consumer threshold test and consumer acceptability test to determine what levels of essential oils are tolerable and objectionable in bissap extract and ice kenkey.
- The antifungal properties of *X. aethiopica* observed in the study in ice kenkey and bissap extract should be further researched.
- The applications of indigenous *X. aethiopica* essential oils to other foodborne organisms especially those listed on the Centre for Disease Control's list of foodborne germs are encouraged. This is because when the susceptibility of these organisms are proven with *X. aethiopica*, it will lead to its use in the combat against food contamination and food poisoning in Ghana.
- The duration of 'essential oil – foodborne organism' challenge test should be extended beyond five days to further ascertain the antibacterial potency of *X. aethiopica* oil.
- Innovative, cost effective and sustainable technology should be researched and adopted for curbing or reducing the overly compelling strong piquant taste of *X. aethiopica* essential oils when they are applied at concentrations that still retain their antibacterial prowess.

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APPENDICES

APPENDIX 1: Presented Abstract – Oral presentation

Abstract presented on 2nd June 2017, at the African Research and Innovation Summit (ARIS) organised by Association of African Universities (AAU)

Chemical and antimicrobial characterization of essential oil extract of Xylopia aethiopica, an indigenous Ghanaian spice

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Abstract

Food poisoning episodes and worse cases of foodborne illnesses in Ghana are seldom traced to their microbial causative agents. The vulnerability of many traditional food products to microbial spoilage and poisoning has hastened the need for a natural and novel antimicrobial source in order to kerb the menace. Essential oil extracts of *Xylopia aethiopica* (hwintia) was therefore characterised and its antimicrobial potential experimented on the foodborne pathogens *Staphylococcus aureus, Escherichia coli* and *Salmonella* Typhimurium.

Essential oils were extracted from dried *Xylopia aethiopica* fruits through the conventional steam distillation method. Essential oil samples were analysed using GC-MS, and further antimicrobial susceptibility test was conducted using the modified Kirby-Bauer Disk diffusion method on Mueller-Hinton agar.

The results of the study show that the essential oil was composed of 105 different chemical compounds which worked synergistically to inhibit the growth of *Salmonella* Typhimurium, *Escherichia coli* and *Staphylococcus aureus*. Twenty-five percent (25%) concentration of hwintia essential oil yielded 12.33±0.58mm and 7.67±0.58mm zones of inhibition for *S.* Typhimurium and *E. coli* respectively. A 7.67±0.58mm zone of inhibition was recorded for *Staphylococcus aureus* at 55% concentration of the hwintia essential oil. The results, however, contrast studies which reveal that Gram positives are inhibited than Gram-negative strains.

Selected bioactive compounds of antimicrobial or pharmacological importance include 1, 2-Benzenedicarboxylic acid, diisooctyl, Kaur-16-ene, and Longipinocarvone.

The inhibitory effect suggests that this essential oil when fully explored, can protect humans against foodborne pathogens and replace the use of synthetic antimicrobials.

Keywords: *Xylopia aethiopica*, Minimum Inhibitory Concentration, Antimicrobial resistance

APPENDIX 2: GC- MS Composition of essential oil of *X. aethiopica*, and indigenous spice from Ghana

