EFFECT OF PROCESSING AND STORAGE ON FLAVOUR RETENTION OF*Xylopia aethiopica* AND *Curcuma longa*

BY

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B.Tech, MSc (FUTO)

REG. NO. 20114910638

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY (**PhD**) DEGREE IN FOOD SCIENCE AND TECHNOLOGY (FOOD CHEMISTRY AND NUTRITION OPTION)

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July 2018

CERTIFICATION

I certify that this work "Effect of Processing and Storage on the Retention of Flavour in *Xylopia aethiopica* and *Curcuma longa* Spice Samples" was carried out by **Ibe Chidiuto**. (Reg. No. 20114910638) in partial fulfillment of the requirement for the award of degree of Doctor of Philosophy (PhD) in Food Science and Technology (Food Chemistry and Nutrition Option) in the Department of Food Science and Technology of the Federal University of Technology, Owerri.

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DEDICATION

This thesis is mostly dedicated to God for his mercies, guidance and protection. And to my entire family and in loving memory of my beloved father, Mr C.M. Ibe.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

The main objective was to determine the effect of processing and storage on flavour retention of *Xylopia aethiopica* and *Curcuma longa*. Spice products were processed into powders and six different solvents (absolute ethanol 98.99%, 40% ethanol, methanol, acetone, n-Hexane and water) were used to extract the bioactive compounds in the spices. The spices were separately processed into six different spice products that included spice powder, liquid spice extract and four encapsulated spice samples with wheat and yam grits as carriers at the ratios of 1:2 and 1:3 spice extract to carrier.They were packaged in amber coloured plastic bottles and stored at ambient temperature (28 ± 3^0C) for six months. The essential oils obtained by different solvent extractions from spiceswere analysed by gas chromatography-mass spectrometry (GC-MS) while the curcumin content of the *C. longa* for the different solvent extracts were analysed with Ultra-fast liquid chromatography (UFLC). Microbiological analysis were conducted on the fresh and dried ground spices for initial microbial load determination. Sensory evaluation were also carried out on the spice samples. The results of phytochemicals on the two spices identified the presence of alkaloids, flavonoids, phytic acid, saponins, tannins and oxalates. Different compounds were obtained from the different solvent extracts of *X. aethiopica* but the most predominant common flavour compounds found were two monoterpenes and one diterpene which included beta-pinene (15.48% in absolute ethanol, 20.28% in 40% ethanol, 8.11% in acetone, 14.03% in methanol but not detected in n-Hexane and water extracts), beta-phellandrene (18.93% in absolute ethanol, 23.93% in 40% ethanol, 21.54% in methanol, 7.38% in acetone, 12.09% in n-Hexane and 4.29% in water) and andrographolide (9.66% in absolute ethanol, 8.06% in 40% ethanol, 7.67% in methanol, 7.29% in acetone, 4.20% in n-Hexane and was not detected in the water extract). The major compounds identified by GC-MS analysis of *C. longa* were sesquiterpenes (40.55%), fatty acids (27.16%), diarylheptanoid (12.02%) and steroids (4.35%). The following curcumin concentrations were obtained in the different solvents extracts; absolute ethanol (53.27%), 40% ethanol (10.74%), methanol (12.46%), acetone (14.15%), n-Hexane (3.77%) and water (2.29%). At the end of the 6 months ambient storage of *X. aethiopica* spice products, the 40% ethanol extract had the highest concentration of beta-phellandrene with 19.56%, the concentration of beta-phellandrene was significantly ($p<0.05$) higher when in wheat grit carrier (13.54%) at ratio 1:2 after 6 months compared to when it was in yam grit carrier (9.77%) at ratio 1:2. However, the 1:2 ratio retained more than the 1:3 ratio in both carriers. But in *C. longa* spice products, at the 6^{th} month of storage the spice powder had the most concentration of Curcumin with 39.67%, the yam grits carrier retained more than the wheat grits while the 1:2 ratio retained more than 1:3 ratio in both carriers. The total fungal counts for *C. longa* ranged from 2.0×10^5cfu/g in the ground sample to 3.2×10^8cfu/g in the raw sample and 2.20×10^5cfu/g in the ground *X. aethiopica* sample to 5.1×10^8cfu/g in the raw sample. The common microflora of the spices were; *Saccharomyces cerevisiae*, *Enterococcus*, *Bacillus*, *Lactobacillus* and *Streptococcus* for *C. longa* and *Saccharomyces cerevisiae*, *Enterococcus*, *Penicilliumnotatum*, *Bacillus*, *Lactobacillus, Staphylococcus* and *E. coli* for *X. aethiopica* The scores of the sensory quality attributes of the spices showed that the 40% ethanol extract was the most acceptable sample. The sample was recommended for a greater number of different foods and drinks such as rice, meat, sauce, tea, akamu/pap, baked products, yoghurt, alcoholic and non-alcoholic beverages for *C. longa* and meat, porridge, pepper soup, Igbo soup, Yoruba soup, Hausa soup and alcoholic beverages for *X. aethiopica.*

Keywords: *Curcumalonga*, encapsulation,flavour principle,grits,liquid flavour extract, microbial load, sensory evaluation, *Xylopia aethiopica*.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Spices are dried plant parts such as seeds, fruits, roots, barks or leaves used for flavouring, colouring or preserving food (Iwu, 1993; Rakesh *et al*., 2012). The spice market is directly influenced by the growing needs of the processed-food industry. The rise in consumption of bakery products, confectionary products, ready-to-eat and fried foods in developed and developing world is driving the market for spices. The global spice market grew from USD 10.7 billion in 2010 to USD 12.5 billion in 2013. The market is further estimated to reach USD 16.6 billion in 2019 (Spice market, 2013).

Curcuma longa (Turmeric) and *Xylopia aethiopica* (African Guinea-pepper) as spices have long been used by ancient civilization for culinary, medicinal and cosmetic purposes. They are used for flavouring, colouring and preserving foods as well as to hide or mask other flavours and also possess antimicrobial properties (Iwu, 1993; Rakesh *et al.*, 2012). These spices are popular among Nigerians, although they are majorly grown in the wild (Adelaja *et al*., 2008).The recognition of the unique flavour and colour qualities of *X. aethiopica* and *C. longa*, along with other native foods, has increased in recent times thus necessitating the need to fully characterize their flavours.

Spice oils and spice oleoresins are indispensable in the food and beverage manufacturing industries, the perfumery, the cosmetic industry and the pharmaceutical industry. Some spices and their derivatives possess antioxidant and antimicrobial properties (Iwu, 1993. This fact has increased interest in the commercial exploitation of aromatic plants for food preservation and crop protection. With the growing demand of natural and organic products and the increasing clamor to dispense with synthetic flavours and artificial food colouring, the future for spices especially *X. aethiopica* and *C. longa* look bright.

The food industry across the globe is turning more and more to spice oils and oleoresins to create newer varieties of food. New flavour systems are being developed to introduce new products in the market and create competitive advantages. The India spice oils and oleoresin industry is engaged in continuous researching, innovation and upgrading of process and product to meet the

new global demands (Spice market, 2013). The world demand for "organic" foods is growing rapidly in Europe, USA, Japan, Australia and even Africa. Therefore, food professionals are continually searching for new and unique spice flavours to meet up with the growing global demand for authentic ethnic and cross-cultural cuisines. Consumers are also seeking for foods with natural preservatives for healthy lifestyle (Raw Materials Research and Development Council [RMRDC], 2013).

1.2 PROBLEM STATEMENT

The major form of trading indigenous spices such as *X. aethiopica* and *C. longa* in local market places is incompletely packaged whole spices. The local alternative form, "ground spice" lack appropriate packaging with respect to insect infestation, moisture penetration and flavour protection, yet spices are known to lose the colour, taste and aroma over time after harvest. Besides, the flavour principles have to be identified and the appropriate processing, packaging and storage techniques investigated/developed for intended quality/flavour retention. These information are lacking for these two spices; consequently these spices have not been optimally utilized in possible industrial products beyond the cultural soups and stews (culinary products).

1.3 OBJECTIVES OF STUDY

The main objective of this study was to determine the effect of processing and storage on flavour retention of *X. aethiopica* and *C. longa*.

The specific objectives are:

i. to produce different guinea-pepper and turmeric spice samples by grinding, liquid extraction and encapsulation.
ii. to package and store the samples for a period of 24 weeks.
iii. to identify the predominant flavour principles in the spices samples.
iv. to evaluate the microbial load on the spices.
v. to conduct sensory evaluation of the spice samples.
vi. to determine the appropriate food for the utilization of each spice or their blend.

1.4 JUSTIFICATION OF STUDY

The findings from this work have helped in the recommendation of the appropriate processing, packaging and storage techniques that enhanced the retention of flavour and colour in *X. aethiopica* and *C. longa*. The flavour principles in the spices have been revealed. It has also opened investment opportunities for cottage/ancillary industries and has renewed interest on these spices as raw materials for production purposes. The findings and application of the recommendations have not only helped in reducing the almost total dependence on imported spice products but have encouraged export market for these spices. This research has also revealed the enormous potentials for local utilization of these spices which have promoted the domestication of *X. aethiopica* and *C. longa* for improved economic development. The findings have aided the penetration and market share of *X. aethiopica* and *C. longa* to international market with the current growing global demand for authentic ethnic and cross-cultural cuisines.

1.5 SCOPE OF STUDY

This work has identified the flavour principles of the two spices and accessed their levels. The processing and packaging methods that guarantee quality and flavour retention have been investigated. Flavour retention has been evaluated in terms of intensity perceived by a random sensory panel who were familiar with the spices, and so have been the appropriate foods for utilization. The micro flora of the spices have been investigated.

CHAPTER TWO

LITERATURE REVIEW

2.1 SPICE OVERVIEW

Spices are grown in diverse geographical regions and climatic conditions. They can be collected from the wild or cultivated in schemes that range from home gardens to monoculture plantations. Nigeria has several indigenous spice resources namely: *X. aethiopica, C. longa, Aframonum longiscarpum, Mondora myristica* among others (Adelaja *et al.,* 2008). Nigerian farmers cultivate spices such as curry (*Muurraya koenigii*), sweet basil (*Ocimum basilicum),* bush tea (*Ocimum gratissimum),* ginger (*Zingiber officinale), X. aethiopica),* African black pepper (*Piper guineense*) and *C. longa*. Ginger and African black pepper are the major Nigerian spices in international market, the rest are consumed locally (RMRDC, 2013).

Spices may be derived from many parts of the plant: bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the entire plant tops. Spices are available in several forms: fresh, whole dried, or pre-ground dried, but generally spices are dried. Plants used as spices and condiments are usually aromatic and pungent (Achinewhu *et al*., 1995). The flavour of a spice is derived in part from compounds that oxidize or evaporate when exposed to air. Processed spices lose flavour more easily than whole ones. This is because spices that have been cut or ground into powder have more surface area exposed to air and so lose flavour more rapidly than the whole spices, therefore, all processed spices have short shelf life.

It is important to acquire knowledge of the chemicals responsible for the characteristic odours, colours, flavours and medicinal properties of spices.

It is widely accepted that spices, fruits and vegetables have many health promoting properties. There are considerable amount of epidemiological evidences revealing an association between those who have a diet rich in fresh fruits, vegetables, spices, and decreased risk of cardiovascular diseases and certain forms of cancer (Salah *et al.*, 1995). The constituents of these spices, fruits and vegetables that contribute to these protective effects are phytochemicals, vitamins and minerals (Okwu, 2003). Phytochemicals as antioxidants play vital roles in human health. Naturally occurring phytochemicals like flavonoids are potentially anti-allergic, anti-carcinogenic, anti-viral and antioxidant agents (Close and McArthur, 2002).

2.2 DESCRIPTION OF *Curcuma longa*

C. longa is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae (Chan, 2009). It is native to tropical South Asia but is now widely cultivated in the tropical and sub-tropical regions of the world. In the South Eastern part of Nigeria, it is known as "ede-ala". It is commonly known as "Ajo", "laali pupa" or "obedo" by the Yorubas (Iwu, 1993). Its Commercial name is Indian saffron or Turmeric. The name appears to be derived from the latin words, terra merita (merited earth) or turmeryte, possibly related to saffron (Royal Botanical Gardens [RBG], 2012).

C. longa grows to a height of 3 to 5 feet with orange-red blossoms resembling lilies (plate 2.1 – 2.1.1 and 2.2). The flowering time is usually in August (Chempakam and Parthasarathy, 2008). Although its flowers are stunning, its rhizome (underground stem) is what attracts the most attention.

India is the largest producer of *C. longa* supplying over 90% of the world's demand (Olojede *et al*., 2009). The country produced about 716,900 metric tonnes (mt) of *C. longa* from approximately 161,300 hectares of crops during 2004 – 2005 (Chempakam and Parthasarathy, 2008). Other producers in Asia include Bangladesh, Pakistan, Sri Lanka, Taiwan, Burma (Myanmar), and Indonesia. *C. longa* is also produced in commercial quantities in the Caribbean and Latin America; Jamaica, Haiti, Costa Rica, Peru, and Brazil (Dahal and Idris, 1999; Weiss, 2002).

Rhizomes come as fingers, bulbs and splits. Fingers are the secondary branches from the mother rhizome while the bulb, and splits are the bulbs cut into halves or quarters before curing (Surwase *et al.*, 2011; Tainter and Grenis, 2001). The fingers are 2 to 8 cm long and 1 to 2 cm wide, and are easier to grind than the more fibrous bulbs and splits, and thus command a higher price (American Spice Trade Association [ASTA], 2002). Rhizome quality is judged by a clean and smooth skin, uniform skin and flesh colours, and a clean snap (or "metallic twang" as described by the Indian Ministry of Agriculture standards, Agmark) when broken (Weiss, 2002). *C. longa* cleanliness specifications for import pertain to whole rhizomes (ASTA, 2000).

Plate 2.1 Plate 2.1.1

Plates 2.1 – 2.1.1: *C. longa* (FAO, 2004).

Plate 2.2: *C. longa* plantation (FAO, 2004).

When not used fresh, the rhizomes are boiled for several hours and then dried in hot oven, after which they are ground into a deep orange-yellow powder (plate 2.3) commonly used as a component of curry and other South Asian and Middle Eastern cuisine, for dyeing and to impart colour to mustard condiments. Its active ingredient is Curcumin and it has a distinctly earthy, slightly bitter, slightly hot peppery flavour and a mustardy smell (National Institute of Health [NIH], 2012). *C. longa* is mostly traded as a whole rhizome, which is then processed into powder or oleoresin by flavour houses and the industrial sector (ASTA, 2002; Surwase *et al.*, 2011).

Plate 2.3: *C. longa* powder (FAO, 2004).

2.3 VARIETIES OF *C. longa*

There are about 70 cultivars or varieties of *C. longa* cultivated in India. Some important regional trade varieties of *C. longa* are; Madras, Rajapuri, Duggirala, Cuddappah, Berham pur, Bengal, Erode, Nizamabad, Koraput, Kasturi, Chaya, Kodur, Salem, Waigon, Alleppey, Karur, Tekurpeta (Sasikumar, 2015). There are two dominant types of *C. longa* found in the world market: 'Madras', and 'Alleppey', both named after the regions of production in India. The orange-yellow flesh Alleppey *C. longa* is predominantly imported by the United States, where users prefer it as a spice and a food colourant (ASTA, 2002). Alleppey *C. longa* contains about 3.5% to 5.5% volatile oils, and 4.0% to 7.0% curcumin (ASTA, 2002; Buescher and Yang, 2000; Weiss, 2002). In contrast, the Madras type contains only 2% of volatile oils and 2% of curcumin (ASTA, 2002). The Madras *C. longa* is preferred by the British and Middle Eastern markets for its more intense, brighter and lighter yellow colour, better suited for the mustard paste and curry powder or paste used in oriental dishes (ASTA, 2002; Surwase *et al.*, 2011). *C. longa* produced in the Caribbean, Central and South America has low curcumin and volatile oil contents, and is darker (Surwase *et al.*, 2011; Tainter and Grenis, 2001). It is not desired by the U.S. importers (ASTA, 2002). The Bengal type is preferred for use in dyes in India (Dahal and Idris, 1999). It is interesting to note that in the United States, *C. longa* is considered as a spice by the food industry, whereas it is classified as a food colourant by the FDA (Tainter and Grenis, 2001).

In medieval Europe, *C. longa* became known as Indian saffron since it was widely used as an alternative to the far more expensive saffron spice. It has regional names based on language and country.

2.4 POST-HARVEST MANAGEMENT OF *C. longa*

2.4.1 Harvest

C. longa readiness for harvest is indicated by the drying of the plant and stem, approximately 7 to 10 months after planting, depending on cultivar, soil and growing conditions. The rhizome bunches are carefully dug out manually with a spade, or the soil is first loosen with a small digger, and clumps manually lifted. It is better to cut the leaves before lifting the rhizomes. Rhizomes are cleaned from adhering soil by soaking in water, and long roots are removed as

well as leaf scales. Rhizomes are then further cured and processed, or stored for the next year's planting (Anandaraj *et al.*, 2001; Dahal and Idris, 1999; Surwase *et al.*, 2011; Weiss, 2002).

Rhizomes for seed purposes must be stored in well-ventilated rooms to minimize rot, but covered with the plant dry leaves to prevent dehydration (Surwase *et al.*, 2011). They can also be stored in pits covered with sawdust, sand, or panal (*Glycosmis pentaphylla*) leaves that may act as insect repellent (Anandaraj *et al.*, 2001). The Indian Institute of Spice Research recommends the following fungicides as a pre-storage dip treatment for rhizome seeds: quinalphos at 0.075%, and mancozeb at 0.3% (Anandaraj *et al.*, 2001). Studies indicate that bulbs (mother rhizomes) are preferred to fingers as a seed stock (Surwase *et al.*, 2011).

2.4.2 Post-Harvest Handling: Curing, Drying and Polishing

C. longa rhizomes are cured before drying. Curing involves boiling the rhizomes until they are soft. It is performed to gelatinize the starch for a more uniform drying, and to remove the fresh earthy odour (Surwase *et al.*, 2011; Weiss, 2002). During this process, the colouring material is diffused uniformly through the rhizome. Recommendations as to the acidity or alkalinity of the boiling water vary by author. The Indian Institute of Spice Research, Calicut, Kerala, and the Agricultural Technology Information Center simply recommend boiling in water for 45 min to one hour, until froth appears at the surface and the typical *C. longa* aroma is released (Anandaraj *et al.*, 2001). They reported that colour deteriorates as a result of over-cooking, but that the rhizome becomes brittle when undercooked.

Optimum cooking is attained when the rhizome yields to finger pressure and can be perforated by a blunt piece of wood (Surwase *et al.*, 2011; Weiss, 2002). Boiling in alkaline water by adding 0.05% to 1% sodium carbonate, or lime, may improve the colour (Surwase *et al.*, 2011; Weiss, 2002). For the curing process, it is important to boil batches of rhizomes that are equal in size since different materials with different sizes would require different cooking times. Practically, fingers and bulbs are cured in separate batches, and bulbs are cut in halves. Cooking may vary from one to four or six hours, depending on the batch size (Surwase *et al.*, 2011). Curing should be done two or three days after harvest, and should not be delayed to avoid spoilage of the rhizome (Anandaraj *et al.*, 2001). Benefits of curing *C. longa* include reduction of the drying time, and a more attractive product (not wrinkled) that lends itself to easier polishing (Surwase *et al.*, 2011). The curing by boiling process has the advantage of sterilizing the

rhizomes before drying. Cooked fingers or bulbs are dried to a moisture level of 5% to 10%. Sun drying may take 10 to 15 days, and the rhizomes should be spread in 5-7 cm thick layers to minimize direct sunlight that results in surface discolouration (Anandaraj *et al.*, 2001). *C. longa* is one of the spices for which it is more advantageous to use mechanical driers because of the sensitivity to light. These can be drums, trays, or continuous parallel or cross-flow hot air tunnels (Weiss, 2002). Like with ginger rhizomes, the optimum drying temperature is 60 ^{o}C. Dried fingers are polished to remove scales and rootlets from the rhizomes by using rotating drums lined with a metallic mesh that abrades the rhizome's surface (Anandaraj *et al.*, 2001). *C. longa* powder suspended in water is sprinkled over the rhizomes at the final stage of polishing to give an attractive colour (Anandaraj *et al.*, 2001).

2.4.3 Grading, Packing and Storage

Quality specifications are imposed by the importing country, and pertain to cleanliness rather than quality of the spice. Proper care must be taken to meet minimum requirements, otherwise a lot may be rejected and need further cleaning and/or disinfection with ethylene oxide or irradiation. Bulk rhizomes are usually graded into fingers, bulbs and splits (ASTA, 2002).

2.4.4 Grinding and Milling

Grinding is a simple process involving cutting and crushing the rhizomes into small particles, then sifting through a series of screens. Depending on the type of mill, and the speed of crushing, the spice may heat up and volatiles may be lost. In the case of *C. longa*, heat and oxygen during the process may contribute to curcumin degradation. Cryogenic milling under liquid nitrogen prevents oxidation and volatile loss, but it is expensive and not widespread in the industry (Tainter and Grenis, 2001). Ground spices are size sorted through screens, and the larger particles can be further ground.

2.4.5 Extraction: Oleoresin Production

Since curcumin is the compound of interest in *C. longa* rhizome, it is important to know the solubility of curcumin in different solvents in order to choose the appropriate solvent. Curcumin is soluble in polar solvents (acetone, ethyl acetate, methanol, ethanol), and quite insoluble in non-polar solvents such as hexane, and insoluble in water (Verghese, 1993). Dried powdered

rhizomes are extracted by percolation with the polar solvent. The particle size, uniform packing in the extractor, temperature and percolation rate of the solvent are all important parameters for optimum extraction (Surwase *et al.*, 2011). If the oleoresin is the desired product, the solvent is completely evaporated by distillation at 45-55 °C (Weiss, 2002). If curcumin is the final product, the solvent is only partially removed, and the colour material is separated from the solvent by freezing, then centrifugation or vacuum-filtration (Verghese, 1993). At this stage, curcumin is further purified with a wash with hexane. Hexane will extract all the gummy matter, oils, fats, and volatile essential oils that would otherwise impart on *C. longa* flavour. The yield of curcumin from dried *C. longa* root is about 5% (ASTA, 2002). Oleoresin composition will vary greatly with the type of solvent, temperature and extraction methods, in addition to the effect due to quality of the raw material (Beuscher and Yang, 2000). The yield of oleoresin from dried root is typically in the range of 10-12% (ASTA, 2002; Beuscher and Yang, 2000; Surwase *et al.*, 2011).

2.5 CHEMICAL COMPOSITION OF *C. longa*

C. longa contains up to 5% essential oils and up to 5% curcumin, a polyphenol (Tahira, 2010). Curcumin (figure 2.1) is the active substance of *C. longa*. Phytochemical investigations of the plants revealed the presence of curcumin, demethoxycurcumin (figure 2.2) and bis-demethoxycurcumin (figure 2.3) (Wuthi-Udolmlert *et al.*, 2000). Tumerone and carvacrol have been reported as the most abundant constituents of rhizome essential oil of yellow and red varieties of Bangladesh grown *C. longa* (Chowdhury *et al.*, 2008). Usman *et al.* (2009) have identified "-phellandrene and terpinolene" as the predominant constituents of leaf oil of South – West Nigerian grown *C. longa*. Earlier work on rhizome essential oil of South – West Nigerian plant also revealed the presence of ar-turmerone as the most abundant constituent (Ajaiyeoba *et al.*, 2008). It has been established that composition pattern of essential oil could be affected by the geographical and climatic conditions (Lahlou, 2004).

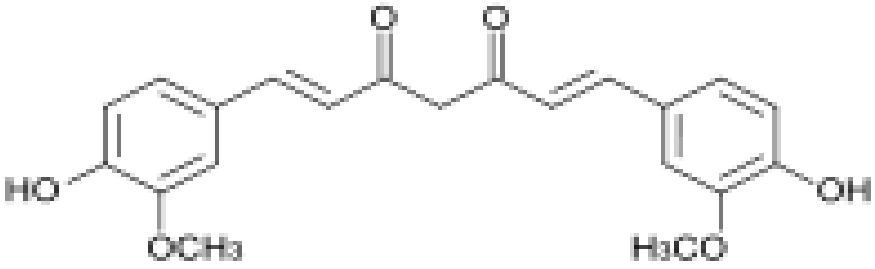
Curcumin can exist at least in two tautomeric forms, keto and enol. The keto form is preferred in solid phase and the enol form in solution.

In acidic and neutral solutions as well as in the solid state, the keto form predominates, and curcumin acts as a potent donor of H-atoms. In contrast, under alkaline conditions (≥pH 8), the

enolic form predominates, and the phenolic part of the molecule plays the principal role as an electron donor (Jovanovic *et al.*, 1999). Curcumin is a pH indicator, in acidic solutions (pH < 7.4) it turns yellow, whereas in basic (pH > 8.6) solutions it turns bright red.

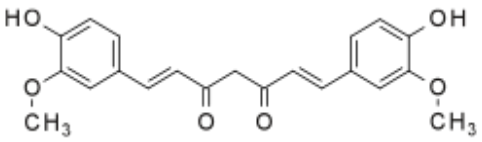
Curcumin, with a molecular formula of $C_{21}H_{20}O_6$ has a molar mass of 368.38g, a melting point of 183^{o}C, is bright yellow to orange in colour (Gregory *et al.*, 2008) while Bis-demethoxycurcumin with a molecular formula of $C_{19}H_{16}O_4$ has a molar mass of 308.33g with a melting point of 222^{o}C and yellow colour. On the other hand, Demethoxycurcumin has a molecular formular of $C_{20}H_{18}O_5$, a melting point of 172^{o}C and also a yellow colour.

Curcumin

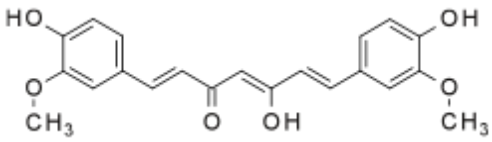


IUPAC name: (1*E*,6*E*)-1,7-bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione.

Other names: curcumindiferuloylmethane, C.I. 75300, Natural Yellow 3



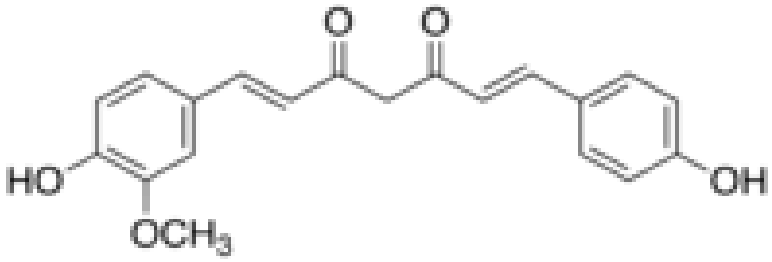
Curcumin keto form



Curcumin enol form

Figure 2.1. Curcumin and its derivatives (Sotanaphun *et al.*, 2009).

Demethoxycurcumin

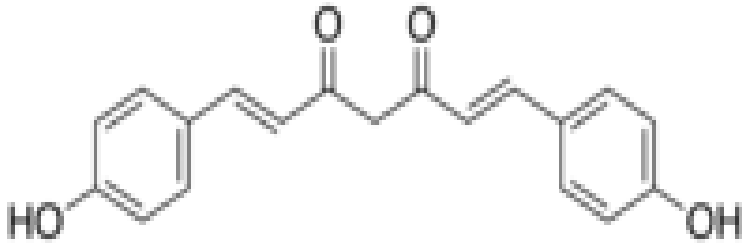


IUPAC name: (1*E*,6*E*)-1,6-Heptadiene-3,5-dione, 1-(4-hydroxy-3-methoxyphenyl) -7-(4-hydroxyphenyl)

Other names: 4-hydroxycinnamoyl(feroyl) methane, curcumin II, BHCFM

Figure 2.2. The structure of Demethoxycurcumin (Sotanaphun *et al.*, 2009).

Bis-demethoxycurcumin



IUPAC name: (1*E*,6*E*)-1,7-bis (4-hydroxyphenyl) hepta-1,6-diene- 3,5-dione

Other names: Bis(4-hydroxycinnamoyl)methane, BHCMT

Figure 2.3. The structure of Bis-demethoxycurcumin (Sotanaphun *et al.*, 2009).

Curcumin is a main colouring substance in *C. longa* and the two related compounds, demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) are altogether known as curcuminoid. The principal colouring component of curcumin exhibit a keto-enol tautomerism and antioxidant properties (Wuthi-Udolmlert *et al.*, 2000).

Curcumin and the two curcuminoids; demethoxycurcumin and bis-demethoxycurcumin are responsible for the yellow colour of *C. longa* (Surwase *et al.*, 2011). Pure Curcumin is a yellow-orange crystalline powder, insoluble in water and ether but soluble in low chain alcohols such as ethanol and methanol, acetone, acetic acid and dichloromethane. The extraction yield of oleoresin from *C. longa* rhizomes using organic solvents such as ethanol, acetone and dichloromethane, lies between 5 and 13 (wt. %) but generally the extraction of oleoresin from *C. longa* rhizomes is performed using ethanol as solvent (Jayandran *et al.*, 2015). According to chemical investigations on *C. longa* as reported by Nahar and Sarker (2007), at least 235 compounds primarily phenolic compounds and terpenoids have been identified, including diarylheptanoids (commonly known as curcuminoids), diarylpentanoids, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, alkaloids and sterols etc. Quantitative estimation of curcuminoids can be carried out photometrically based on its absorbance at 420nm.

2.5.1 Phenolic Compounds

2.5.1.1 Diarylheptanoids and Diarylpentanoids

Over 300 diarylheptanoids have been reported in the family Zingiberaceae and some non – closely related families (Lv and She, 2010). Curcuminoids belong to the group of diarylheptanoids (or diphenylheptanoids) having an aryl-C7-aryl skeleton. These yellow pigments are usually used as food colouring agents and they are the main active compounds in *C. longa*. Usually, these polyphenols are present in 3-15% of *C. longa* rhizomes with curcumin as the principal compound. Curcumin ($C_{21}H_{20}O_5$) also known as diferuloyl methane or 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E), was isolated in 1815 (Chempakam and Parthasarathy, 2008) and its chemical structure was determined in 1910. The compound is a yellow-orange powder with a molecular weight of 368.37. Commercial "curcumin" is usually a mixture of three curcuminoids. For example, the composition of a

commercial “curcumin” is about 71.5% curcumin, 19.4% demethoxy-curcumin and 9.1% bisdemethoxy-curcumin (Pfeiffer *et al.*, 2003). These three major curcuminoids are also found in some other species of curcuma but have lower concentrations eg C. *amada* Roxb., C. *aeruginosa* Roxb., C. *aromatica* (Nahar and Sarker, 2007).

2.5.1.2 Terpenes

The chemical structure of the terpenes was established by Wallach in 1887 (Christmann, 2010). He proposed the so-called “isoprene rule” by which terpenes were considered to be fusion products of two or more isoprene (C_5H_8) moieties. The general formula of the terpenes is therefore $(C_5H_8)n$, where n=2, the hydrocarbons are known as monoterpenes or simply as terpenes; where n=3 they are called sesquiterpenes and where n=4 as diterpenes. Triterpenes (C_{30}) and tetraterpenes (C_{40}) are also known. At least 185 compounds of terpenes have been isolated or detected from leaves, flowers, roots and rhizomes of *C. longa*, including 68 monoterpenes, 109 sesquiterpenes, 5 diterpenes and 3 triterpenes (Leela *et al.*, 2002).

2.5.1.3 Monoterpenes

The volatile oils from leaves and flowers of *C. longa* were usually dominated by monoterpenes, particularly ρ-cymene, β-phellandrene, terpinolene (terpenoline), ρ-cymen-8-ol, cineole and myrcene while the major part of the oil from roots and rhizomes contained sesquiterpenes (Leela *et al.*, 2002). The chemical characteristic can be useful in identification of leaves or flowers of *C. longa* used to substitute its rhizome for *C. longa* oil production. In total, 68 monoterpenes have been identified from various tissues of C. longa (Leela *et al.*, 2002).

2.5.1.4 Sesquiterpenes

Dried *C. longa* rhizomes usually yield 1.5 to 5% essential oil which are dominated by sesquiterpenes and are responsible for its aromatic taste and smell (Li *et al.*, 2011). Ar-turmerone, α-turmerone and β-turmerone are major ketonic sesquiterpenes of essential oils, and

these compounds may account for at least 40% of essential oils of *C. longa* rhizomes (Li *et al.*, 2011).

2.5.2 Active Ingredients Variation of Curcuminoids

Curcuminoids in *C. longa* are primarily accumulated in rhizomes of *C. longa* (Kobayashi *et al.*, 2010). The contents of curcuminoids in *C. longa* rhizomes vary often with varieties, locations, sources and cultivation conditions (Kobayashi *et al.*, 2010). Plant maturity has significant impact on chemical constituents of *C. longa* rhizomes of curcuma. In Sri lanka, both total curcuminoids and curcumin (I) in rhizome reach the highest yield at 5.5 months and maturity results in decline of the pigments but essential oils will not reach maximum yield until 7.5 to 8 months (Asghari *et al.*, 2009). Plants grown in different habitants may also affect curcumin yield in rhizomes. A recent Japanese study showed that curcumin content in rhizomes from the plants cultivated in dark-red soil is about 100% higher than in those from gray soil and more than 200% higher than those from red soil (Hossain and Ishimine, 2005). It was also reported that potassium in soil positively affected curcumin yield in rhizomes (Karthikeyan *et al.*, 2009). Post- harvest processing of *C. longa* is also an important factor to affect the content of curcuminoids. However, some reports are controversial, for example, one study found that concentration of curcumin was reduced by 27-53% from heat processing of *C. longa* (eg curing with boiling water) (Suresh *et al.*, 2007), but another investigation indicated that heat treatment of *C. longa* prior to dehydration increased curcuminoid levels (Bambirra *et al.*, 2002). It is believed that curcumin is produced in leaves and is then translocated to rhizome (Behar, Tiwari & Jadhav, 2016). However, the mechanism of metabolism and accumulation of curcuminoids remain elusive and induced production of the active ingredients has not been addressed.

2.5.3 Curcumin Safety and Toxicity

Small doses of *C. longa* (curcumin) are taken daily as a spice by the population in many Asian countries. In one epidemiologic survey, in terms of its dietary use in Nepal, *C. longa* consumption was found to be up to 1,500 mg per person per day, equivalent to approx. 50 mg/day of curcumin (Eigner and Scholz, 1999). In India, where the average intake of *C. longa* can be as high as 2,000–2,500 mg per day (corresponding to approximately up to 100 mg of curcumin), no toxicities or adverse effects have been reported or studied at the population level

(Chainani-Wu, 2003). It was also reported that amounts of up to 8g, administered per day for three months, were not toxic to humans (Cheng *et al.*, 2001). However the doses administered in clinical trials are expected to be rather higher than those normally consumed in the diet. This fact underlines the need for systematic safety and toxicity studies. Based on repeated studies, *C. longa* is Generally Recognized As Safe (GRAS) by the US FDA, and curcumin has been granted an acceptable daily intake level of 0.1–3 mg/kg-BW by the Joint FAO/WHO Expert Committee on Food Additives, 1996 (National Cancer Institute [NCI], 1996).

Although *C. longa* is often used to treat inflammatory skin conditions in traditional Asian medical systems, it should be noted by potential laboratory and clinical investigators that a few reports of allergic dermatitis after contact with curcumin have been published in the scientific literature (Babu, 2013; Thompson and Tan 2006). An allergic reaction to *C. longa*-related products was also described in one healthy volunteer enrolled in a phase I study testing the safety of *C. longa* oil and *C. longa* extract (Joshi *et al.*, 2003). Despite the lack of systematic testing on the interaction between curcumin with other commonly used drugs, the US Department of Health and Human Services has recommended, based on published laboratory and animal studies, that co-administration of curcumin with nonsteroidal anti-inflammatory drugs (NSAIDs) or anti-coagulant drugs (heparin, clopidogrel, aspirin) may result in an increased risk of bleeding.

2.5.4 Recent Advancements on Curcumin Formulations and Delivery Systems

Like many other natural polyphenols, curcumin is also poorly soluble in water. It is well established fact that the main limitation of broader use of curcumin-based formulations is its poor solubility and fast metabolism (Bansal *et al.*, 2001). Therefore, in order to increase its solubility, stability and pharmacological activities, further research on improved formulations and delivery systems is needed to achieve its optimum therapeutic effects. In search for enhanced bioavailability of curcumin, the first step would be to improve its solubility. For that purpose, various classical techniques based on physical parameters such as heat, pH, and complexations with metal ions, polymers or serum have been applied to prepare more soluble curcumin formulations. In addition, chemical modifications of curcumin are carried out to prepare curcumin derivatives or analogues. Kurien and Scofied (2009) claimed that the solubility of curcumin and *C. longa* can be increased by 12-fold and 3-fold, respectively, by the use of heat without heat-mediated disintegration of curcumin. For delivery of drugs *in vivo*, water is

indisputably the simplest and the safest vehicle, therefore possibility of considering heat-solubilized curcumin for future *in vivo* and *in vitro* studies might be interesting (Kurien and Scofied, 2009). Zebib *et al*. (2010) prepared complexes of curcumin with metal ions (Zn^{2+}, Cu^{2+}, Mg^{2+} and Se^{2+}) that were found to be readily soluble in water-glycerol (1:1; w/w) and quite stable towards light and heat. Several studies have indicated that the amount of curcumin in the serum after an intake of 4 -8g is only 0.4 -3.6 μM (Anand *et al.*, 2007).

2.6 UTILIZATION OF *C. longa*

C. longa has been widely used throughout Southeast Asia as an aromatic spice in cooking and as a medicinal instrument in complementary health practices for over 6000 years. *C. longa* is eaten as food both raw and cooked throughout Asia. It is remarkable that while *C. longa* root looks much like ginger root, it is less fibrous and is more chewable, crunchy and succulent. The fresh root (not the powder) has a somewhat sweet and nutty flavour mixed with bitter flavour. As a result, it is not unpleasant to eat and not difficult to chew. It is sometimes chewed plain or chopped up and put in salads raw. *C. longa* was regarded as "the herb of the sun" because of its purifying yellow-orange colour, like the fire element which is symbolic for eliminating impurities (Reddi, 2013). *C. longa* has been traditionally recognized as a therapeutic agent in both the Indian and Chinese systems of medicine (Gupta *et al.*, 2013). In Traditional Chinese Medicine (TCM), *C. longa* has been historically used in herbal formulae to treat conditions including flatulence, liver problems, menstrual difficulties, bloody urine, hemorrhage, toothache, bruises and sores, chest pain and colic (Kumar *et al.*, 2013). Ayurveda is the ancient medical practice of the Indian system of medicine and *C. longa* has been documented as a therapeutic agent to treat all kinds of disease states: acne, psoriasis, dermatitis, rash, ulcers, and wounds (Kumar *et al.*, 2013).

Because of the Ayurveda principle that *C. longa* carries medicinal properties, a pinch of *C. longa* is added to most of the foods cooked in India (Thomas-Eapen, 2009). *C. longa* is a major ingredient in many curry powders and it is added as a flavouring agent to vegetables, lentils, beans, meat, fish, and buttermilk (Thomas-Eapen, 2009). In the popular curry powder, curcuma is added to the mix of spices which usually consist of: coriander, cumin seeds. Fenugreek seeds, chili, mustard seeds, black peppercorns and salt (Tayyem *et al.*, 2006). As a tea (for example in

Okinawa), *C. longa* is added to hot water and then strained (with touch of ginger and lemon juice). It can also be added to milk and simmered. It has traditionally been used as a colouring agent in Asian cuisine, as well as in cheese, butter, yoghurt and other kinds of food (Arun and Nalini, 2002). In addition to the use of *C. longa* in Indian cuisine, *C. longa* paste is applied on the face and skin to improve skin appearance and to remove unwanted hair and as a facial for good complexion (Kumar *et al.*, 2013). It is also worn as an amulet or charm to ward off evil spirits, and used during weddings as a nuptial necklace and other religious ceremonies where a yellow and deep-red powder are present at the altar (Reddi, 2013). The pigments are largely used in the food industry as substitutes for synthetic dyes like tartrazin.

Curcumin is now marketed in several countries including the United States, India, Japan, Korea, Thailand, China, Turkey, South Africa, Nepal, and Pakistan in the forms of capsules, tablets, ointments, energy drinks, soaps, and cosmetics (Gupta, *et al.*, 2013). It has also been used as a popular stomach soother and pain reliever. Traditionally the earlier South Eastern Nigeria used raw *C. longa* paste to create a beauty skin design which could last for up to four days before fading away. Sharma *et al.* (2006) and Weisberg *et al.* (2008) also indicated that curcumin has a protective role against diabetic nephropathy especially type 2 diabetes as it causes a decrease in blood glucose and glycosylated hemoglobin level. In 1870's chemists discovered *C. longa*'s orange-yellow root powder turned reddish brown when exposed to alkaline chemicals. This discovery led to the development of *C. longa* paper …..to test for alkalinity (Micheal, 1995).