GC-MS COMPOSITION OF ESSENTIAL OIL OF XYLOPIA AETHIOPICA, INDIGENOUS TO GHANA

No.	Retention Time	Peak Name	Res Type	Quan Ions	Area	Area %
1	4.45	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl	Id.	41.2	10982	0.02
2	4.972	3,3,5,5-Tetramethylcyclopentene	Id.	109	20269	0.03
3	5.308	Ethanone, 1-(1-cyclohexen-1-yl)	Id.	81	8603	0.01
4	5.435	Cyclopentanone, 2,2-dimethyl	Id.	56.1	12471	0.02
5	5.69	Methanol, (1,4-dihydrophenyl)	Id.	79.1	190216	0.28
6	5.946	Cyclohexane, (1-methylethylidene)	Id.	81	4374	0.01
7	6.269	Pentalene, octahydro-2-methyl	Id.	81	17213	0.03
8	6.677	Borazine, 2,4,6-triethyl	Id.	81	6.797E+06	10.03
9	6.854	7-Propylidene-bicyclo[4.1.0]heptane	Id.	93.2	298137	0.44
10	6.941	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1	Id.	91.2	638251	0.94
11	7.463	(E,Z,Z)-2,4,7-Tridecatrienal	Id.	81	2.921E+06	4.31
12	7.515	(E,Z,Z)-2,4,7-Tridecatrienal	Id.	80.7	3.753E+06	5.54
13	7.639	(4-Dimethylaminomethyl-1H-pyrrol-3-ylmet	Id.	136.1	3.418E+06	5.04
14	7.684	3-Cyclohexene-1-methanol,.alph., alpha	Id.	137.1	905211	1.34
15	7.797	6-Isopropenyl-3-methoxymethoxy-3-methyl	Id.	91.2	204003	0.30
16	7.793	1,4-Cyclohexadiene, 1-methyl-4-(1-methyl	Id.	93	179224	0.26

17	7.989	Decahydronaphtho[2,3-b]furan-2-one, 3-[	Id.	136	1.407E+06	2.08
18	8.306	4-Hydroxy-3-methoxybenzyl alcohol	Id.	137.3	7.571E+06	11.17
19	8.309	2-Propanol, 1-[(1-ethynycyclohexyl)oxy]	Id.	93.2	162557	0.24
20	8.681	3-Benzylsulfonyl-2,6,6-trimethylbicyclo	Id.	136	1.437E+06	2.12
21	8.799	2-Cyclohexen-1-ol, 1-methyl-4-(1-methyle	Id.	43.2	57572	0.08
22	8.962	.alpha.-Methyl-.alpha.-[4-methyl-3-pente	Id.	43	77196	0.11
23	8.965	3,7-Dimethyl-2,6-nonadien-1-ol	Id.	81.2	459947	0.68
24	9.129	1-Oxaspiro[4.5]deca-3,6-diene2,6,10,10	Id.	136	524017	0.77
25	9.337	Cyclohexane, 1-methyl-3-(1-methylethenyl	Id.	81.2	969013	1.43
26	9.337	Fenchyl acetate	Id.	81.2	969013	1.43
27	9.468	Cyclohexanone, 2-(1-methylethylidene	Id.	137.8	58647	0.09
28	9.724	Cyclohexanone, 2-mythyl-5-(1-methylethen	Id.	95	150618	0.22
29	9.724	Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-me	Id.	95	150618	0.22
30	9.724	Cyclopentanecarboxylic acid, 2,4-dimethy	Id.	108.1	576359	0.85
31	10.049	Bicyclo[4.4.0]dec-5-ene-1-acetic acid	Id.	135	1.931E+06	2.85
32	10.123	8a-Methyl-5-methylene-3--([(pyridin-2-ylm	Id.	93.2	1.923E+06	2.84
33	10.358	Benzene, 1-[(2-bromophenoxy)methyl]-3,4	Id.	150.9	2.582E+06	3.81
34	10.471	2-(3,4-Dibromo-4-methylcyclohexyl)propan	Id.	94.1	833251	1.23
35	10.626	1-(2,4-Dihydroxybenzoyl)-3-ethyl-5-trifl	Id.	136.9	4.417E+06	6.52
36	10.733	11-Methylene-tricyclo[5.3.1.1(2.6)]dodec	Id.	134.9	197410	0.29
37	10.907	2,4,6-Tris(1-(2-methoxycarbonylpyrrolidi	Id.	135.1	2.413E+06	3.56
38	10.071	Benzenaminium, 3-hydroxy-N,N,N-trimethyl	Id.	150.9	399203	0.59
39	11.163	(2R,4R)-p-Mentha-6,8-diene, 2-hydroperox	Id.	109.1	252787	0.37
40	11.29	O-Trifluoroacetyl-isopulegol	Id.	81	58570	0.09
41	11.463	Benzaldehyde, 4-(1-methylethyl)	Id.	148.9	80573	0.12
42	11.612	cis-p-Mentha-2,8-dien-1-ol	Id.	91.1	50834	0.08
43	11.965	2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-oct	Id.	109.1	34224	0.05