The anti-oxidant property of curcumin can prevent rancidity of foods and provide food stuffs containing less oxidized fat or free radicals and this powerful anti-oxidation property of curcumin has an important role in keeping "curry" for a long time without it turning rancid. Anti-inflammatory effect of curcumin and its derivatives are because of the hydroxyl and phenol group in the molecules. These groups are essential for inhibition of prostaglandin synthetase and leukotrienes synthesis.

2.7 DESCRIPTION OF *X. aethiopica*

X. aethiopica (*"*African guinea pepper" or "Ethiopian pepper") is an angiosperm of the family *Annonaceae*. It is a native to the lowland rainforest and moist fringe forest in the savanna zones

of Africa, but largely located in West, Central and Southern Africa. In Nigeria, it is found all over the lowland rain forest and most fringe forest in the savanna zones of Nigeria (Uzodike and Onuoha, 2010). These trees (plate 2.4) are widely distributed in the humid forest zones of West Africa especially along rivers in the drier area of the region (Tairu *et al*., 1999). In tropical and highlands of Africa (from Ethiopia to Ghana), both species of *X. aethiopica* and *X. striata* occur and both are used for the preparation of indigenous soups. In South America, a third species of interest, *X. aromatica* (burro pepper), has found similar applications among Brazilian Indios. While *X. aethiopica* thrives in the forest regions, the tree can also be found in transitional zones. It is a slim, tall, evergreen tree, growing up to 30m high and about 60 to 70cm in diameter with straight stem, many –branched crown and sometimes buttressed. Its other Commercial names include Ethiopian/Guinea pepper, Spice tree and Negro pepper. In Nigerian Arabic, it is called "kyimba", "kumba" in Arabic-shuwa, "kenya" in Bokyi, "akada" in Degema, "unie" in Edo, "ata" in Efik, "kimbaahre" in Fula-Fulfulde, "kimbaa" in Hausa, "ata" in Ibibio, "uda" in Ibo, "tsunfyanya" in Nupe, "kimbill" in Tera, "eeru" in Yoruba. The flowers are bisexual, up to 5.5 by 0.4 cm and creamy-green. Fruiting takes place in December to March and June to September. The fruits are small with carpels 7-24, forming dense cluster, twisted bean-like pods, which are dark brown, cylindrical, 1.5-6cm long and 4-7 mm thick. The seeds are black, kidney-shaped numbering about 5-8 per pod, approximately 10 mm length with a yellow papery aril. The hull is aromatic, but not the grain itself (Poitou *et al.*, 1996). The mature fruit of green colour takes a brown-black colouration after drying and are used as spices. It is aromatic, quite pungent and slightly bitter, comparable to a mixture of cubeb pepper and nutmeg. *X. aethiopica* is often smoked during the drying process which results in a desirable smoky-spicy flavour. Typical fruit yields are about two to three metric tons per annum per hectare (Tairu *et al.*, 1999). It is used as a spice and possesses great medicinal values in traditional medicine (Oluwatosin *et al*., 2010).

Plate 2.4. *X. aethiopica* (African Guinea-pepper) plant.

2.8 CHEMICAL COMPOSITION OF *X. aethiopica*

Among the compounds reported to confer *X. aethiopica* its biological properties are the diterpenes belonging to the kauranes, the trachylobanes and the kolovanes families (Harrigan *et al.*, 1994; Soh *et al.*, 2013).The essential oil of *X. aethiopica* is constituted of monoterpenes hydrocarbon. These compounds are predominantly β-pinene (9-42%) and Sabinene (36.0%) as reported by (Ayedoun *et al.*, 1996; Jirovetz *et al.*, 1997; Keita *et al.*, 2003; Poitou *et al.*, 1996; Tomi *et al.*, 1996).

Germacrene D is the most sesquiterpene in the spice and the oxygenated compounds are mainly the 1, 8-cineole and the terpinen-4-ol. A survey undertaken on *X. aethiopica* essential oil from Egypt showed very peculiar composition with more than two third of the oxygenated compounds; 23.4% of terpinen-4-ol, 16.3% of 1,8-cineole and 11.1% of a terpineol (Mohamed, 2016). A similar composition with oxygenated monoterpenes (15.1% of 1, 8-cineole, 6.6% of terpinen-4-ol) has been reported in its essential oil from Nigeria (Asekun and Adeniyi, 2004). The main chemical constituents isolated from *X. aethiopica* comprised mainly of xylopic acid (15β-acetoxy (-) Kauran-16-ene-19-oic acid), three diterpenic acids, fats and essential oils. The various acids are responsible for the hot taste which characterizes *X. aethiopica*. In other works by Tairu *et al.* (1999) results obtained showed that linalool, β-trans-ocimene, α-farnesene, α-pinene, β-pinene, myrtenol, β-phellandrene, and 3-ethylphenol were the most important odourants present in the volatile oil of the fruit with linalool being the most intense giving the pepperish note, characteristic of the ground, dried, smoked fruits of *X. aethiopica.*

Among the non-volatile constituents, tetracyclic diterpenes of the Kaurane type have been identified; the kaurane structures are based on a tetracyclo hexadecane skeleton. Kauranes are structurally similar. Kolavanes and trachylobanes also appear in the bark (Tairu *et al.*, 1999). The essential oils of the stem bark (0.85%) and the leaves (0.5%) of *X. aethiopica* have also been investigated. The bark oil consists mainly of α-pinene, trans-pinocarveol, verbenone and myrtenol and differs remarkably from the leaf oil compounds, spathulenol, cryptone, β-caryophyllene and limonene (Tairu *et al.*, 1999). According to Chinwe *et al.*, (2012) the extracted lipid when examined for fatty acid composition, Linoleic (45.1g/100g) and Oleic (26.5g/100g) acids were the predominant unsaturated fatty acids, while palmitic acid

(18.0g/100g) was the major saturated acid. The iodine value of 97g/100g indicates that the seed oil is a non-drying type.

2.9 UTILIZATION OF *X. aethiopica*

The comparative study of nutritional and electrolyte qualities of *X. aethiopica* in novel hydro-alcoholic formulation by Chinwe *et al.* (2012) revealed that *X. aethiopica* might be a major source of iron needed for good health and nutritionally this might explain its traditional local use as post-partum tonic and lactation aid in women who have given birth newly. The iron content could also significantly contribute to reduction of anaemia caused by iron deficiency and correspondingly lead to the production of high oxygen blood level and transport (Bhaskaram, 2001). The fruits are widely used to prepare pepper soup for nursing mothers to accelerate blood flow leading to the elimination of blood clots from her blood system (Inyang, 2003).

X. aethiopica is used as both spice and medicine. The annonacea family species such as *Xylopia* has some multiple biologically active compounds, specifically with microbicidal effect (Lopez *et al.*, 2009). In Nigeria, they are used as a carminative and stimulating additive to other medicines and also as cough medicines (Asekun and Adeniyi, 2004). The powdered root is employed as a dressing in the local treatment of wounds (Asekun and Adeniyi, 2004; Muanya, 2016). Other uses include treatment for stomach ache, bronchitis, biliousness and dysentery (Uyovwiesevwa *et al.*, 2011). It has also been reported to be used to modify flavour in palm wine. The bark of *X. aethiopica* tree when steeped in palm wine is given for attacks of asthma and rheumatism (Smith *et al.*, 1996). The fruit extract has been reported to be active as antimicrobial agent against gram positive and gram negative bacteria, though it is not known to be effective against *Escherichia coli* (Iwu, 1993). *X. aethiopica* has anti-spirochoectal properties such that it works both as a preventive measure and in treatment of primary, secondary and tertiary stages of syphilis (Iwu, 1993). It has been used for treating rheumatism and arthritis as well as other inflammatory conditions. Numerous research studies have confirmed the spice's anti-inflammatory and antipyretic (fever reducing) properties (Okwu, 2001; Uzodike and Onuoha, 2010). Indian researchers reported anti-arthritic and anti-inflammatory actions of one of the compounds of *X. aethiopica* called nimbidin (Uzodike and Onuoha, 2010). The seeds are mainly used by the traditional medicine healers but can also serve as an alternative to pepper. The constituents of these fruits that contribute to nutritional effects are phytochemicals, vitamins and minerals

(Close and McArthur, 2002). Phytochemicals as antioxidants play vital roles in human health. Researchers in India, Europe and Japan have found that polysaccharides and limonoids found in *X. aethiopica* reduce tumors and cancers (Muanya, 2016; Uzodike and Onuoha, 2010). Flavonoids represent the most common and widely distributed of plant phenolics found in *X. aethiopica*. Flavonoids prevent oxidative cell damage, have strong anti-cancer, anti-prostate activity and protects against all stages of carcinogensis (Del-Rio *et al.*, 1997; Muanya, 2016). Anonecaine, an alkaloid constituent of *X. aethiopica* is known to have anti-pyretic effect (Aguoru *et al.,* 2016). Saponins another phytochemical constituent of *X. aethiopica* have wide range of biological properties; they are used to recover homeostasis, and are associated with anti-inflammatory and anti-cancer actions (Sparg *et al.,* 2004). Several studies have reported the biological activity of *X. aethiopica* fruit essential oil (Kouninki *et al.*, 2005; Ngamo *et al.*, 2001; Okonkwo and Okoye, 1996) and its leave essential oil (Asawalam *et al.*, 2006) indicating that they can be used against cowpea *Bruchid callosobruchus* Maculatus (fab) (Coleoptera: bruchidae) or maize weevil *Sithophylus zeamais Motsch*. (Coleoptera: curculionidae). *X. aethiopica* is also active against the termites and other bugs which tackle wood (Lajide *et al.*, 1995). It is also noticed that the features of the ether extract of *X. aethiopica* are favorable to its incorporation in the resins used for the manufacture of the paintings (Ajiwe *et al*., 1998).

2.10 FLAVOURING CONSTITUENTS OF PLANT MATERIALS

Most of the characteristic aromatic profiles of plants used as a source of odourous or flavouring materials depends on a complex mixture of chemicals which have been formed in the plant tissues during the normal growth cycle and remain there, more or less intact, after harvesting with a few exceptions where the flavour is formed enzymatically (eg onions, garlic, mustard etc) (Schwab *et al*., 2008).The biogenesis and synthesis of aromatic compounds in live tissues which are metabolically active is a dynamic process which depends on the plant, its variety, its environment during growth, maturation and fruiting, and its ultimate death by which most but not all of the process are terminated (Schwab *et al.*, 2008). Notwithstanding, not all the chemicals found in plant tissues contribute to a desirable aromatic profile; some are odourless and tasteless, others give rise to objectionable attributes or may even be toxic (Basear and Demirci, 2007).

2.10.1 Flavour Chemistry

Fisher and Scott (1997) listed some benefits of flavour research to include the understanding of biosynthetic pathways so as to improve on nature, obtain better yields of flavour components, or remove objectionable flavour features in food stuffs. It also enables the reconstruction of flavours using synthetic materials identical to those found in nature giving the Food technologist an ability to improve, enhance or regulate the flavour of food products, to rectify processing flavour defects or even to create novel food flavours. Although the flavour profile of natural components containing flavour materials is determined by the balance between the taste of non-volatile and the odour of volatile components. It is the latter which has attracted most attention of researchers. Aromatic components and their precursors are generally present in aqueous solutions or as droplets in the cell sap, although some essential oils may exist discretely in oil sac, glandular hairs etc (Schwab *et al.*, 2008).

It is not surprising that most flavour research has been directed to separating the components from the tissues in which they exist in the living plant, concentrating the isolates as necessary, separating the concentrate first into fractions and then into individual chemicals (Fisher and Scott, 1997).

To be of real practical value, any flavour research has to be orientated towards determining the key marker component which characterizes any odour or flavour, establishing their organoleptic attributes, rating their contribution to the total profile, studying their biogenesis and biosynthesis to enable the growing of more flavour produce, synthesizing these marker chemicals for use in imitation flavourings and studying the nature of flavour precursors and their chemical modification on heat processing.

Odour and flavour are purely subjective properties of food determined by the coordinated interaction of the total complex of chemicals present, not just those associated with the volatile fraction (Fisher and Scott, 1997). Sensory assessment is also inevitable in such flavour analysis.

There is collaboration in tackling the biological/physicochemical problems associated with isolation, separation and identification of components and determining their sensory values and their relative contribution to the odour and flavour profile of the start material. Whether naturally occurring, induced by processing or cooking, or intentionally added, flavour in food and related

consumables is due to the chemicals embracing some inorganic compounds as well as a whole spectrum of aliphatic and aromatic organic chemicals (Fisher and Scott, 1997). The characteristic odour of natural materials in *C. longa* and *X. aethiopica* is due to the presence of volatile chemicals; that taste (eg sweetness, bitterness, etc) and certain other attributes (eg pungency, astringency, etc) are due to non-volatile components and the flavour of the material depends on the balance between these characteristics and their combined effect upon the receptors (Schwab *et al.*, 2008).

The odour of food stuffs is the predominant contributor to their distinctive and often diagnostic flavour profile and hence, a study of the volatile components is of relatively greater importance. There are however, certain flavouring materials which have unique and quite distinctive component (eg esters of trans-2-cis-4-decadienoic acid in Bartlett pears) (Versini *et al.*, 1995). These chemicals are often present in only trace quantity making isolation and positive identification very difficult posing considerable problems in establishing the chemical pathways involved in their biosynthesis. In most cases; sensory assessment is confined to odour evaluation.

Flavours may be classified in many ways; but from the point of view of their chemistry, the most realistic classification is that based on their mode of formation either naturally by biogenetic pathways from known precursors or by processing in which biological, chemical or physical conditions are imposed on natural or artificial start materials (Fisher and Scott, 1997). Natural flavours are mostly metabolites such that their formation in living tissues is complex and dependent on genetic factors and is influenced by environmental conditions during the natural growth cycle (Schwab *et al.*, 2008). Flavours produced by processing may be direct breakdown products or complex interaction products depending on the start materials and the processing conditions imposed.

At various times, two different points of view have been expressed concerning the nature of flavouring constituents namely; that basically all flavour is composed of more or less the same chemical compounds present in different proportions or that flavourings are specific and dependent on distinctive chemical entities and structure for their characteristic profiles (Fisher and Scott, 1997). Indeed, there are many examples of the same chemicals being present in a variety of flavours having quite different profiles, most of the common organics being present in almost all foods (Fisher and Scott, 1997). From the many similarities and differences which are

evident throughout natural flavours, it is probably that both explanations are partially correct, depending on the flavour and its degree of specificity. The quantity of a given component present is not always an indication of its effective contribution and many characteristic profiles are determined by trace constituents (Fisher and Scott, 1997). Determining odour and taste impact values for the different compounds that contribute to aroma, along with their actual flavour attributes helps to join the dots between analytical chemistry and the actual consumer experience (Barrett *et al*., 2010).

2.10.2 Sensory System

Flavour is considered to be a combination of taste and aroma. Taste and aroma are both determined by the simultaneous stimulation of the chemical sensors in the nose and mouth, where sensory receptors send signals for integration by higher brain centres. Different flavour compounds stimulate, and are recognized differently by both the aromatic and taste sensory systems in humans. The perception of flavour may also be affected by what we see. In fact, the initial visual assessment greatly determines whether a food material is chosen for consumption. Once this visual assessment has been made and the decision is taken to eat the food or beverage the real sensory flavour analysis begins (Laing and Jinks, 1996; Small and Prescott, 2005). A wide variety of chemical stimulants are released during the chewing (mastication) process. The volatile components move up into the nasal cavity, stimulating odour receptors, while the saliva in the mouth moves the non-volatile components to oral receptors in the mouth and tongue. Odourants stimulate receptors in the nose, whereas tastants stimulate areas in the mouth. Irritants stimulate receptors in both the mouth and nose (Laing and Jinks, 1996). The neural processing involved in determining odourants is highly complex. In brief, the odourant stimulates receptors that send action potentials that meet in bulbar cells found in the olfactory bulb in the brain and other brain structures. A characteristic spatial ‘map’ for the odourant is produced, which is then sent to other parts of the brain, including memory, which then triggers motor responses to the odourant. Understanding this process inevitably lead to the development of the electronic nose (Small and Prescott, 2005).

Odourants can be classified into a vast array of categories, whereas tastants can be broken down into the five simple categories of sweet, salt, sour, bitter and umami. Each tastant has a point of

sensitivity within the mouth, whether it is at the front, rear or sides of the tongue, cheeks or oesophagus.

2.10.3 Essential Oils

The range of essential oils which are obtained by steam distillation, expression or solvent extraction from many odourous plant sources (Murray, 2009; Padmakumari *et al*., 2009; Sasidharan, 2010) are of prime importance to the flavour chemist as well as forming major articles of commerce for use in the flavour and fragrance industries. These products which give to the plant its distinctive and often diagnostic odour are complex mixtures of organic chemicals , the nature and relative proportions of which are determined by the plant species and agricultural factors such as environment, climate, soil conditions, time of harvesting and post harvesting handling prior to distillation.

Although steam distillation is effective and remains a key technology in the essential oil industry, some more elaborate techniques, such as supercritical fluid extraction, were developed in the early 1990's. These new technologies and methods for essential oil extraction have since become an adopted and popular alternate option in the essential oils industry (Parthasarathy *et al*., 2008). With the significant market for essential oils, the more efficient and effective the extraction process is, the greater the economic benefit to the industry will be. Essential oils play both a large role in the fragrance and cosmetic industries because of their unique health and aromatic qualities. They are also used in the food industry (Sangwan *et al*., 2001).

The enthusiasm shown towards essential oil characterisation really came to life with the entrance and development of gas chromatograph. Combined with improved extraction techniques, this aided in the more complete and accurate collection and separation of compounds. With the introduction of spectrometric techniques for compound identification and the gradual increase in spectral libraries, the rapid separation and identification of essential oil compounds became easier and more time and cost effective (Augusto *et al*., 2003).

The chemicals present in essential oils may be classified as follows: Hydrocarbons of the general formula $(C_5H_8)n$ -- the terpenes, Oxygenated derivatives of these hydrocarbons, Aromatic compounds having a benzenoid structure or Compounds containing nitrogen or sulphur.

These constituents are synthesized by the plant during its normal development and the chemical composition of the oil is generally characteristic of any given plant species. The proportion of the chemicals present in any given essential oil remains within predictable limits so that the quality of oil may often be expressed in terms of one or more specific component (Husnu-Can-Baser and Buchbauer, 2010). Although, the terpene hydrocarbons are quantitatively significant in the composition of many essential oils, they pose little flavour value. It is probably inaccurate to say that they have no odour or flavour but, relative to their oxygenated derivatives, their contribution is small other than to convey a definite "freshness" to the odour profile (Tripathi *et al.*, 2013).

Oxygenated derivatives of the terpene hydrocarbons include alcohols, aldehydes, ketones and esters. These occur widely throughout the essential oils and are major contributors to their distinctive odours and flavours (Vidita *et al*., 2013). The aromatic compounds based on benzene form another important group of flavouring and fragrance components. As with the terpenes, these constituents of an essential oil are the result of biochemical reactions directly associated with the plant's metabolism. They include the whole range of organic functional groups (ie alcohols, acids, esters, aldehydes, ketones, phenols, phenol ethers, lactones etc) their biogenesis and biosynthetic pathways seem to stem from n-propyl benzene as a precursor which is enzymatically activated (Murray, 2009).

Organics containing either nitrogen or sulphur are not widely distributed in the essential oils themselves but plant materials containing much albuminous matter do yield compounds on distillation such as ammonia, trimethylamine, hydrocyanic acid and hydrogen sulfide (Murray, 2009).

2.10.4 Oleoresin

Oleoresins are solvent extracts from foods or spices that are usually extracted for use in food technology and food flavouring (Borges and Pino, 1993). Oleoresins are usually prepared by exposing the flavouring constituents of the product to a solvent through grinding. Grinding assists in the more rapid and efficient extraction of volatile and non-volatile components of the spice (Murthy *et al*., 1999). Guinea pepper has historically been extracted using acetone or chlorinated solvents. However, with the health issues associated in using chlorinated solvents,

their use has been banned in many countries. Food-safe acetone and ethanol have been used as safe alternatives (Borges and Pino, 1993).

Once the solvent has extracted the compounds from the spice, the solvent can be distilled-off to leave the volatile oil and non-volatile fractions. After removing the solvent, the oleoresin goes through decolourisation, blending and addition of oil to preserve the flavour. This allows for the production of a standard industrial food product (Borges and Pino, 1993).

Spice oleoresin extractions have a few main benefits when compared to freshly ground or prepared spice. Firstly, the shelf life of spices is a huge issue due to evaporation and oxidation of important volatile components. The storage requires strict climate control to ensure there is no spoilage or rapid degradation. Oleoresins are more stable, resulting in less net loss of volatiles over time. They are also more concentrated and easy to store and transport. Oleoresin extracts (or alcohol extracts) are most commonly associated with the food industry, where they are added to process and preserve foods because of their positive flavour attributes and potential to increase shelf life (Tipsrisukond *et al*., 1998).