44	12.077	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2	Id.	95	44991	0.07
45	12.19	Benzenemethanol, 4-(1-methylethyl)	Id.	135	76947	0.11
46	12.294	1-Cyclohexene-1-methanol, 4-(1-methleth	Id.	93.2	32175	0.05
47	12.612	Cyclohexanone, 2-(2-butynyl)	Id.	135	6967	0.01
48	12.7	1,4-Cyclohexadiene, 1-methanol, 4-(1-meth	Id.	79.2	85201	0.13
49	12.825	Cyclohexane, 1-ethenyl-1-methyl-2-(1-met	Id.	121.2	960745	1.42
50	12.975	.alpha.-Cubebene	Id.	161	107763	0.16
51	13.363	Copaene	Id.	160.9	665280	0.98
52	13.363	Napthalene, 1,2,3,4,4a,5,6,8a-octahydro	Id.	161.1	665280	0.98
53	13.538	1H-Cycloprop[e]azulene, decahydro-1,1,7	Id.	160.9	207319	0.31
54	13.73	Ethanone, 1-[5-[(5-methy-2-furanyl)meth	Id.	204	2.543E+06	3.75
55	13.954	Napthalene, 1,2,3,4,4a,5,6,8a-octahydro	Id.	161.1	697577	1.03
56	13.954	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0	Id.	161.1	697577	1.03
57	14.073	Napthalene, 1,2,3,4,4a,5,6,8a-octahydro	Id.	161	552719	0.82
58	14.073	Napthalene, 1,2,4a,5,6,8a-hexahydro-4,7	Id.	161	597854	0.88
59	14.427	1R,4S,7S11R-2,2,4,8-Tetramethyltricyclo	Id.	147.1	539724	0.80
60	14.493	1H-3a,7-Methanoazulene, octahydro-1,9,9	Id.	108.2	142040	0.21
61	14.802	.gamma.-Himachalene	Id.	161.3	3.844E+06	5.67
62	14.802	1,4-Methanoazulene, decahydro-4,8,8-trim	Id.	161.1	3.378E+06	4.98
63	15.13	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro	Id.	161.1	19290	0.03
64	15.224	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7	Id.	204	396919	0.59
65	15.367	1H-Cycloprop[e]azulen-7-ol, decahydro-1	Id.	205	38908	0.06
66	15.346	Cyclohexanemethanol, 4-ethenyl-,alpha	Id.	161.1	20664	0.03
67	15.613	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahy	Id.	131.2	61833	0.09
68	15.8	Diepicedrene-1-oxide	Id.	123.2	69034	0.10
69	15.803	Isoaromadendrene epoxide	Id.	39.2	58610	0.09
70	16.122	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bic	Id.	123.2	143090	0.21