A reliable, consistent and hygienic food product can be created using oleoresin extraction techniques. Oleoresins contain higher levels of natural antioxidants when compared to essential oils, which make them naturally more stable over time and less prone to flavour damaging oxidation (Borges and Pino, 1993).

2.11 METHODS FOR FLAVOUR EVALUATION AND CHARACTERIZATION

There are many innovative and technical instruments for analyzing food components, including high performance liquid chromatograph (HPLC) and gas chromatograph (GC) coupled with mass spectrometry (MS). However issues remain with sampling and samples analyzed as the sample matrix itself can rarely be directly injected. Typically, more than 80 % of time is spent on sample preparation steps in these analytical methods. Finding new, efficient and effective sampling methods is a constant focus of current research (Kataoka *et al*., 2000).

There are various methods that have been established for analyzing and characterizing flavour. All have advantages and limitations, so when choosing a method to analyze a sample, it is important to have a good understanding of what you want to know. Some methods such as HPLC are designed for looking at certain specific compounds or groups and this method is not usually used for a complete profiling of a product (Merken and Beecher, 2000).Gas

chromatograph is the most common equipment for the separation of essential oil extracts and freshly captured aroma compounds. The Gas chromatographic techniques have the potential to rapidly separate and identify a wide array of different important flavour compounds in a gas or liquid sample (Grob and Barry, 2004).

2.12 FOOD ENCAPSULATION

Following the first commercial use of microencapsulation in 1954 to create a carbonless copy paper (Shahidi and Han, 1993), different encapsulation techniques were developed and accepted within the pharmaceutical, chemical, cosmetic, and food industries (Gibbs *et al.*, 1999; Madene *et al.*, 2006). Encapsulation or micro encapsulation of flavours consists in protecting a flavouring agent or a mixture of molecules with a dedicated envelope. It is a process to entrap active agents within a carrier material and it is a useful tool to improve delivery of bioactive molecules and living cells into foods. Encapsulation can limit the degradation or loss of flavour during the various product processes and storage. The envelope can also provide functional properties, such as a controlled release of aromatic molecules in a given environment like water (if flavoured tea), mouth (solid food: candy, toothpaste) or spray. Microencapsulation is the process by which active ingredients (core materials) such as food oils and flavours are packaged within a secondary (wall) material (Arshady, 1993; Augustin *et al.*, 2001; Desai and Park, 2005).

The size of particles formed through encapsulation may be classified as: macro (>5000 mm): micro (1.0–5000 mm); and nano (<1.0 mm) (Kings, 1995). Capsules below 1.0 mm in size are frequently referred to as nanocapsules, which are often made by very specialized Nano encapsulation methods (Legrand *et al.*, 1999; Shapiro, 2004).

Encapsulation can turn liquids and other ingredients into dust free powders, making it simpler to process and easier to dose, offering much more effective cost in use. It can also be used to improve the freeze and thaw ability of sensitive ingredients as well as providing protection from moisture and cross contamination.

Materials used for design of protective shell of encapsulates must be food-grade, biodegradable and able to form a barrier between the internal phase and its surroundings. Among all materials, the most widely used for encapsulation in food applications are polysaccharides. Proteins and lipids are also appropriate for encapsulation.

2.12.1 Microencapsulation of Food Flavours and Oils

The initial step in encapsulating a food ingredient is the selection of a suitable wall material, basically a film-forming biopolymer, from a wide variety of natural or synthetic polymers, depending on the core material and the characteristics desired in the final microcapsules (Badee *et al.*, 2012; King, 1995; Re, 1998). For flavour and oil encapsulation in particular, the ideal wall material should have emulsifying properties; be a good film former; have low viscosity at high solids levels; exhibit low hygroscopicity; release the flavour when reconstituted in a finished food product; be low in cost; bland in taste; stable in supply; and afford good protection to the encapsulated flavour and oil (Brazel, 1999; Desai and Park, 2005; Seid *et al.*, 2008). Because almost no wall material can meet all the properties listed, in practice they are used in combination with each other. Some types of wall materials along with their needed properties are presented in figure 2.4.

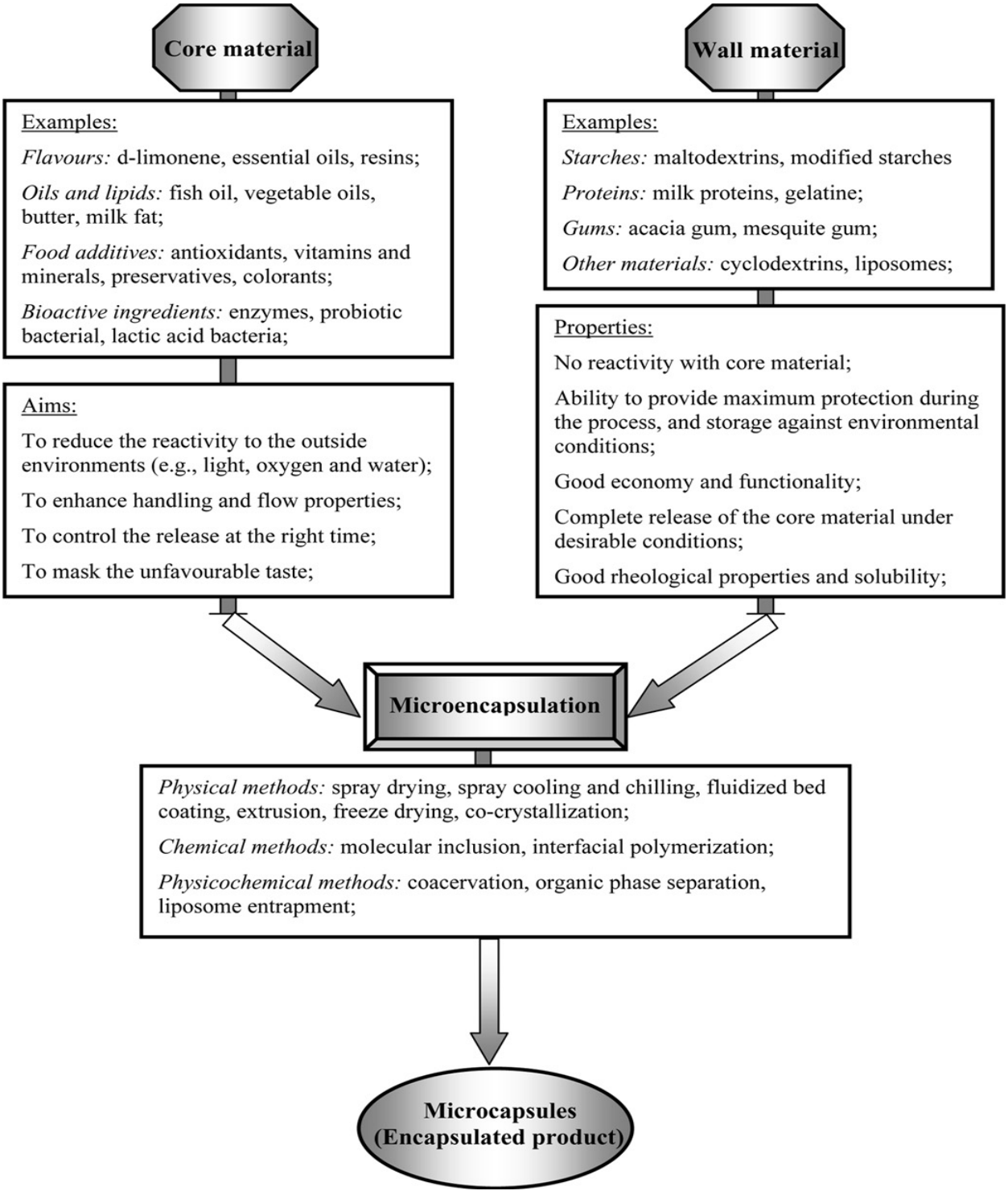


Figure 2.4. Schematic description of the microencapsulation of food ingredients along with some example of core and wall materials, wall material properties, aims, and different techniques of the microencapsulation process (Seid *et al.*, 2008).

Microencapsulation can potentially offer numerous benefits to the food ingredients being encapsulated. Various properties of active materials may be changed by encapsulation. For instance, handling and flow properties can be improved by converting a liquid to a powdered encapsulated form. Hygroscopic materials can be protected from moisture and stability of ingredients that are volatile or sensitive to heat, light or oxidation can be maintained. Materials that are otherwise incompatible can be mixed and used safely together (Augustin *et al.*, 2001; Brazel, 1999; King, 1995; Re, 1998; Sharma, 1999). There are many different types of microcapsules being used as food additives such as encapsulated food flavours and edible oils. Some examples are given in figure 2.4.

A vast majority of the flavour compounds used in the food industry are mainly in the form of liquid at room temperature. Also there is a need to incorporate some edible oils such as fish oil and many other vegetable oils into food products to increase the nutritional value of these products (Kolanowski *et al.*, 1999; Kolanowski & Laufenberg, 2006). Most of these food oils exhibit considerable sensitivity to air, light, irradiation and elevated temperature (Baik *et al.*, 2004; Reineccius, 1994). Conversion of liquid flavours and edible oils to dry powders is an important application of microencapsulation in the food industry (Madene *et al.*, 2006; Reineccius, 1994; Sharma, 1999). Also, one of the key aims for the microencapsulation of food oils and flavours is to control the release of these active ingredients until the right time (Madene *et al.*, 2006; Reineccius, 1995). Microencapsulated oils provide the convenience of a solid powder, with reduced volatility and less oxidation, and can be used in many different finished products such as cakes, beverages, etc (Shahidi and Han, 1993; Sharma, 1999; Vetsic, 1998). Examples of commonly used encapsulated flavours and oils are citrus oils, artificial or natural flavours, essential oils and spices, tuna oil, fatty acids, soy oil, and sunflower oil (Ascheri *et al.*, 2003; Baranauskiene *et al.*, 2006).

2.12.2 Parameters Affecting Encapsulation Efficiency

2.12.2.1 Properties of the Wall Materials

There are numerous wall materials available for use as flavour and oil encapsulating agents. For spray drying microencapsulation, in particular, the choice of wall material is critical as it will influence emulsion properties before drying, retention of the volatiles during the process and

shelf-life of the encapsulated powder after drying (Seid *et al.*, 2008). Among the available ingredients, the major wall materials used for spray drying applications are carbohydrates including modified and hydrolysed starches, cellulose derivatives, gums, and cyclodextrins; proteins including whey proteins, caseinates, and gelatine; and new emerging biopolymers such as products of Maillard reaction. A brief summary of these wall materials along with their properties and applications is presented in Table 2.1

Table 2.1: Different wall materials used in spray-drying microencapsulation of food oils and flavours

Wall materials		Properties	Examples	Encapsulated Flavour and oil
Carbohydrate	Hyhrolyzed starches	Very good oxygen-barrier, cheap, low viscosity at high solids; no/limited emulsion stabilization	Corn syrup solids, maltodextrins	Citral and linalyl acetate; ethyl caprylate; cheese aroma; linoleic acid; orange peel oil; lemon oil
	Modified Starches	Very good emulsion stabilization, inexpensive; sometimes varying quality, not universally usable owing to regulatory situation	Capsul, N-lok, Hi-Cap	Meat flavour; fish oil; orange oil; d-limonene; l-menthol; butter oil; cream; black pepper oleoresin; vitamin E
	Gums	Good emulsions, very good retention of volatiles; varying quality, price depends on availability, sometimes impurities	Arabic gum, mesquite gum	Essential oils; monoterpens; orange peel oil; cardamom oil; vegetable oils; cardamom oleoresin; linoleic acid; bixin; short-chain fatty acids; lipids; acetyl pyrroline; soy oil; d-limonene; ethyl butyrate
	Cyclo-dextrins	Very good inclusion of volatiles, excellent oxygen barrier; relatively expensive	α-, β-, α-Cyclodextrins	Pine flavour; shiitake flavour; d-limonene; ethyl hexanoate; caraway fruit oil; lemon oil
Proteins	Milk proteins	Very good emulsions; expensive, being dependent on other factors such as pH and ionic strength, allergenic potential Caseinates Skim milk powders	Whey proteins	fat; linoleic acid; soy oil; ethyl butyrate; ethyl caprylate
	Other proteins Soluble soy			Fish oil; soy oil Oregano and marjoram flavours; caraway essential oil
Other Biopolymers	polysaccharides, chitosan, alginates, Maillard products, modified celluloses, gelatine			Orange oil Wheat germ oil; evening primrose oil; PUFAs; fish oil

Source: Seid *et al.*, (2008).

2.12.2.2 Properties of the Core Materials (Retention of Volatiles)

The loss of some volatiles including flavours during spray drying encapsulation is inevitable. Other than properties of the used wall material, some features of the core material will also affect the retention during the process. The fact that both ‘‘molecular weight’’ and ‘‘vapour pressure’’ of the flavour compounds have an influence on their retention during spray drying is both obvious and well documented in the literature (Goubet *et al.*, 1998; Re, 1998; Reineccius, 2001; Reineccius, 2004).

Molecular weight is a reasonable representation of molecular size, which actually is the primary factor determining diffusion (Dalgleish, 2006; Goubet *et al.*, 1998). The increase of molecular size generally results in slower diffusion rate, subsequently, the molecules will take more time to reach the atomized droplet surface during drying, particularly initial stages, and retention will increase (Charve and Reineccius, 2009). A second factor promoting the retention of large flavour molecules is that the surface of the droplet becomes impermeable to them more quickly during drying, when diffusions effectively stops at low moisture content. Both of these factors favour the retention of larger molecular weight (size) volatiles.

Relative volatility plays a secondary role in determining flavour retention owing to its influence in controlling flavour loss until the droplet surface becomes semi-permeable. Volatility reflects the ability of a compound to reach the gaseous phase and can be evaluated by measuring the vapour pressure of the pure compound (Goubet *et al.*, 1998; Reineccius, 2001).

The retention of volatiles also depends on their polarity the more polar, the less retention (Ewa and Iwona, 2008; Re, 1998; Voilley, 1995). This could be explained by the greater solubility of polar compounds in water. As the water solubility of the volatile increases, the volatile losses increase due to the ability of the water fraction to diffuse through the selective membrane, even at late stages of the drying process.

It should be noted that individual volatiles can be retained at different rates during spray drying encapsulation. Goubet *et al.* (1998) revealed that the retention of aroma compounds with various functional groups is in the order of acids < aldehydes < esters ≤ ketones ≤ alcohols with acids having the minimum retention. Therefore, it can be seen that retention of volatiles depends on their molecular weight, relative volatility, polarity, and type. These different parameters act on the capacity of the volatile to diffuse through the droplet surface and on its ability to form small pools. The final result is that small, very volatile and water soluble flavours are lost to a greater

extent than the larger, less volatile and water-insoluble flavourings (Reineccius, 1998; Reineccius, 2001; Reineccius, 2004).

Besides factors such as volatility, solubility and diffusivity of the volatile compound through the droplet, another factor that should be taken into account in spray drying, microencapsulation is the possible interactions between the volatiles and the wall material (Re, 1998). This may involve physical or physicochemical interactions including formation of insoluble complexes, and molecular association of the wall material with the volatile through hydrogen bonds.

2.12.2.3 Conditions of the Spray-Drying Process

If the infeed emulsion is stable enough with optimum conditions such as viscosity and droplet size, encapsulation efficiency could be maximized by the right choice of spray drying parameters including inlet and outlet drying air temperatures, infeed temperature, atomization type and conditions, drying air flow rate and humidity, and powder particle size.

2.13 PACKAGING OF SPICES AND SPICE PRODUCTS

Packaging used for food is often different from non-food product or industrial packaging. Packaging for food has to be compatible with the food product packed within, and food standards and regulations must be met. For instance, the migration of harmful substances from packaging material in direct contact with food must be avoided. Thus, materials used for food packaging, handling and storage must be chosen accordingly.

Much attention has not been paid on the primary packaging of spices notwithstanding the large production of spices. Some countries importing spices are particular about standards relating to safety and hygiene. The regulatory authorities are always more concerned about biological infestation, pesticide residues and the presence of toxins (Sadecka, 2007).

Proper functional packaging can be expected to play an important role in maintaining quality of spices during storage and transportation (Peter, 2006). In the subcontinent, whole spices are bought mostly in loose form. Packaged spices are being sold only in cities where incomes are higher and convenience of a product is a priority (Pura-Naik *et al.*, 2001). Bulk packed whole spices which are being transported across the country in gunny bags, tend to lose the oil content but not pungency due to heat. The relatively high cost of spices and overwhelming importance of

retaining their aromatic and special flavour components fully justify the requirement of a functional retail package even for international market (Peter, 2006).

2.13.1 Packaging Method and Material for Spice and Spice Products

The spices are ground to release the flavour. The finer the powder, the more flavour is readily available. So powdered spices, masala mixes (mixture of different ground spices), and curry powders are available. Due to the grinding as the cell membrane and the natural protection ruptures, and due to tiny particle size, the surface area available for reaction increases, hence the rate of deterioration of ground is much higher than that of whole spices (Peter, 2006). To avoid spoilage, they are packed and stored in multiwall paper bags, lined textile sacks, lined HDPE sacks, glass/metal containers.

In a small unit pack, all the more care is needed to offer long shelf life. The relatively high cost of ground spices and the overwhelming importance of retaining their aromatic and special flavour components justify the selection of expensive glass or metal containers.

Among plastics, PET containers and PET –G co polymer containers meet the requirements and are good replacement for metal and glass at places where relative humidity is below 70% (Peter, 2006). For oleoresins which are highly volatile with good export demand, they have to be packed in tightly closed bottles, suitably lined tin or aluminium containers. They need to be protected from light, heat and oxygen, hence they need to be stored in cool, dark place. Of late, Oleoresins are also packed in thick food grade HDPE (high density polyethylene) containers (Indiramma, 1995).To prevent or slow down the deteriorative characteristics of spices and spice products during storage, for easy handling, transportation and to have export potential for spices, Peter (2006) suggested that the package: should have the ability to protect the contents from spoilage and spillage, should offer protection against physico-chemical and microbiological spoilage due to environmental contitions like humidity, temperature, light and oxygen, should be a good aroma barrier to prevent loss of flavour substance from the product and pick up of foreign odours, should have good oil and fat resistance characteristics, should have good machineability characteristics and possess the required mechanical strength properties, should have good resistance to insect and mites, should be compatible with the product packed as regards tainting and migration and conform to the food laws of the importing and exporting countries and should have good appearance and printability to assist in selling by

suitable attractive graphics. It should act as a 'silent salesman' for the product. Sales fall into two categories: first-time sales and repeat sales. The package must attract first-time buyers and build brand loyalty for repeat sales. In addition, it should be economical, easily available and disposable.

2.15 NATURE AND DETERIORATIVE CHARACTERISTICS OF SPICES AND SPICE PRODUCTS

The main quality contributing factors of spices like aroma, flavour and colour are sensitive to vagaries of climate and during storage. According to Peter (2006) they are affected by factors like high temperature and humidity, oxygen, respiration and heating, insects, pests, microorganisms, rodents and birds which work in combination to cause the following deteriorations: Loss of aroma and flavour caused by the loss of volatile oil content due to evaporation, seepage and oozing out through packaging material and /or due to oxidation of some aroma components. This is accelerated by temperature. Bleaching of colour occurs in spices like *C. longa*, green cardomon, red chillies etc which contain natural pigments. This deterioration is caused by oxygen and accelerated by light, humidity and temperature and favoured by oxygen. Loss of free flowing nature: the spice powders become soggy and lose their free flowing nature due to moisture ingress from the surroundings through the package. Caking and lumping problems do not arise in whole spices, however, development of musty odours does occur at higher relative humidity.

2.15. INSECT INFESTATION AND MICROBIAL CONTAMINATION IN SPICES

Important problem in spice packaging relates to insect infestation and microbial contamination during storage and distribution. The extent of damage depends on factors like initial contamination of the product, the type of insect and / or microbe and the packaging materials used (Pura-Naik *et al.*, 2001). Insect infestation may occur or develop in spice during any of the post-harvest operations such as harvesting, drying, threshing, transportation or storage. The problem of insect infestation in spices is quite worrisome. As many as 55 species of insects like drug store beetle, cigarette beetle, coffee bean weevil attack spices and spice powders. These insects require congenial atmosphere for their life. The tropical climate is very conducive for their activity and results in qualitative and quantitative losses.

The product must be free from any insect or its stages before packaging. The initial microbial load and insect contamination can be reduced to a great extent by adopting some methods like heating, sterilization, microwave treatment, fumigation and γ-irradiation (Peter, 2006; Sadecka, 2007). But since first three methods cause loss of volatile oil, other two methods are followed for spices. Usually, spice products are fumigated either using liquid fumigants like methyl bromide at a concentration of 32g/m^3 with an exposure time of 24 to 48 hours or using commercially available solid fumigant aluminium phosphide at concentration of 3 to 6 g/m^3 as liberated phosphine with an exposure time of seven days (Peter, 2006; Sadecka, 2007). γ-irradiation is effective for commercial decontamination of spices. A dose of 3-10 kGy can reduce the viable cell count to an acceptable level and does not affect the sensory quality of spices (Peter, 2006). The limitation of irradiation of spices is the high cost of irradiation.

2.16 ECONOMIC IMPACT AND TRADE

The spice market is directly influenced by the growing processed food industry. The rise in consumption of bakery products, confectionary products and ready-to-eat and fried food in the developed and developing economies is driving the market for spice. The global spice market grew from USD 10.7 billion in 2010 to an estimated USD 12.5 billion in 2013. The market is further expected to reach USD 16.6 billion in 2019. The market sizes for spice were also analyzed for the key regions - such as North America, Europe, Asia-Pacific, and Rest of the World (ROW) (figure 2.5). The North American region accounted for the largest share for spices in 2013. The market for spice in the Asia-Pacific region is emerging with substantial opportunities for the snacks, ready-to-eat food products, and confectionery industries.

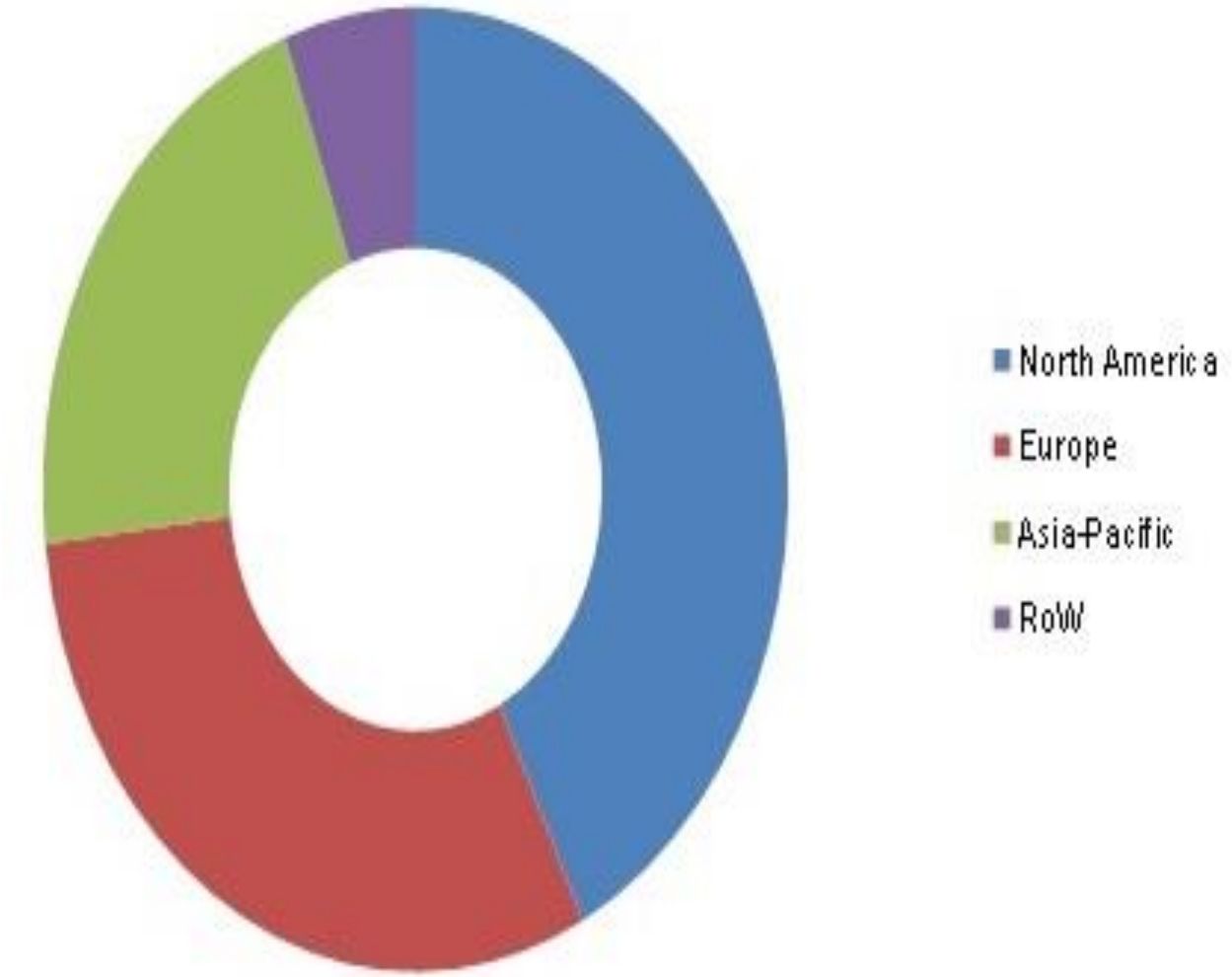


Figure 2.5: Spice Market Share (Value), 2013 By Region

Source: (Spice market, 2013).

There are around 40 to 50 spices of global economic and culinary importance. There are also many other species that are used in traditional cooking in the region of their natural occurrence but have yet to reach any significant trade (Green *et al*., 1999). Countries such as India are moving into the value-added sector, producing spice, essential oils, oleoresins, powders, specialty extracts and blends.

The trade distribution structure in the spice and herb trade can be divided into lines of supply to the three broad market sectors – industrial, catering and retail. The structure of the supply tree shows there are a number of different routes to market, and the most direct is the producer supplying directly to the industrial sector. It is estimated that about 85% of the international trade spices is dried and cleaned for use in a crude form without further processing. Greater interest in a wide range of international and ethnic dishes has been stimulated in recent years by extensive foreign travel, the establishment of a diverse range of ethnic restaurants, and the effects of immigration on food markets and supply chains. The food industry has been very active in promoting an interest in exotic foods as a promising growth sector. Advertising and media promotion by means of television cooking programmes, radio and magazines has stimulated demand. Ethnic groups have shops dedicated to their national foods, as well as supermarkets selling authentic ethnic products which are quick and easy to prepare. These developments have stimulated a wider range of food choice in home cooking and created an increased demand for spices. It is important to note that it is generally true that the more affluent the market, the more demanding is its 'entry standards'.

2.16.1 Processing

The role of processors in Europe includes sourcing, cleaning, treating against bacteria and spores, processing, grinding, storing, blending and selling. Spices are imported whole and ungrounded. Processors purchase about 350-400 different spices and herbs. Each product has its own specific supply chain (partly because of the importance of the origin of the products).

2.16.2 Final Use

Final users are: (1) The food processing industries (55-60% of the total use) that integrate spices in food and beverages manufactured for customers. Flavouring companies are another channel that utilizes spices dedicated to food and beverage products. (2) The retail sector (35-40%) where consumers purchase branded spices sold for home consumption. These spices could be in many forms, powder mixtures, fresh/dried or essential oils and oleoresins. (3) The catering sector (10-15%): restaurants, bakeries, confectioneries purchase spices for their preparations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Material collection

The *X. aethiopica* fruits, *C. longa* rhizomes (Alleppey variety) and white yam variety used for the study were obtained from local farms in Umulogho, Obowo LGA in Imo State and the National Root Crop Research Institute, Umudike, Abia State. The wheat grains were purchased from Owerri municipal main market, Imo state, Nigeria.

The packaging materials; amber plastic bottles were purchased from A&J pharmaceutical Ltd, Egbu town, Owerri while High Density Polyethylene (HDPE) bags were bought from a local supplier located at the Industrial layout, Irete, Owerri, Imo State.

The microbial media; Nutrient agar, Potato Dextrose agar, MacConkey agar and Brain Heart Infusion agar (BHIA) used for the isolation of bacteria, yeasts and moulds, were all purchased from Finlab Laboratory Chemical Supplier, Owerri, Imo State.

The solvents, De-ionized water, Peptone water, Ethanol (98% assay), Methanol, Acetone, n-Hexane and HCl were procured from Finlab Laboratory Chemical Supplier, Owerri, Imo State, while the UFLC reagents (analytical grade), Acetonitrile, acetic acid (glacial, 100% anhydrous) and tetrahydrofuran were purchased from Labstock Nig. Ltd., Lagos State.

The flavour principle standards (UFLC analytical grade) used including Curcumin for *C. longa* samples, and Beta-phellandrene for African guinea-pepper, were purchased from SIGMA Chemical Supplier Inc., USA.

3.2 METHODS

3.2.1 Raw materials preparation

3.2.2 Ground *C. longa* sample

The procedure described by Weiss (2002) was used for the preparation of ground *C. longa* rhizome sample. This procedure involved cleaning of the rhizome before drying. The cleaned rhizome (plate 3.1.a) was sliced into 3-5mm thick flakes and blanched at 75^{o}C for 15 min. The

blanched rhizome was subjected to sun-drying until a moisture content of 10% was obtained and this

Plate 3.1a: Dried *C. longa* rhizomes

operation lasted for up to 4-days. The dried rhizome flakes were milled into powder using a Warring blender (model: HGB2WTG4) and packaged in an air tight plastic container for further use. The processing operations are shown in figure 3.1.

3.2.3 Ground *X. aethiopica* sample

The procedure described by Ilusanya *et al.* (2012) was modified and used for the preparation of *X. aethiopica* ground sample. The mature ripe fruits of *X. aethiopica* (plate 3.1b) were cleaned using hot potable water (65^{o}C) and sun-dried for 4 days. The dried fruits (plate3.1c) were ground using a Warring blender (model: HGB2WTG4). The meal was sieved through a 120 µm -mesh sieve to separate the needed granular particles from the fibrous spice materials and was packaged in an air tight plastic container for further use. The processing operations are shown in figure 3.2.

3.2.4 Wheat grits (carrier)

The method described by Anderson (1982) was modified for the processing of high quality wheat grit. The wheat grains were parboiled at 70^{o}C for 15 min and then dried in a hot air oven at 60^{o}C for 8h. It was passed several times through an abrasive dehulling machine to remove the bran, and then milled into grits with a hammer mill. All the grits were sieved until they did not pass through a 120µm-mesh sieve after passing140 µm-mesh (120 grit size) and then stored in an air tight polyethylene bag for further use (Jillavenkatesa *et al.*, 2001). The processing operations are shown in figure 3.3.

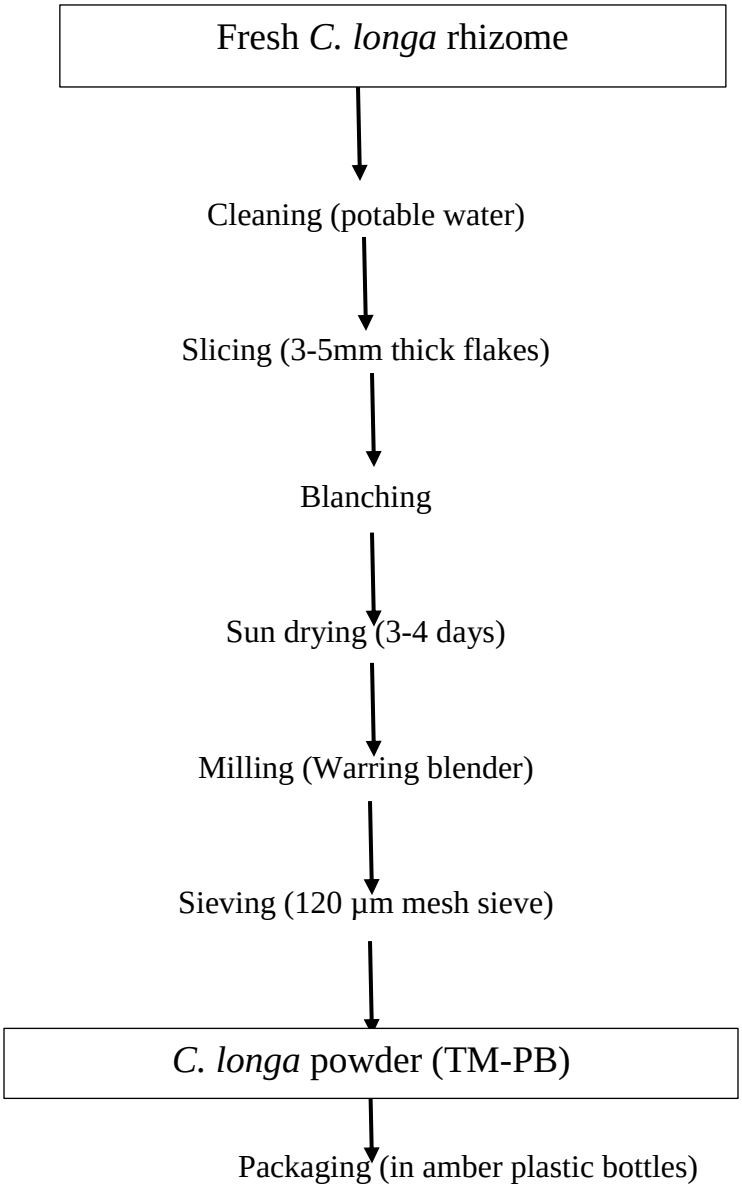


Figure 3.1: Flow diagram for the production of *C. longa* powder

Plate 3.1b: Fresh ripe fruits of *X. aethiopica*

Plate 3.1c: Dried *X. aethiopica* fruits (pods)

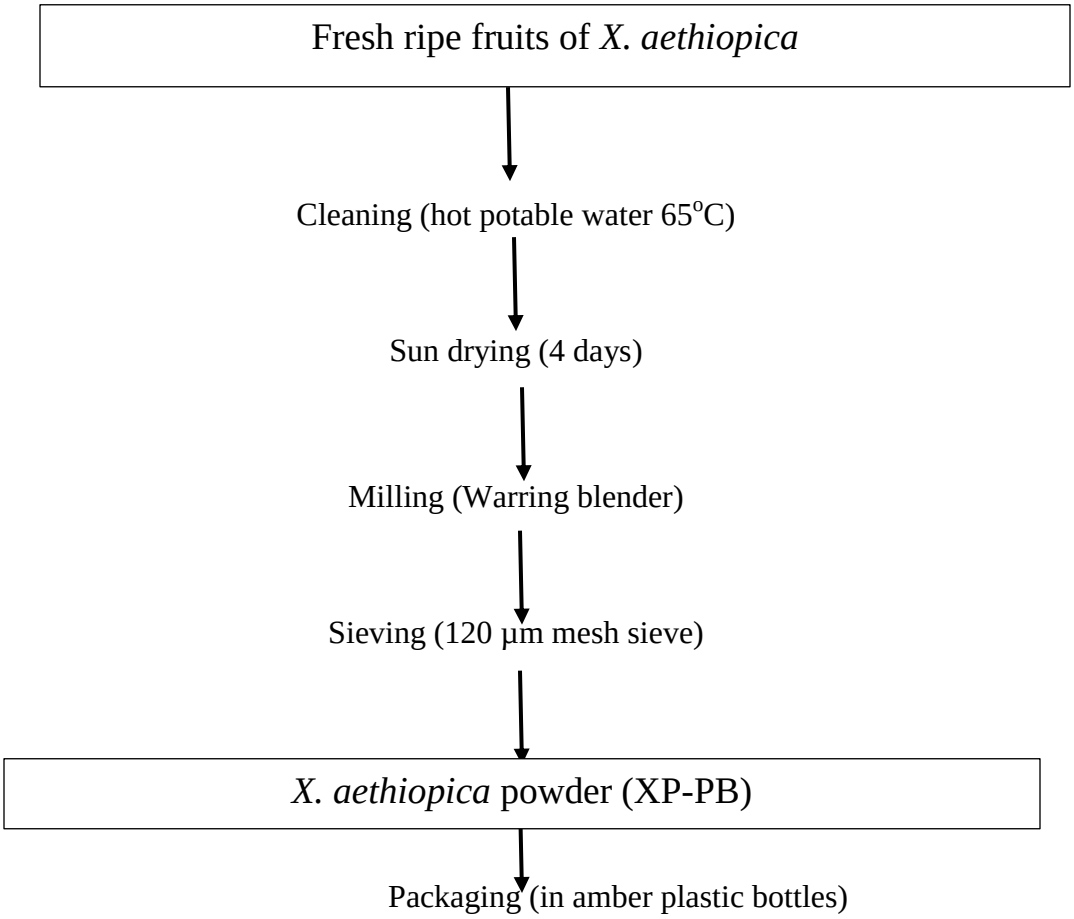


Figure 3.2: Flow diagram for the production of *X. aethiopica* powder

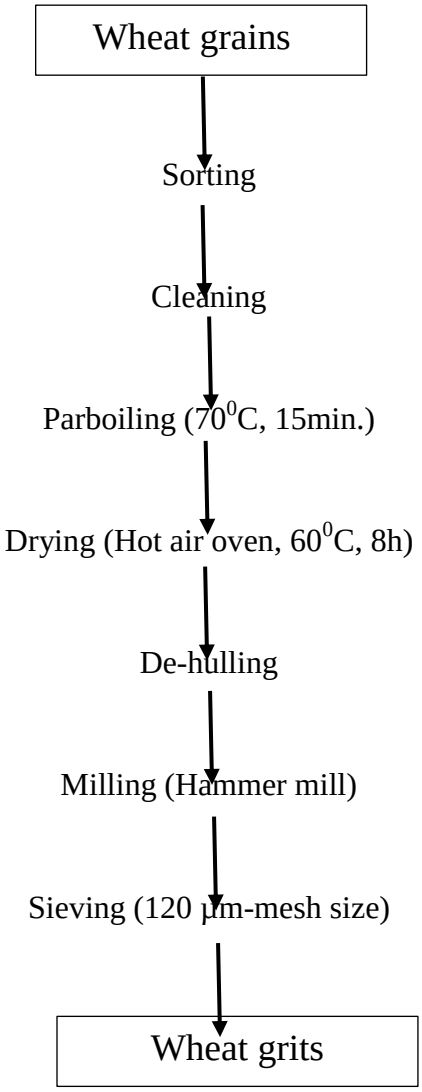


Figure 3.3: Flow diagram for the production of wheat grits (flavour carrier)

3.2.5 White yam grits (carrier)

The method described by Oluwole *et al.* (2013) was used for the processing of high quality white yam grit. The white yam (*Discorea rotundata*) was subjected to some preliminary operations such as peeling, washing, slicing (5mm thick), parboiling at 70^{o}C for 20min and drying at 60^{o}C in a hot air oven for 8h. The dried yam slices were milled using a hammer mill and sieved into grits by sieving until they did not pass through a 120 µm -mesh sieve after passing140 µm-mesh (120 grit size) and then stored in an air tight polyethylene bag for further use (Jillavenkatesa *et al.*, 2001). The processing operations are shown in figure 3.4.

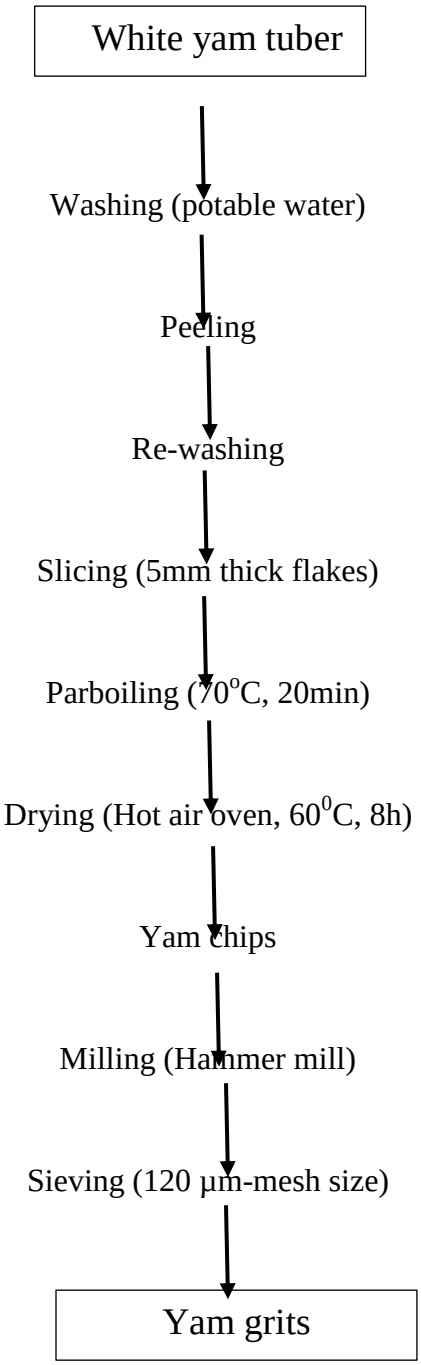


Figure 3.4: Flow diagram for the production of yam grits (flavour carrier)

3.3 PRODUCTION OF SPICE SAMPLES

3.3.1 Liquid extracts

The procedure described by Chang *et al.* (1988) was modified for the extraction of spice bioactive flavour components. The ground spice samples were mixed at a ratio of 1:5 (w/v) with each of the solvents; 98% ethanol, 40% ethanol, acetone, n-Hexane, methanol, and de-ionized water (as control) respectively. The suspensions were each centrifuged at 3000rpm for 10min and kept under ambient condition for 48h. The liquid extracts were passed through Whatman [No. 2] filter papers and the filtrates were further concentrated by evaporation in a rotary vacuum evaporator at 40^{o}C to obtain concentrated flavour extract of the solvents. The liquid extracts from the different solvents were evaluated for intensity of flavour principles perceived, desirability of the aroma, cost and compatibility of solvent to food and the most effective and safe solvent was chosen for the continuation of the study. The processing operations are shown in figure 3.5.