71	16.307	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-me	Id.	159.2	46463	0.07
72	16.308	Ledene alcohol	Id.	105.2	63773	0.09
73	16.525	Isoaromadendrene epoxide	Id.	123.2	24191	0.04
74	16.526	1H,4H-Pyrazolo[3,4-b]pyran-5-carbonitril	Id.	203.1	933010	1.38
75	16.686	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-me	Id.	177	16568	0.02
76	16.807	Columbin	Id.	159.2	41956	0.06
77	17.17	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-me	Id.	39.2	32889	0.05
78	17.169	Longipinocarveol, trans-	Id.	159.2	68806	0.10
79	17.311	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahyd	Id.	218	37464	0.06
80	17.516	2-Naphthalenemethanol, 1,2,3,4,4a,8a-hex	Id.	220	8669	0.01
81	17.766	Longipinocarvone	Id.	91.1	8679	0.01
82	17.907	9-Isopropyl-1-methyl-2-methylene-5-oxatr	Id.	91.2	14463	0.02
83	18.437	5,6-Azulenedimethanol, 1,2,3,3a8,8a-hex	Id.	159.2	5440	0.01
84	18.625	3,9-Dimethyltricyclo[4.2.1(2,5)]decan	Id.	81	10680	0.02
85	18.716	Phosphonous dichloride, (1,7,7 trimethyl	Id.	81.2	30663	0.05
86	18.793	2(1H)Naphthalenone, 4a,5,6,7,8,8a-hexah	Id.	191	11570	0.02
87	18.986	1,2-Benzenedicarboxylic acid, butyl octy	Id.	149.2	194102	0.29
88	19.139	Bornyl bromide	Id.	81.2	8064	0.01
89	19.237	Methyl (Z)-5,11,14,17-eicosate	Id.	81	23345	0.03
90	19.236	6-(1-Hydroxymethylvinyl)-4-8a-dimethyl-3-	Id.	93.2	5702	0.01
91	19.966	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a	Id.	203	13363	0.02
92	20.188	5,8,11,14,17 -Eicosapentaenoic acid, meth	Id.	147.1	2269	0.00
93	20.593	1,3,6,10-Cyclotetradecatetraene, 3,7,11	Id.	79.2	5436	0.01
94	20.593	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecah	Id.	257.2	57497	0.08
95	20.881	Kaur-16-ene	Id.	257.2	6977	0.01
96	21.116	Alloaromadendrene oxide-(1)	Id.	41.3	4592	0.01
97	21.116	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1	Id.	41.2	4592	0.01

98	21.281	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a	Id.	203.2	4669	0.01
99	21.436	4,8-Propanoborepino[1,2-b][1,2,5]oxadibo	Id.	203.2	3602	0.01
100	21.658	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-me	Id.	81	6367	0.01
101	22.489	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl	Id.	278.2	73156	0.11
102	22.903	Docosahexaenoic acid, 1,2,3-propanetriyl	Id.	91.2	3201	0.00
103	23.127	5-Benzofuranacetic acid, 6-ethenyl-2,4,5	Id.	205.3	1958	0.00
104	25.658	1,2-Benzenedicarboxylic acid, diisooctyl	Id.	149.3	225495	0.33
105		Unknowns, TIC				

APPENDIX 3a: Statistical Analysis of pH values

pH for samples under ambient temperature

Two-Sample T-Test and CI: Control (Bissap e, Treatment (Spike

Two-sample T for Control (Bissap extract without vs Treatment (Spiked with EO and c

	N	Mean	StDev	SE Mean
Control (Bissap e	5	2.6140	0.0744	0.033
Treatment (Spike	5	2.6140	0.0926	0.041

Difference = mu (Control (Bissap extract without) - mu (Treatment (Spiked with EO and c)
Estimate for difference: 0.000000
95% CI for difference: (-0.125615, 0.125615)
T-Test of difference = 0 (vs not =): T-Value = 0.00 P-Value = 1.000 DF = 7

pH reading of bissap samples under refrigerated temperature

Two-Sample T-Test and CI: Control (Bissap e, Treatment (Spike

Two-sample T for Control (Bissap extract withou_1 vs Treatment (Spiked with EO and_1

	N	Mean	StDev	SE Mean
Control (Bissap e	5	2.6360	0.0945	0.042
Treatment (Spike	5	2.6300	0.0883	0.039

Difference = mu (Control (Bissap extract withou_1) - mu (Treatment (Spiked with EO and_1)
Estimate for difference: 0.006000
95% CI for difference: (-0.130781, 0.142781)
T-Test of difference = 0 (vs not =): T-Value = 0.10 P-Value = 0.920 DF = 7

Two-Sample T-Test and CI: Control (Ice ken, Treatment (Ice k

Two-sample T for Control (Ice kenkey without EO vs Treatment (Ice kenkey spiked wi

	N	Mean	StDev	SE Mean
Control (Ice ken	5	3.792	0.417	0.19
Treatment (Ice k	5	3.876	0.247	0.11