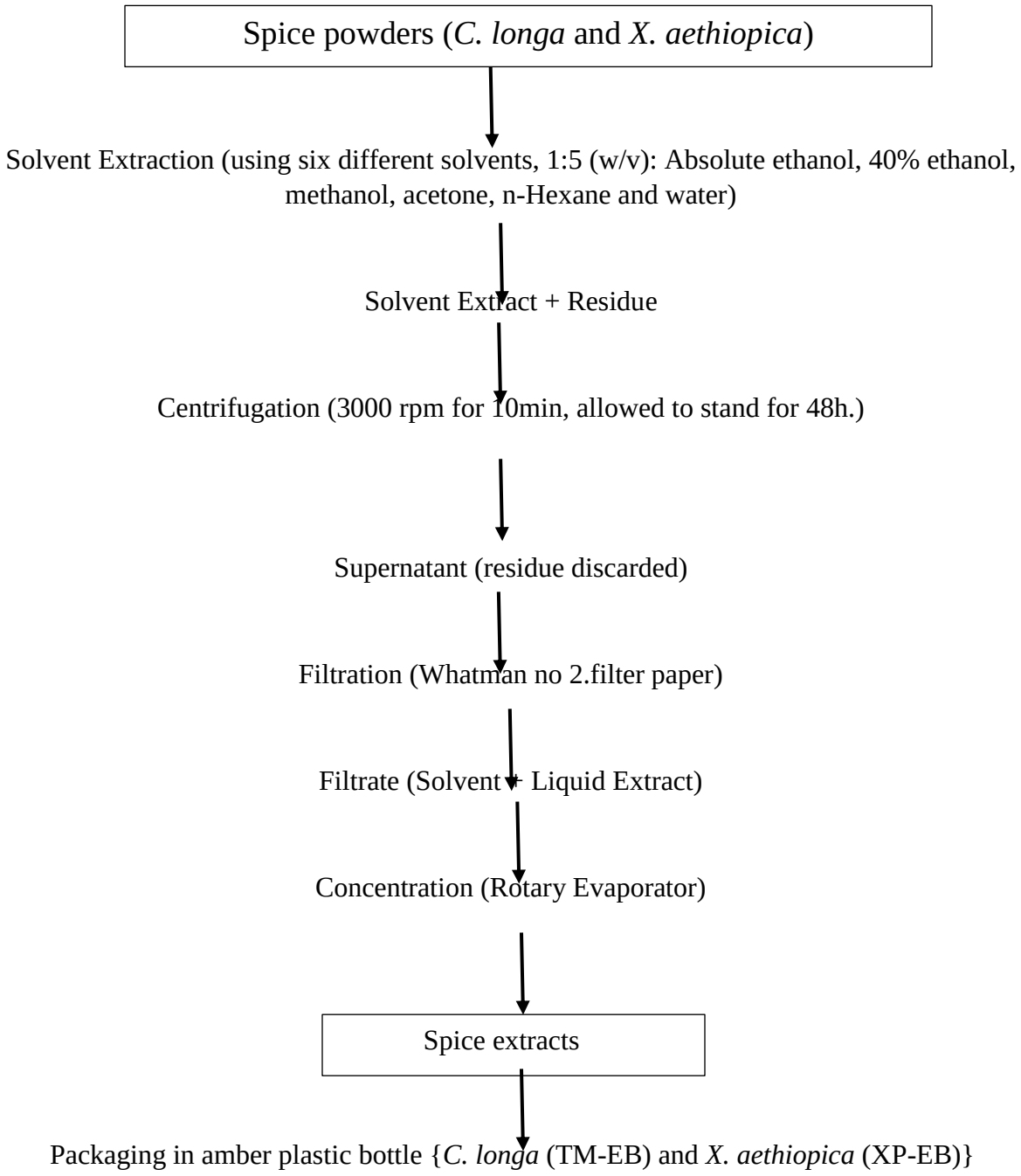


Figure 3.5: Flow diagram for the solvent extraction of *C. longa* and *X. aethiopica* (production of *C. longa* and *X. aethiopica* liquid extracts)

3.3.2 Encapsulated spice flavour samples

The method described by Jung and Sung (2000) was modified for the production of encapsulated flavour extracts from each of the spices (Table 3.1).Two edible carriers (gelatinized grits from white yam and wheat) were used for these samples. The formulation was done using varied ratios (2:1, 3:1, w/v) of edible carrier to liquid flavour extract. The mixture was homogenized in a shear homogenizer for 5 min at 800-1000rpm until the carrier and extracts were mixed completely. The resultant slurry was lyophilized in a Telstar freeze-dryer (model: Bomb as de Vacio-2G6). The lyophilized sample was packaged and stored for further examination and study. The processing operations are shown in figure 3.6.

Table 3.1: Formulation of encapsulated spice extract product

Sample code	Spice	Extraction	Carrier type	Encapsulation ratio
TUM_{EYC1}	*C. longa*	40% Ethanol	White yam grit	2:1
TUM_{EYC2}	*C. longa*	40% Ethanol	White yam grit	3:1
TUM_{EWC1}	*C. longa*	40% Ethanol	Wheat grit	2:1
TUM_{EWC2}	*C. longa*	40% Ethanol	Wheat grit	3:1
AGP_{EYC1}	*X. aethiopica*	40% Ethanol	White yam grit	2:1
AGP_{EYC2}	*X. aethiopica*	40% Ethanol	White yam grit	3:1
AGP_{EWC1}	*X. aethiopica*	40% Ethanol	Wheat grit	2:1
AGP_{EWC2}	*X. aethiopica*	40% Ethanol	Wheat grit	3:1

Key:

TUM_{EYC} = *C. longa* extract with white yam-grit carrier

TUM_{EWC} = *C. longa* extract with wheat-grit carrier

AGP_{EYC} = *X. aethiopica* extract with white yam-grit carrier

AGP_{EWC} = *X. aethiopica* extract with wheat-grit carrier

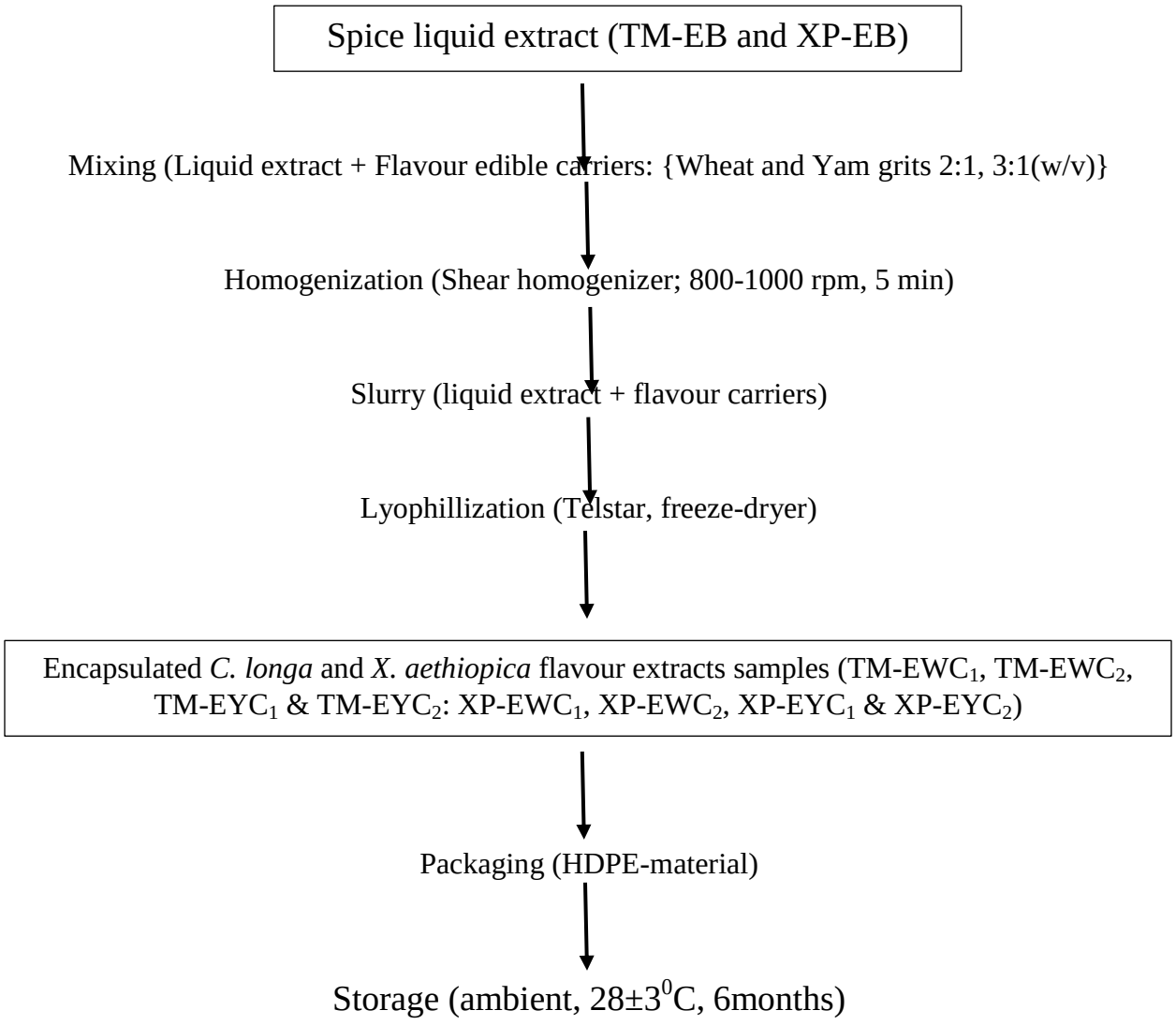


Figure 3.6: Flow diagram for the encapsulation of spice flavour samples

Key:

$TM\text{-}EWC_1$ = Wheat grit encapsulation at ratio 1:2 *C. longa* extract to carrier

$TM\text{-}EWC_2$ = Wheat grit encapsulation at ratio 1:3 *C. longa* extract to carrier

$TM\text{-}EYC_1$ = Yam grit encapsulation at ratio 1:2 *C. longa* extract to carrier

$TM\text{-}EYC_2$ = Yam grit encapsulation at ratio 1:3 *C. longa* extract to carrier

$XP\text{-}EWC_1$ = Wheat grit encapsulation at ratio 1:2 *X. aethiopica* extract to carrier

$XP\text{-}EWC_2$ = Wheat grit encapsulation at ratio 1:3 *X. aethiopica* extract to carrier

$XP\text{-}EYC_1$ = Yam grit encapsulation at ratio 1:2 *X. aethiopica* extract to carrier

$XP\text{-}EYC_2$ = Yam grit encapsulation at ratio 1:3 *X. aethiopica* extract to carrier

3.4 PACKAGING AND STORAGE OF SPICE PRODUCTS

3.4.1 Packaging of Spice Products

Each of the product samples prepared from each spice (ground, liquid extract and encapsulated spice product) was packaged in two types of packaging materials; plastic bottles (amber coloured type) and high density poly ethylene (HDPE) bags. The schematic representations are shown in figures 3.7 and 3.8 while some of the stages of operation are shown in plates 3.2a, 3.2b, 3.2c, 3.2d, 3.2e and 3.2f.

3.4.2 Storage of Spice Products

The samples were stored at ambient temperature of $\leq 28 \pm 3^{o}C$ for six months and analyzed for flavour principles at two weeks interval during the first two months and monthly for the remaining four months.

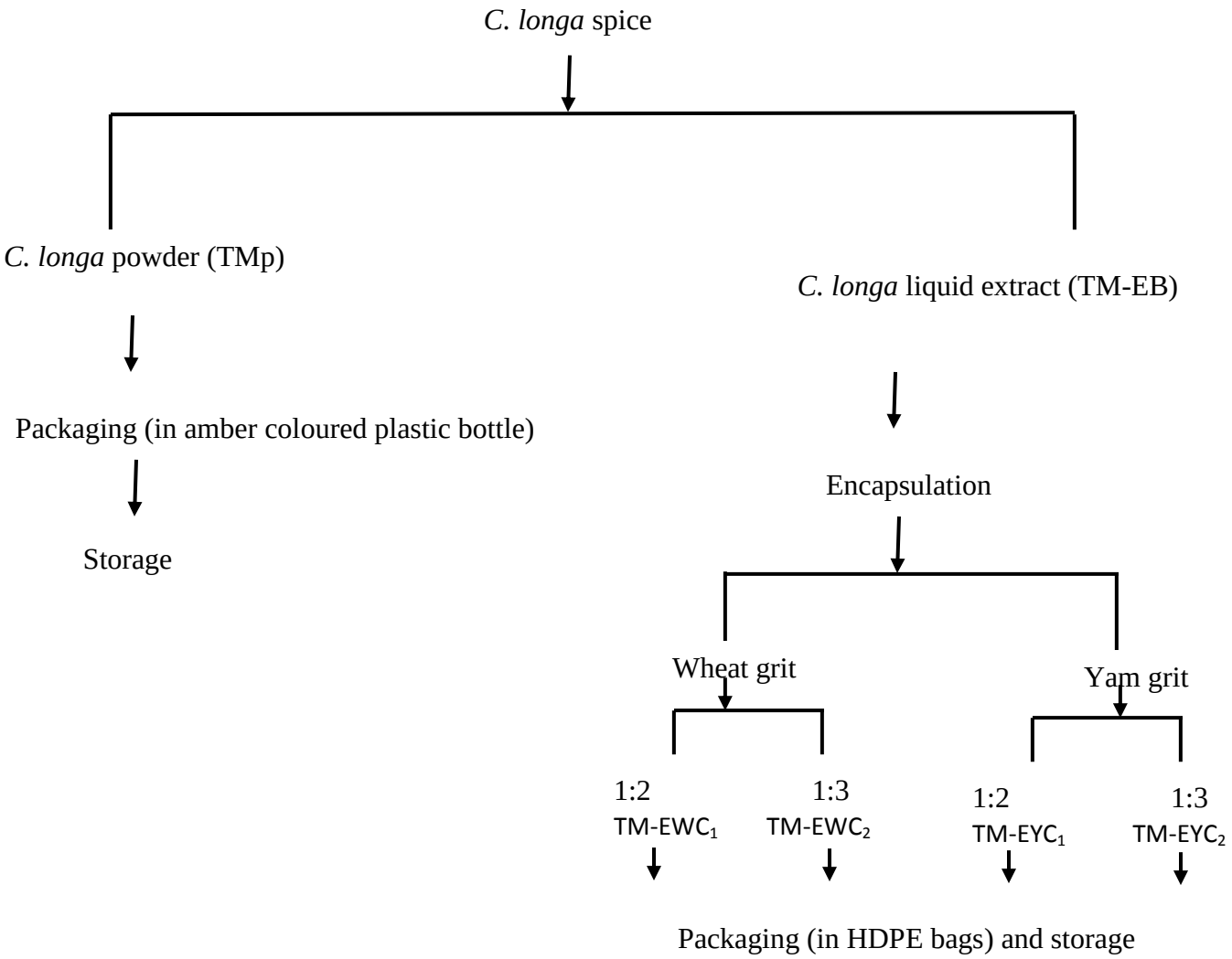


Figure 3.7: Schematic representation of the processes for production of *C. longa* spice products

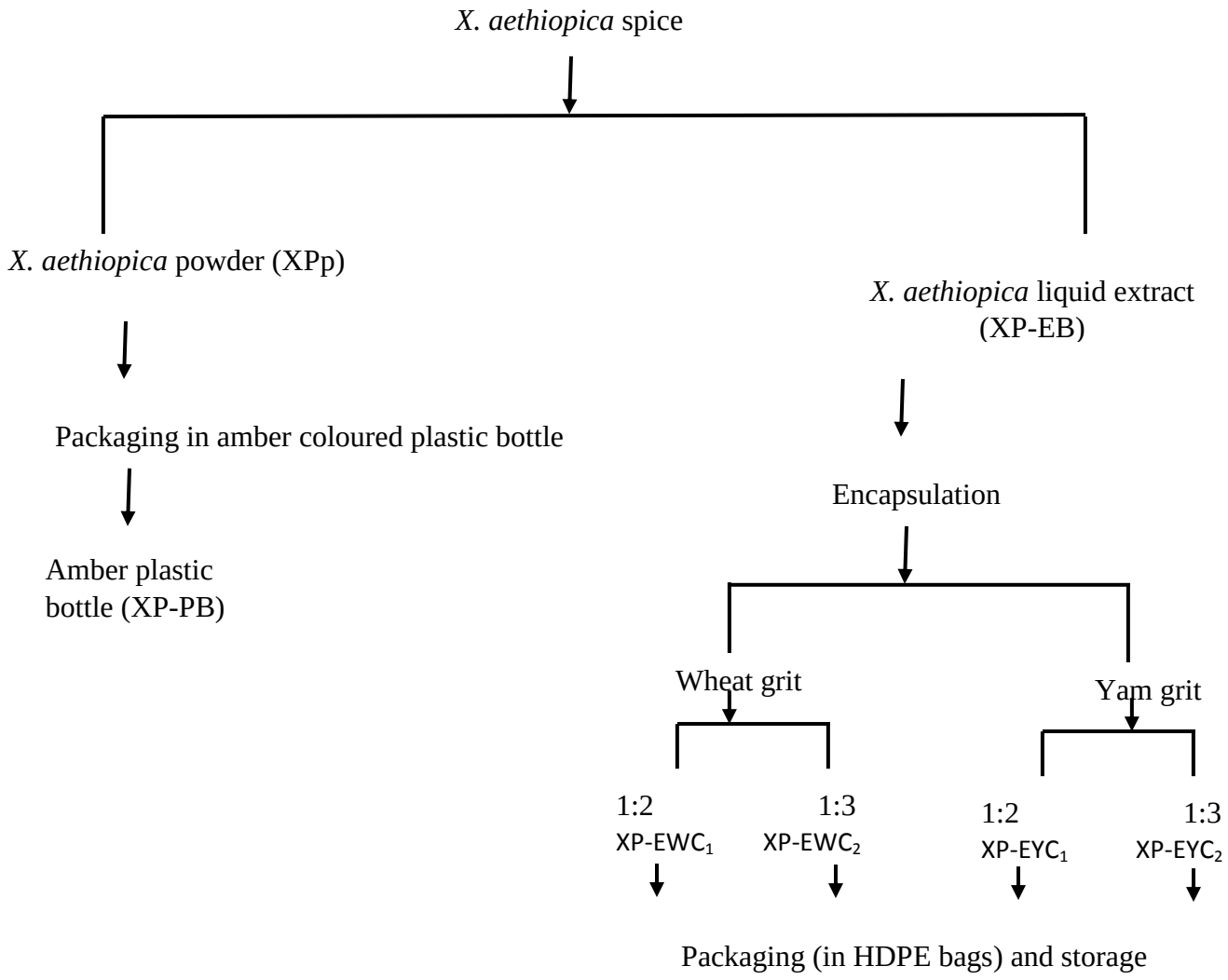


Figure 3.8: Schematic representation of the processes for production of *X. aethiopica* spice products

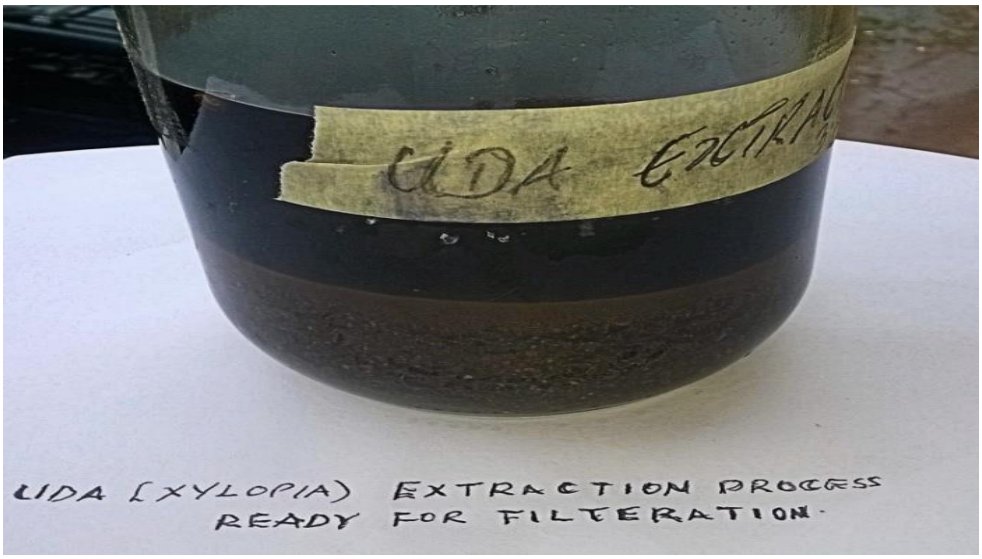


Plate 3.2a: Extraction of *X. aethiopica* (Uda) ready for filteration

Plate 3.2b: *X. aethiopica* (Uda) liquid flavour extract

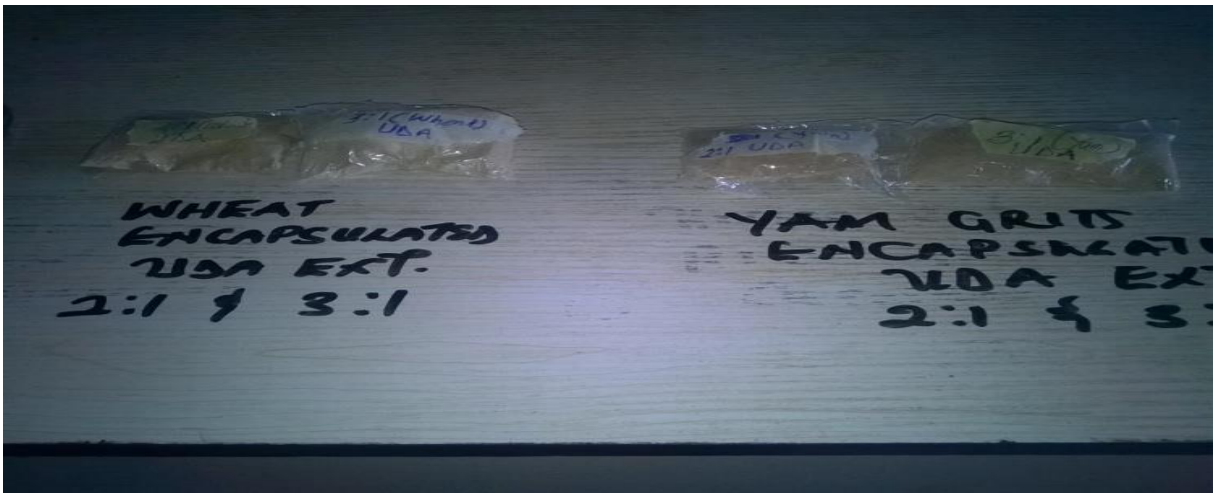


Plate 3.2c: *X. aethiopica* (Uda) encapsulated samples (with wheat grits and yam grits at ratio of 2:1 and 3:1 carrier to 40% ethanol extract)

Plate 3.2d: Packaging of *X. aethiopica* spice samples (40% ethanol extract and spice powder samples in amber coloured plastic bottles and encapsulated samples in HDPE bags)

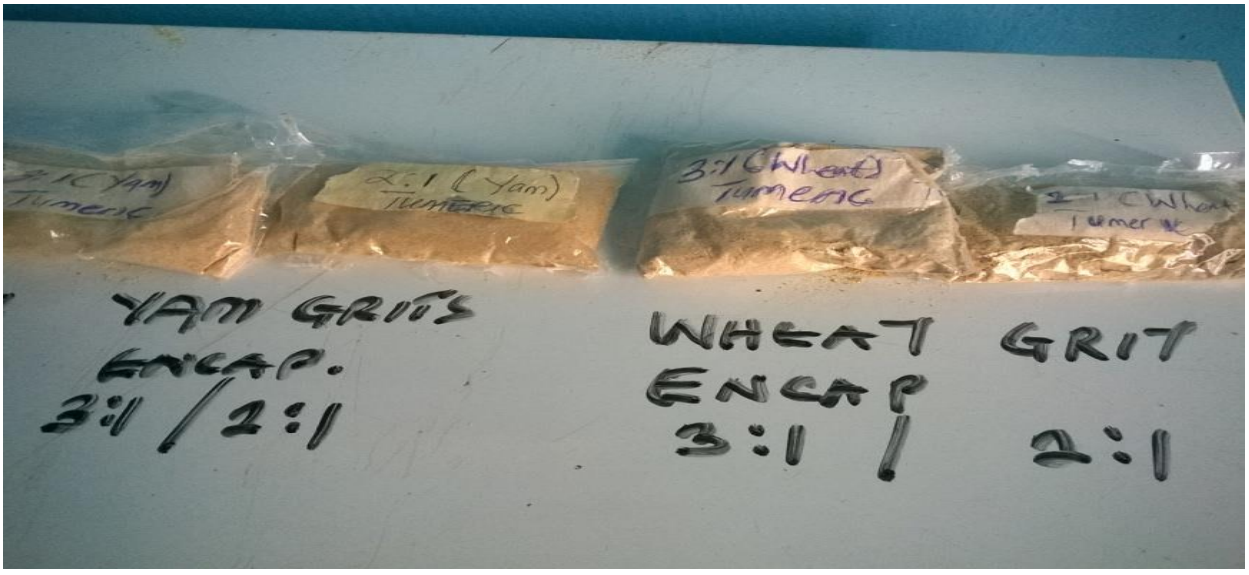


Plate 3.2e: *C. longa* encapsulated samples (with wheat grits and yam grits at ratio of 2:1 and 3:1 carrier to 40% ethanol extract)

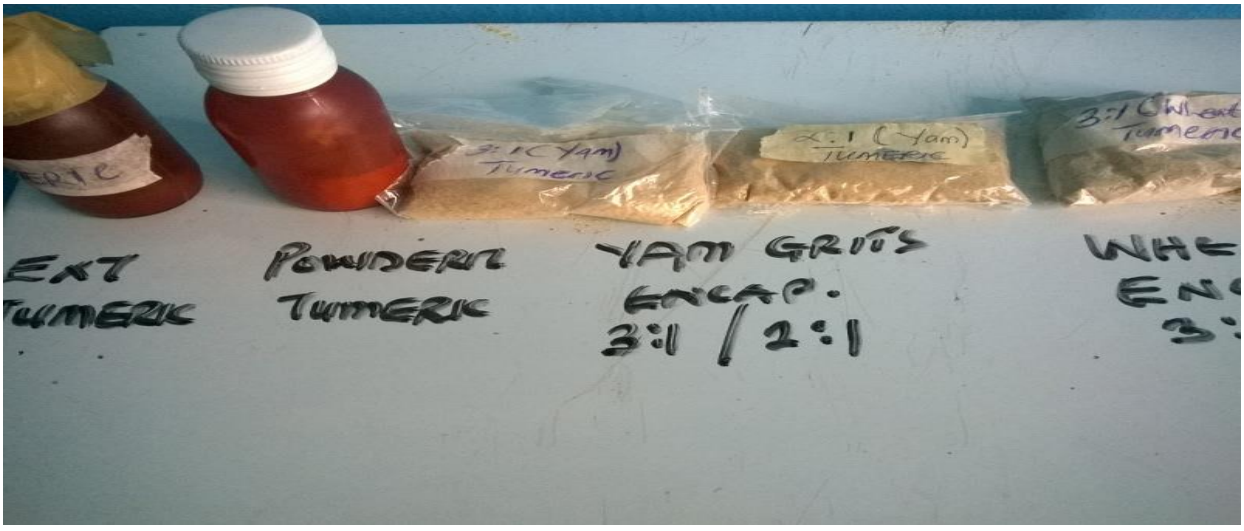


Plate 3.2f: Packaging of *C. longa* spice samples (40% ethanol extract and spice powder samples in amber coloured plastic bottles and encapsulated samples in HDPE bags)

3.5 DETERMINATION OF CHEMICAL COMPONENTS

3.5.1 Moisture Content

The standard AOAC method (2010) was used to determine the percentage moisture content of the samples. Two grams (2g) of the spice products were weighed into a well dried and weighed crucible and placed in an air-tight oven (Gallen Kamp Hot box size 1). The samples were dried at 60^{o}C for 24h, cooled in a dessicator and weighed. Each crucible with content was transferred back to the oven, dried, cooled for another 1h, and re-weighed. This process of drying, cooling and re-weighing continued until a constant weight was obtained. The loss in weight obtained represented the moisture content and was calculated with the following formula:

$$\% \text{ Moisture content} = \frac{W_1 - W_2}{W_1} \times \frac{100}{1} \quad \text{.....Eqn. 3.1}$$

Where:
W_1 = the weight of sample before drying.

W_2 = the weight of sample after drying.

Moisture content of each sample was obtained and the mean of duplicate determination calculated for each sample.

3.5.2 Determination of Specific gravity of the Liquid Extract

This was done according to the method of AOAC (2010). The specific gravity of the spice liquid extract was determined by weighing the dried empty specific gravity (density) bottle; filling it to mark with the liquid spice extract and re-weighing to determine the weight of the liquid extract, then filling the density bottle with water and weighing to determine the weight of the water. The specific gravity of the solvent extract was calculated with the formula:

$$\text{Specific gravity} = \frac{W_3 - W_1}{W_2 - W_1} \quad \text{.....Eqn. 3.2}$$

Where:
W_1 is the weight of empty density bottle.

W_2 is the weight of bottle plus water

W_3 is the weight of bottle plus liquid extract

3.5.3 Determination of Phytochemicals

The phytochemical components were determined on the raw spices (as control), liquid extracts and dried residues obtained from the extraction process. The phytochemicals evaluated included alkaloids, tannins, flavonoids, saponins and phytates (AOAC, 2010) and oxalates (Singleton and Rossi, 1999).

3.5.3.1 Determination of alkaloids

This was done by the alkaline precipitation gravimetric method described in AOAC (2010). Two grams (2g) of the sample was weighed and dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28^{o}C. It was later filtered with Whatman No. 42 filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH_4OH until the alkaloids were precipitated. The alkaloids precipitated were received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80^{o}C. The alkaloids content was calculated and expressed as a percentage of the weight of sample analyzed.

The percentage alkaloid was calculated using the formula:

$$\text{Alkaloid (\%)} = \frac{\text{Weight of alkaloid precipitate}}{\text{Weight of sample}} \times \frac{100}{1} \quad \text{..... Eqn. 3.3}$$

3.5.3.2 Determination of flavonoids

This was determined according to the method of AOAC (2010). Five grams of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through Whatman No. 42 filter paper. A measured volume (10ml) of the extract was treated with equal volume of ethyl acetate by drops. The flavonoids precipitated were recovered

by filtration using weighed filter paper. The resulting weight difference was used to get the weight of flavonoids in the sample.

The percentage flavonoids was calculated using the formula:

$$\text{Flavonoids (\%)} = \frac{W_2 - W_1}{W_3} \times \frac{100}{1} \quad \text{..... Eqn. 3.4}$$

Where

W_1 = Weight of filter paper

W_2 = Weight of filter paper + flavonoids precipitate

W_3 = Weight of sample

3.5.3.3 Determination of phytates

This was done according to the method of AOAC (2010). One gram of sample material was added with 0.2N HCl such that 3.30µg ml^{-1} phytate solution was obtained, then 0.5ml of extract was pipetted into a test tube fitted with a ground glass stopper. One milliliter (1ml) of the extract was added and the tube covered with a stopper and fixed with a clip. The tube was heated in a boiling water bath for 30 min. Care was taken to ensure that the tube remained well stoppered in the first 5 min. After cooling in ice water for 15 min, it was allowed to adjust to room temperature. When the tube reached the room temperature, the content of the tube was mixed and centrifuged for 30 min at 3000rpm. One milliliter (1ml) of the supernatant was transferred into another test tube and 1.5ml of solution added. The absorbance was measured at 519nm wavelength against distilled water (using a spectronic 20 England spectrophotometer).

Preparation of the calibration curve was carried out by plotting the concentrations of the reference solutions against their corresponding absorbance. Then the absorbance of each test sample was used to obtain its concentration from the calibration curve.

3.5.3.4 Determination of saponins

The Spectrophotometric method of AOAC (2010) was used for saponin analysis. One gram (1 g) of finely ground sample was weighed into a 250ml beaker and 100ml Isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker and 20 ml of 40% saturated solution of magnesium carbonate added. The mixture obtained with saturated $MgCO_3$ was again filtered through a Whatman No 1 filter paper to obtain a clear colourless solution. Then 1 ml of the colourless solution was pipetted into a 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water. The solution was allowed to stand for 30 min for a blood-red colour to develop. Some 0-10ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2ml of 5% $FeCl_3$ solution as done for 1 ml of the sample above. The absorbances of the samples as well as the standard saponin solutions were read (after colour development) on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm.

The percentage saponin was calculated using the formula:

$$\text{Saponin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x 10,000}} \times \frac{100}{1} \quad \ldots\ldots \text{Eqn. 3.5}$$

3.5.3.5 Determination of tannins

The method of AOAC (2010) was used for the determination of tannin contents of the differently prepared samples. A zero point two grams (0.2 g) portion of the finely ground sample was measured into a 50 ml beaker. Then 20 ml of 50% methanol was added, covered with paraffin and placed in a water bath at 80^{o}C for 1h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. One milliliter (1 ml) of the sample extract was pipetted into 50 ml volumetric flask, then 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2C0_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20min when bluish-green colouration developed. Some standard tannic acid solutions of 0-10ppm were treated similarly as the 1ml of sample above. The absorbances of the tannic acid standard solutions as well as the samples were read (after colour development) on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm.

The percentage tannin was calculated using the formula:

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x 10,000}} \times \frac{100}{1} \quad \text{…….. Eqn. 3.6}$$

3.5.3.6 Determination of oxalates

This determination was carried out using the method described by Singleton and Rossi (1999). This method involves three major steps; digestion, precipitation and permanganate titration.

Sample digestion:

Two grams (2g) of sample was suspended in 190ml of distilled water in a 250ml volumetric flask. It was followed by the addition of 10ml of 6M HCl and the suspension digested at 100°C for 1h. The suspension was cooled and made up to 250ml mark before filtration.

Oxalate precipitation:

Duplicate portions of 125ml of the filtrate were measured into beakers and four drops of methyl red indicator added. This was followed by the addition of Conc. NH_4OH solution (drop wise) until the test solution changed from salmon pink colour to a faint yellow colour (pH 4 - 4.5). Each of the duplicate portions was then heated to 90°C, cooled and filtered to remove precipitates containing ferrous ion. The filtrate was again heated to 90°C and 10ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 5°C. The solution was then centrifuged at 2500 rpm for 5 min. The supernatant was decanted and the precipitates completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

Permanganate titration:

At this point, the total filtrate resulting from the digestion of 2g of sample was made up to 300ml. Aliquots of 125mL of the filtrate was heated until near-boiling and then titrated against 0.05M standardized $KMnCO_4$ solution to a faint pink colour which persists for 30s. The calcium oxalate content was calculated using the formula.

$$\text{Oxalate (\%)} = \frac{T \times (Vme)(Df) \times 10^5}{(ME) \times Mf} \quad \text{(mg/100g)} \quad \text{.....Eqn. 3.7}$$

Where T is the litre of $KMnO_4$ (ml), Vme is the volume - mass equivalent (i.e $1cm^3$ of 0.05M $KMnCO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid), Df is the dilution factor V_T/A (2.4 where V_T is the total volume of titrate (300ml) and A is the aliquot used (125 ml), ME

is the molar equivalent of $KMnO_4$ oxalate ($KMnO_4$ redox reaction) and Mf is the mass of sample used.

3.6 DETERMINATION OF FLAVOUR PRINCIPLES

3.6.1 Preparation of samples for GC-MS analysis

A 3g weight of each powdered samples was accurately transferred into a 50ml centrifuge tube and extracted with 7ml of the particular extraction solvent (absolute ethanol, 40% ethanol, n-Hexane, acetone, methanol and distilled water). The mixture was shaken for 20min using Stuart vortex mixer (SA7) and allowed to stand for 72h. The mixture was then centrifuged at 4000rpm for 40min using Hanil MF80 centrifuge (Korea). The supernatant was taken with the aid of a micro pipette into a vial for GC-MS analysis.

3.6.2 GC-MS analysis of *X. aethiopica* samples

The beta-phellandrene and other flavour components were determined using the GC-MS procedure described by Owokotomo and Ekundayo (2012).

The GC-MS analysis was conducted using a Shimadzu QP2010 Ultra GC-MS (Japan) instrument equipped with reference libraries (NIST/EPA/NIH). One microliter (1µl) of the sample was injected at 250^{o}C into a capillary column, type Rtx.5MS, 30m x 0.25µm x 0.25mm stationary phase using helium (99.999%) as carrier gas at a flow rate of 1.5ml/min and the injection mode used was split 2:1. The column oven temperature was programmed from initial 100^{o}C (2min holding time) to final 250^{o}C at an increase rate of 15^{o}C/min. The detector temperature was 250^{o}C. The mass spectrometer (MS) was operated at an ion source temperature of 250^{o}C and

ionization voltage of 70eV. The acquisition mode was scan mode (35-600) m/z. Library search was carried out using Wiley GC-MS library. The individual identifications were made by the comparison of fragmentation pattern with those found in the library of the mass spectrometer and literature (Adams, 2001).

3.6.3 GC-MS analysis of *C. longa* samples

The GC-MS analysis was conducted using a Shimadzu QP2010 Ultra GC-MS (Japan) instrument equipped with reference libraries (NIST/EPA/NIH). The initial temperature was 40^{o}C and final temperature was 250^{o}C. One microliter (1µl) of the sample was injected at 40^{o}C into a capillary column, type Rtx.5MS, 30m x 0.25µm x 0.25mm stationary phase using helium (99.999%) as carrier gas at a flow rate of 1.5ml/min and the injection mode used was split 10:1. The column oven temperature was programmed from initial 40^{o}C (2min holding time) to final 250^{o}C at an increase rate of 6^{o}C/min. The detector temperature was 250^{o}C. The mass spectrometer (MS) was operated at an ion source temperature of 200^{o}C and ionization voltage of 70eV. The acquisition mode was scan mode (35-600) m/z. Library search was carried out using Wiley GC-MS library. The individual identifications were made by the comparison of fragmentation patterns with those found in the library of the mass spectrometer and literature (Adams, 2001).

3.6.4 Curcumin

The spice samples were analyzed for the Curcumin component using an Ultra-Fast Liquid Chromatograph (UFLC). The procedure described by Vipul *et al.* (2013) was modified for the identification of flavour compounds and quantification of the values in the spice samples.

Preparation of standard solution of curcumin

A standard stock solution of curcumin was prepared by accurately weighing 5mg of standard curcumin into a 10ml stoppered test tube and dissolved with 5ml of mobile phase solution (acetonitrile, 2% tetrahydrofuran and 2% acetic acid in the ratio of 50:30:20 v/v). The solution in the stoppered test tube was shaken properly for about 1min with a votex mixer to obtain 1mg ml^{-1}or 1000µg ml^{-1} of stock solution. The standard solution was obtained by diluting 1ml of the stock solution up to 10ml with mobile phase solution to obtain 100 µg ml^{-1}. The solution was filtered through a 0.45-micro syringe filter and the resultant solution was used as standard solution for UFLC analysis.

Preparation of samples for UFLC analysis

A 2g portion of each powdered *C. longa* sample was accurately weighed into a 50ml centrifuge tube and was dissolved with 6ml of a particular extraction solvent (absolute ethanol, 40% ethanol, n-Hexane, acetone, methanol and distilled water). The mixture was shaken for 20min using Stuart vortex mixer SA7 and allowed to stand for 24h. The mixture was then centrifuged at 4000rpm for 30min using Hanil MF80 centrifuge (Korea). The supernatant was taken with the aid of a micro pipette into a vial for UFLC analysis.

Determination of appropriate wavelength

A curcumin solution (100µg ml^{-1}) was ran in the UFLC and multiple wavelengths were monitored in the UV region (400-500nm) by using photodiode array detector (PDA). The optimum absorption (highest intensity) was achieved at 425nm, and the spectrum view also showed curcumin peak at the same 425nm.