Ice kenkey samples under ambient temperature

Difference = mu (Control (Ice kenkey without EO) - mu (Treatment (Ice kenkey spiked wi)
Estimate for difference: -0.084000
95% CI for difference: (-0.613970, 0.445970)
T-Test of difference = 0 (vs not =): T-Value = -0.39 P-Value = 0.712 DF = 6

Ice kenkey samples under refrigerated temperature

Two-Sample T-Test and CI: Control (Ice ken, Treatment (Ice k

Two-sample T for Control (Ice kenkey without EO vs Treatment (Ice kenkey
spiked _1

	N	Mean	StDev	SE Mean
Control (Ice ken	5	4.1820	0.0915	0.041
Treatment (Ice k	5	4.1940	0.0950	0.042

Difference = mu (Control (Ice kenkey without EO) - mu (Treatment (Ice kenkey
spiked _1)
Estimate for difference: -0.012000
95% CI for difference: (-0.151493, 0.127493)
T-Test of difference = 0 (vs not =): T-Value = -0.20 P-Value = 0.845 DF = 7

APPENDIX 3b: One Way Anova Table (Between organisms)

ANOVA Table (S. aureus, E. coli, S. Typhimurium) Vs Mean zone of inhibition

Mean zone of inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.009	2	30.005	1.012	.381
Within Groups	622.583	21	29.647		
Total	682.593	23			

Multiple Comparisons

Dependent Variable: Mean zone of inhibition

Tukey HSD

(I) Organism	(J) Organism	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Escherichia coli	Salmonella Typhimurium	-3.33333	2.72244	.452	-10.1954	3.5288
	Staphylococcus aureus	.04167	2.72244	1.000	-6.8204	6.9038
Salmonella Typhimurium	Escherichia coli	3.33333	2.72244	.452	-3.5288	10.1954
	Staphylococcus aureus	3.37500	2.72244	.444	-3.4871	10.2371
Staphylococcus aureus	Escherichia coli	-.04167	2.72244	1.000	-6.9038	6.8204
	Salmonella Typhimurium	-3.37500	2.72244	.444	-10.2371	3.4871

APPENDIX 3c: One Way Anova Table (Between Different concetrations)

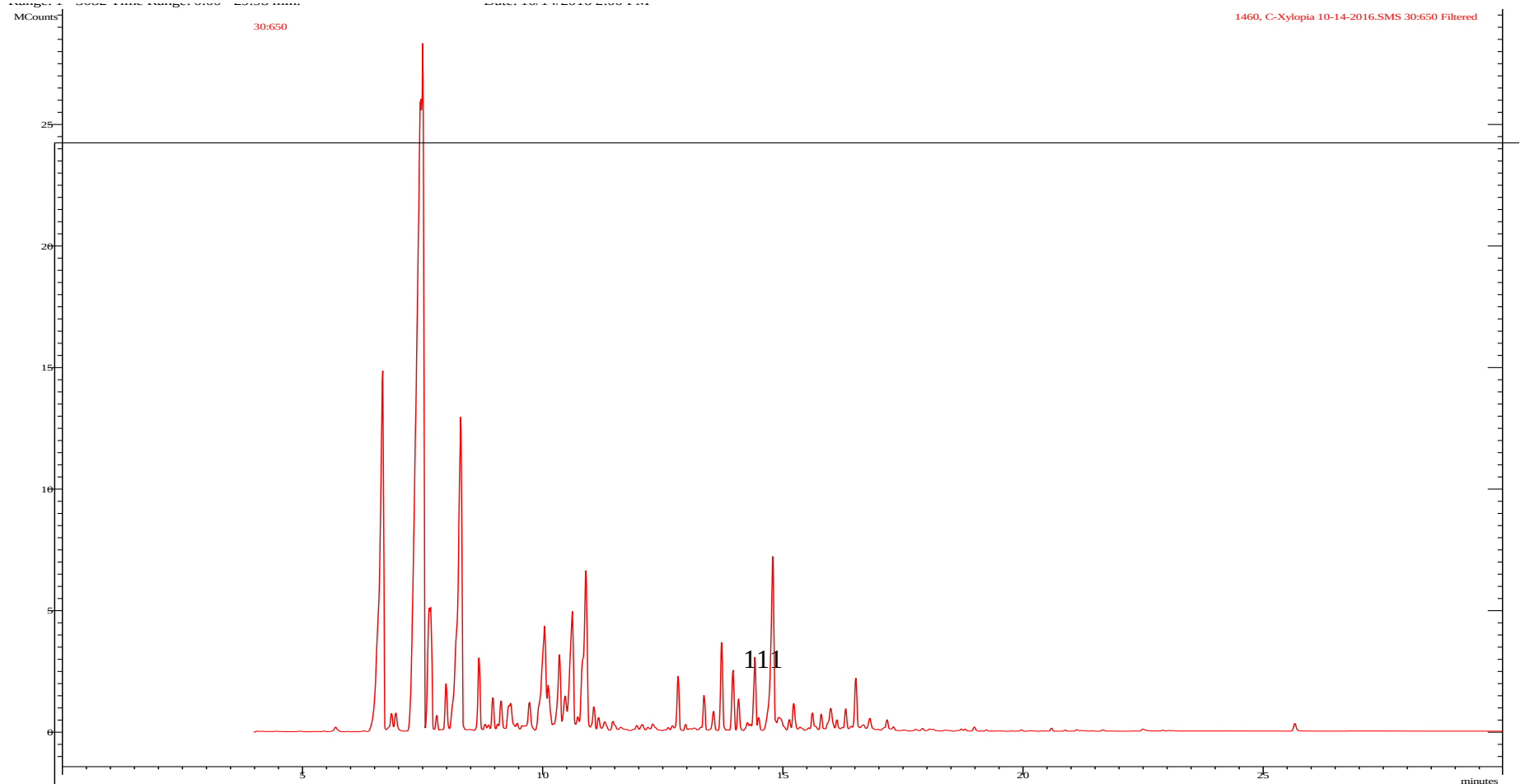
ANOVA Table (Different concentration of X. aethiopica) Vs Mean Zone of inhibition

Mean zone of inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	565.556	7	80.794	11.045	.000

Within Groups	117.037	16	7.315		
Total	682.593	23			

APPENDIX 4: GC-MS Chromatograph or spectrum of all chemical compounds in X. aethiopica essential oils



APPENDIX 5: Spice Retailer, Food Vendors and Herbalist Questionnaire on spice usage

RESPONDENT ID: ……

QUESTIONNAIRE TO EXPLORE RESPONDENTS' KNOWLEDGE ON SPICES, THEIR ORIGIN AND PLACE OF CULTIVATION

Dear Respondent,

The researcher is a Food Science student of the Department of Nutrition and Food Science, University of Ghana, Legon. This questionnaire seeks to collect data on the "**Knowledge, on indigenous Ghanaian spices, their origin, place of cultivation, and perceived benefits and usage in food and herbal medicine**" towards his/her Master of Philosophy degree in Food Science. It is a subsidiary of the **SPICE ESSENTIAL OIL PROJECT** currently ongoing in the department. Thank you in advance for your candid opinion on the subject matter. Be assured of the confidentiality and anonymity for all the responses you will provide. I count on your co-operation.

Please direct any enquiry to:

Dzesi Kwame Torku

Department of Nutrition and Food Science.

University of Ghana, Legon

Mob: 0245673185

Email: dktorku@gmail.com

A. RESPONDENT DETAILS [DEMOGRAPHY]

Please circle answers where applicable for the following questions: ◯

1. Sex a. Male b. Female

2. Age: ……..

3. Highest educational status a. None b. Primary c. JSS/ JHS d. Middle School e. SHS f. Vocational school g. Polytechnic h. University

4. Occupation a. Spice retailer b. Herbalist c. Food vendor d. Other Specify ……

5. Do you have any alternative occupation? a. Yes b. No

6. How long have you been trading in spices or using spices to cure ailments or cooking a. 0 – 2 years b. 2 – 4 years c. 4 – 6 years d. 6 – 8 years e. Above 8 years

B. IDENTIFICATION OF SPICES

You have been provided with an album containing pictures of different spices? Kindly mention the ones you know in any language? There is no wrong or right answer. Kindly

suggest or mention the names of spices you know, but are not included in the photo album or list.

***Insert pictures of spices as documented on the checklist**

The surveyor should enter a response on the A3 sheet provided. However, spaces have been provided on the questionnaire for some responses to be recorded.

C. INDIGENOUS GHANAIAN SPICES: Talking about the commonly grown spices (commonly sold spices) in Ghana.

General description.

1. How will you describe the spice you mentioned to a novice, in order to help them identify such a spice? Give a brief description; shape, colour, parts of the plant; leave, root, stem etc.

Origin

1. Where are these spices cultivated? Provide names of villages, town, districts or regions.
2. Do you know of any spices grown around Obuasi, Sikamang, Mensonso, Brofoyedru [Ashanti Region] or Mim-Goaso, [Brong Ahafo]

……

Availability

1. Where can large quantities of the spices be purchased?
2. How available are the spices? [all-year-round, seasonal, readily available, weekly, monthly]
3. Do you know of any spices that used to be common, but now very scarce?
    i. Can you mention the name(s) of such a spice?

……

    ii. Have you heard of Nsensam, semanini, adowa wisa before?

……

The surveyor should enter a responses on the A3 sheet provided.

D. Usage, parts of spice used and cost of spices

1. Do you know any group of people, e.g. ethnic, association, or family that takes delight in the usage of particular spices? If yes, name the group, and where they can be found
2. What is unique about the spice? (flavour, taste, aroma, medicinal value)

Packaging and Cost of the spices

3. Do the spices come packaged? What form of packaging?

4. Do you have a rough idea about the cost of the spice per package, or unit of measurement? E.g. cost per bowl, cost per sack, cost per unit?
5. Do you know how some of the mentioned spices are processed or handled from the farm to the market or consumer? E.g. sun drying, open air drying
6. How is the spice presented for use? (dry, fresh, powdered, grits, mixes, liquid)

Usage

7. Mention some of the uses of the spices mentioned?
8. Do you know of any nutritional value or perceived medicinal value? Mention spices with such properties
9. What are some perceived benefits of spices?
   ……
   ……
   ……
10. Sort the commonly known spices we have been discussing based on their perceived benefits
    ……
    ……
    ……
11. Can spices be used to mask off flavours/taste in decaying foods?
12. What are some negative effects of spices used in foods?
13. Which spices produce some negative effects when used in food?

Other relevant comments not outlined:

……

……

……

……

……

……

……

……

……

……

……

Name of spice	**Botanical name**	**Common local name**	**Another local name (respondents mentioned)**	**Commonly grown in Ghana (YES/NO)**	**Commonly sold /used in Ghana (YES/NO)**	**Remark**
Aniseed	*Pimpenella anisum*	Nketekete				
Black pepper	*Piper guineesis*	soro wisa				
Calabash nutmeg	*Monodora myristica*	Awedeaba				
Galbanum	*Tertrapleura tetraptera*	Prekese				
Ginger	*Zingiber officenalis*	Akakaduro				
Negro pepper	*Xylopia aethiopia*	Hwintia				
White pepper	*Piper nigrum*	Famu wisa				
Cinnamon	*Cinnamomum zeylanicum*					
Clove	*Eugenia caryophylata*	Pepre				
Chili pepper	*Capsicum frutescens*					
Caper	*Capparis spinosa*					
Sage	*Salvia officinalis*					
Rosemary	*Rosmarinus officinalis*					
Corianda	*Coriandrum sativum*					
Thyme	*Thymus vulgaris*					
Oregano	*Origanum vulgare*					
Cumin	*Cuminum cyminum*					
Vanilla	*Vanilla planifolia*					
Parsley	*Petroselinum sativum*					
Basil	*Ocimum basilicum*					
Adowa wisa	*Afromomum hanburyi*					
Nsensam	*Afromomum geocarpum*					
Semanini	*Costus afer*					