Development of calibration curve for curcumin

Some working solutions for a calibration curve were prepared from the standard stock solution by appropriately diluting the standard stock solution to obtain concentrations ranging from 2.5 $\mu g\ ml^{-1}$ - 40 $\mu g\ ml^{-1}$.

Some of the *C. longa* liquid extract samples (from acetone, 40% ethanol, absolute ethanol and n-hexane) were further diluted by taking 0.5ml and making it up to 5ml with the individual solvents used for its extraction while the methanol and water (control) samples were analysed without further dilution. A calibration curve was plotted for each corresponding peak area against each standard concentration and the slope value and correlation coefficient (R^2) value were calculated using the equation;

$y=20302x$ ……Eqn. 3.8

where y is the peak area of curcumin and x is the concentration.

Appropriate dilution factors were applied where dilution was done.

$$\text{Dilution factor (D.F)} = \frac{\text{Volume of diluted sample solution}}{\text{Volume of aliquot taken for dilution}} \quad \ldots\ldots\ldots\ldots\ldots \text{Eqn. 3.9}$$

D.F = 5ml/0.5ml =10

Instrument and chromatographic conditions

A Shimadzu (Kyoto, Japan), prominence ultra-fast liquid chromatograph (UFLC) instrument equipped with LC-20AD solvent delivery gradient pump with an injection loop volume of 5 µl, 7725i Rheodyne injector attached to a data solution of LC solution software using a Photodiode array detector (PDA) was utilized for the development and validation (Shanmugam *et al.*, 2014). Separation was achieved on a Phenomenex Gemini-Luna 5u C_{18}(2)100A column using a degassed mobile phase of acetonitrile, 2% tetrahydrofuran and 2% acetic acid in the ratio of

50:30:20 v/v respectively but in two phase combinations; Mobile phase A {2% tetrahydrofuran (30%) and 2% acetic acid (20%)} and Mobile phase B {acetonitrile (50%)} with a flow rate of 0.7ml min^{-1} on a gradient elution. The standard and samples were monitored for UV absorption at a wavelength of 425nm with injection volume of 5µl and column oven temperature of 35^{o}C. Curcumin was identified in each sample by comparing the retention time (RT) and spectra obtained from the sample and standard solutions.

3.7 MICROBIOLOGICAL ANALYSIS

3.7.1 Determination of Microbial load

The microbial count was done according to ICMSF (2002). Five hundred grams (500g) of each fresh *X. aethiopica* and *C. longa* were soaked in 1L of peptone water for 2h respectively. Then 10ml of the aliquot from each sample was dispersed in 90ml of sterile peptone water. Again, in a separate container, ten grams (10g) of each dried ground spice was dispersed in 90 ml of sterile peptone water. Each sample was homogenized by shaking vigorously and serially diluted to 10^{-10} dilution. An aliquot portion (0.1ml) of the 3rd up to 9th serial dilution was inoculated in duplicate onto the Potato Dextrose agar (PDA), Nutrient agar (NA) and MacConkey agar (MA) for the isolation of fungi, bacteria and coliforms respectively. Again, 0.1ml from the 2nd up to 5th dilution was inoculated in duplicate onto Brain Heart Infusion agar (BHIA) for isolation of spore formers. Potato Dextrose agar plates were incubated for 48h at ambient temperature (28$\pm$2^{o}C) for fungal growth. The nutrient agar plates were inoculated using the spread plate method and incubated for 48h at 37^{o}C for total viable count (Pelezar *et al.*, 1993). The MacConkey agar plates were incubated at 37^{o}C for 24h for coliform bacteria (Cheesbrough, 2000; Pelezar *et al.*, 1993). The Brain Heart Infusion agar plates were incubated at 50^{o}C for 48h for detection of vegetative cells (Hauchild, 1989; Kramer and Gilbert, 1989). The microbial colony count was done using the Gallenkamp electronic colony counter.

3.7.2 Isolation into pure cultures

From the incubated plates, a sterile wire loop was used to pick on the different colonies and streaked on the surface of fresh agar plates; Nutrient agar for bacteria and Potato Dextrose agar for yeast and moulds. The plates for bacteria were incubated at 37^{o}C for 24h while the plates for yeast and moulds were incubated at 30$\pm$2^{o}C for 72h.

At the end of incubation, the plates were retrieved and with a sterile wire loop little portions of the colonies were taken and streaked on the surface of freshly prepared agar slants; Nutrient agar slant for bacterial cultures and Potato Dextrose agar slant for yeast and mould cultures. The bacterial slants were incubated at 37^{o}C for 18h while the yeast and mould slants were incubated at 30^{o}C for 72h. After incubation, the bijou bottles were firmly closed and were subsequently stored in the refrigerator at 4^{o}C until required. The bijou bottles were appropriately labelled.

3.7.3 Identification of Microflora

The identity of the bacterial and yeast isolates was determined based on the colonial, microscopic and biochemical characteristics (Cheesbrough, 2000; Harrigan and McLance, 1990). The characteristics of the bacterial isolates were matched against those in Buchannan and Gibbon (1994) while those of the yeast were matched with features presented in Harrigan and McLance (1990).Cultures of moulds were identified based on macro and micro morphology, together with reverse and surface colouration of colonies grown on Potato Dextrose agar (PDA) (Harrigan and McLance, 1990).

3.7.4 Spore Staining Test

The isolates were heat-fixed on a slide and flooded with 5% malachite green. It was heated for 3 min (without allowing it to boil), dried, cooled and then rinsed off, and stained with Safranin for 30 sec. This was also rinsed, dried with filter paper and viewed under the microscope using oil

immersion lens (x 100) where the positive spores appeared green while the vegetative cells stained pink (ICMSF, 2002).

3.7.5 Biochemical Tests

Catalase Test

This was done to determine the ability of the isolates to produce an enzyme – catalase that splits hydrogen peroxide to oxygen and water. A drop of H_2O_2 was placed on the surface of a clean, grease- free slide and mixed thoroughly with a loop full of the isolate. The effervescence due to the liberation of oxygen indicated a positive catalase reaction.

Coagulase Test

This test differentiates *Staphylococcus aureus* which produces the enzyme, coagulase from the non-coagulase producing Staphylococcus (*S. epidermidis* and *S. saprophyticus*). The enzyme coagulase causes plasma to clot by converting fibrinogen to fibrin (Benson, 1998). A drop of physiological saline was placed on a clean slide and a colony of the isolate was emulsified in the drop which resulted to a thick suspension. A drop of rabbit plasma was added to the suspension and mixed gently for 10 seconds. A clumping (coagulation) showed a positive result.

Oxidase Test

The test depends on the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and ∞-naphthol. This method uses N, N-dimethyl-p-phenylenediamine oxalate. In the presence of the enzyme cytochrome oxidase the N, N-dimethhyl-p-phenylenediamine oxalate and ∞-naphthol react to produce indophenol blue. Ten

drops of freshly prepared oxidase reagent (1% tetramethyl-p-phenylene-diamine dihydro-chloride) were placed on a sterile filter paper to soak it. The isolates were streaked on the soaked filter paper. The appearance of a purple colour after 10 seconds indicated a positive oxidase reaction.

Sugar Fermentation/Oxidation

This test was used to differentiate between bacterial groups that oxidize carbohydrate. One milliliter (1ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately transferred into duplicate tubes containing 9ml of sterile Hugh and Leifson's medium under aseptic conditions to obtain a final concentration of 1% of each of the sugars. The tubes were stab-inoculated in duplicates while two uninoculated tubes served as control. Vaseline was used to cover one set of the duplicate tubes, to discourage oxidative utilization of sugar. All tubes were incubated at 37^{o}c for 48h. After the incubation, they were observed for acid production in the culture. Yellow colouration indicated acid production in the open tubes (oxidative utilization of the sugar) while acid production in the sealed tubes suggested a fermentative reaction.

Hydrogen Sulphide Production (H_2S) Test

The test isolates were aseptically inoculated into a tube containing Triple Sugar Iron agar, by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37^{o}c for 72h and was examined daily. Black precipitation and yellow colouration was checked for. Black precipitate indicated H_2S production and yellow colouration indicated sucrose, lactose and glucose fermentation.

Urease Test

Urease Agar slant in McCartney bottle was inoculated with the bacterial isolate and incubated at 30^{o}C for 4 h and then overnight. A pink colour in the medium indicated a positive result.

IMViC Tests

These test consisted of four different tests; namely Indole production test, Methyl-Red test, Voges- Proskaeur test and Citrate utilization test. These tests were specifically designed to determine the physiological properties of microorganisms. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

Indole Test

This test was done to demonstrate the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacterial isolates were inoculated into the medium and incubated at 37°C for 48 h. At the end of the incubation period, 3 drops of Kovac's reagent was added and then shaken. A red colour ring at the interface of the medium denoted a positive result.

Methyl Red Test/ Voges-Proskaeur Test

Methyl red (MR) and Voges-Proskaeur (VR) test were considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+. Into one milliliter of the culture, one milliliter of six percent alcoholic solution of alpha-naphtol and one milliliter of 16% KOH were added and left to stand for 20 minutes. The development of red to pink colour was a positive result for Methyl red test which

showed that the organism can produce acid from the fermentation of sugar (dextrose) while negative result (Voges-Proskaeur) showed that the organism produced acetoin from the glucose.

Citrate Utilization Test

This is one of the several techniques used to assist in the identification of Enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon. The test was carried out using Simmon's citrate agar.

The slants of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to streak on the slant with a saline suspension of the test organisms before stabbing the butt. The bottles were incubated at 35^{o}C for 48 h. Bright blue colour in the medium meant a positive result while no change in colour of medium indicated negative citrate test (Cheesbrough, 2000).

3.8 SENSORY EVALUATION

The sensory evaluation of the experimental spice food samples was carried out using the scoring test method described by Iwe (2002). The sensory characteristics of the samples such as taste, aroma (flavour) and general acceptability were done by a team of seven (7) semi-trained panelists for product-oriented test and twenty (20) panelists for consumer preference test. This test was done in order to determine the perceptible differences on the sensory attributes of the samples during and after development. Consumer-oriented test was conducted to determine product`s acceptability and preferences on selected food dishes {rice, meat, porridge, Yoruba

local soup, pepper soup, ugba, stew, Igbo local soup, sauce, tea, akamu (pap), Hausa local soup, baked bread, ice cream, yoghurt, non –alcoholic beverage (pineapple juice) and alcoholic beverage (palm wine)} using the 7-point hedonic scale (7 = very much liked, 6 = moderately liked, 5 = slightly liked, 4 = neither liked nor disliked, 3 = slightly disliked, 2 = moderately disliked, 1 = very much disliked) and the spice products from *X. aethiopica* were tried on pepper soup while those from *C. longa* were tried on akamu (pap) as they were highly recommeneded in those foods by the panelists. The panelists were drawn from staff and students of the Department of Food Science and Technology, Federal University of Technology, Owerri, who are familiar with the spices used in this research work.

Three of the *X. aethiopica* spice products (the powder, the 40% ethanol extract and the wheat grit encapsulation product at a ratio of 1:2 spice extract to carrier) were used to spice pepper soup. One litre of pepper soup broth was made by boiling 500g of cat fish, a pinch of ground fresh pepper and one grated onion in 1.5L of portable water and a pinch of salt was added to taste. The mixture was cooked for 30min to form the pepper soup stock. A 200mL portion of the pepper soup stock was added with 5g of each of the three spice products and stirred thoroughly, then allowed to cool to 40^{o}C prior to sensory evaluation.

The *C. longa* spiced *akamu* (pap) was prepared by making a slurry of 500g of freshly prepared white maize pap with 250mL cold water and stiring thoroughly. One liter of potable water was boiled to 100^{o}C and the boiled water was added into the slurry in a stainless bowl and stirred consistently to avoid formation of lumps. The prepared *akamu* (pap) was divided into three portions of 200mL each. Then, 5g of each of the three *C. longa* spice products (the powder, the 40% ethanol extract and the yam grit encapsulation product at a ratio of 1:2 spice extract to

carrier) was added into each portion of *akamu*, stirred thoroughly and allowed to cool to 40^{o}C before serving for sensory evaluation.

3.9 EXPERIMENTAL DESIGN

A 2x6 factorial design comprising of 2 types of indigenous spices (*C. longa* and *X. aethiopica*) and six solvents giving a total of twelve (12) samples, was used at the preliminary phase (extraction process). A 2x6x2 factorial design comprising of 2 spices, 6 spice product forms and 2 packaging materials giving rise to a total of 24 samples, was adopted during the product development phase of this research work. Therefore, the total number of samples was 36 in all. The data obtained were presented as tables, graphs and charts where necessary.

3.10 STATISTICAL ANALYSIS

The data obtained from the different analyses were subjected to various statistical analyses which included simple descriptive mean and standard deviation, Analyses of variance (ANOVA), while multiple comparison tests (Turkey and Duncan's tests) were used to separate means where significant differences exist. The statistical package for social science (SPSS) 20.0 Software Inc. USA was used.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

The results obtained in the study are presented in the tables below.

4.1.1 Moisture content of the spice products

Table 4.1: The Moisture Content of *X. aethiopica* and *C. longa* Flavour Samples Encapsulated in Wheat and Yam Grits at different Ratios

Spice samples	Moisture content (%) (*X. aethiopica*)	Moisture content (%) (*C. longa*)
Powder	$9.22^{a}\pm0.02$	$10.01^{a}\pm0.01$
Wheat grit encapsulated with spice extract at 1:2 spice to carrier	$7.45^{c}\pm0.03$	$6.45^{e}\pm0.02$
Wheat grit encapsulated with spice extract at 1:3 spice to carrier	$7.88^{b}\pm0.01$	$6.72^{d}\pm0.02$
Yam grit encapsulated with spice extract at 1:2 spice to carrier	$6.98^{d}\pm0.02$	$7.33^{b}\pm0.01$
Yam grit encapsulated with spice extract at 1:3 spice to carrier	$7.01^{d}\pm0.01$	$7.01^{c}\pm0.02$
LSD	0.02550	0.01789

($\pm$) = Means and standard deviation of triplicate sample, a,b,c,… = mean scores with different superscripts down the column are significantly different ($p<0.05$). LSD = Least significant difference

4.1.2 Specific gravity of spice flavour extraction

Table 4.2: The Specific gravity Values of *X. aethiopica* and *C. longa* Extracts with different Solvents at 1:5 Spice: Solvent Ratio

Solvent	Specific gravity	Specific gravity of *X. aethiopica* extract	Increase in Specific gravity of X. aethiopica	Specific gravity of *C. longa* extract	Increase in Specific gravity of *C. longa*
Absolute ethanol	0.795	1.079	$0.284^{c}\pm0.02$	1.007	$0.212^{e}\pm0.001$
40% ethanol	0.935	1.361	$0.426^{a}\pm0.002$	1.505	$0.570^{a}\pm0.001$
Methanol	0.791	1.043	$0.252^{d}\pm0.003$	1.023	$0.232^{c}\pm0.001$
Acetone	0.790	1.166	$0.376^{b}\pm0.001$	1.082	$0.292^{b}\pm0.001$
n-Hexane	0.659	0.859	$0.20^{f}\pm0.0$	0.887	$0.228^{d}\pm0.001$
Water	1.000	1.235	$0.235^{e}\pm0.001$	1.200	$0.20^{f}\pm0.0$
LSD			0.00227		0.00096

($\pm$) = Mean and standard deviation of triplicate sample, a,b,c,… = mean scores with different superscripts down the column are significantly different ($p<0.05$). LSD = Least significant difference

4.1.3: Phytochemical Content of Solvent Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spices

Table 4.3.1: The Alkaloids Content (%) of Liquid Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spice Samples

Spice extract sample	*X. aethiopica* samples			*C. longa* samples		
	Liquid	Residue	Total	Liquid	Residue	Total
Ethanol (absolute) extract	$20.93^{a}\pm.0.1$	$2.24^{c}\pm0.0$	$23.16^{a}\pm0.01$	$3.05^{d}\pm0.03$	$1.50^{c}\pm0.02$	$4.55^{d}\pm0.01$
Ethanol (40%) extract	$21.50^{b}\pm0.0$	$1.49^{f}\pm0.02$	$22.56^{a}\pm0.01$	$5.76^{c}\pm0.02$	$1.61^{b}\pm0.02$	$7.41^{b}\pm0.0$
Methanol extract	$7.54^{c}\pm0.02$	$2.79^{a}\pm0.0$	$10.33^{b}\pm0.0$	$6.05^{b}\pm0.02$	$1.06^{f}\pm0.03$	$7.12^{c}\pm0.03$
Acetone extract	$2.61^{e}\pm0.01$	$2.15^{d}\pm0.01$	$4.76^{d}\pm0.03$	$2.39^{e}\pm0.01$	$1.46^{d}\pm0.01$	$3.86^{e}\pm0.04$
N-Hexane extract	$3.59^{d}\pm0.01$	$2.53^{b}\pm0.02$	$6.12^{c}\pm0.03$	$6.54^{a}\pm0.02$	$2.10^{a}\pm0.01$	$8.65^{a}\pm0.01$
Water extract	$1.90^{f}\pm0.02$	$1.57^{e}\pm0.02$	$3.47^{e}\pm0.02$	$1.93^{f}\pm0.03$	$1.16^{e}\pm.02$	$3.10^{f}\pm0.02$
LSD	0.00957	0.01000	0.23733	0.02958	0.00707	0.02958

($\pm$) = Mean and standard deviation of triplicate sample, a,b,c,.. = mean scores with different superscripts down the same column are significantly different ($p<0.05$). LSD = Least significant difference

Table 4.3.2: The Flavonoids Content (%) of Liquid Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spice Samples

Spice extract sample	Samples from *X. aethiopica*			Samples from *C. longa*		
	Liquid	Residue	Total	Liquid	Residue	Total
Ethanol (absolute) extract	$3.63^{c}\pm0.02$	$2.49^{f}\pm0.01$	$6.12^{d}\pm0.01$	$3.40^{d}\pm0.02$	$2.36^{d}\pm0.03$	$5.76^{d}\pm0.01$
Ethanol (40%) extract	$0.35^{f}\pm0.02$	$6.04^{b}\pm0.0$	$6.40^{c}\pm0.02$	$5.05^{c}\pm0.01$	$3.71^{b}\pm0.03$	$8.76^{b}\pm0.0$
Methanol extract	$8.80^{a}\pm0.01$	$5.49^{c}\pm0.02$	$14.29^{a}\pm0.02$	$6.05^{b}\pm0.02$	$2.64^{c}\pm0.01$	$8.70^{b}\pm0.02$
Acetone extract	$4.11^{b}\pm0.02$	$9.85^{a}\pm0.01$	$13.96^{b}\pm0.02$	$2.40^{e}\pm0.0$	$0.66^{f}\pm0.01$	$3.07^{e}\pm0.02$
N-Hexane extract	$2.41^{d}\pm0.0$	$3.06^{e}\pm0.02$	$5.47^{e}\pm0.01$	$6.55^{a}\pm0.03$	$1.09^{e}\pm0.01$	$7.65^{c}\pm0.03$
Water extract	$0.59^{e}\pm0.01$	$3.50^{d}\pm0.01$	$4.10^{f}\pm0.01$	$1.93^{f}\pm0.03$	$7.58^{a}\pm0.02$	$9.57^{a}\pm0.03$
LSD	0.01118	0.00707	0.01258	0.02958	0.00866	0.04378

($\pm$) = Mean and standard deviation of triplicate sample, a,b,c,… = mean scores with different superscripts down the same column are significantly different ($p<0.05$). LSD = Least significant difference.

Table 4.3.3: The Phytic acid Content (%) of Liquid Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spice Samples

Spice extract sample	Samples from *X. aethiopica*			Samples from *C. longa*		
	Liquid	Residue	Total	Liquid	Residue	Total
Ethanol (absolute) extract	$2.90^{e}\pm0.02$	$1.47^{d}\pm0.01$	$4.37^{f}\pm0.03$	$6.93^{f}\pm0.01$	$1.78^{c}\pm0.01$	$8.71^{f}\pm0.02$
Ethanol (40%) extract	$4.26^{c}\pm0.03$	$3.72^{a}\pm0.02$	$8.14^{c}\pm0.01$	$12.85^{b}\pm0.03$	$3.63^{b}\pm0.0$	$16.49^{a}\pm0.02$
Methanol extract	$3.71^{d}\pm0.01$	$3.42^{b}\pm0.02$	$7.13^{d}\pm0.03$	$14.95^{a}\pm0.01$	$1.34^{d}\pm0.02$	$16.29^{b}\pm0.02$
Acetone extract	$4.11^{c}\pm0.01$	$0.91^{e}\pm0.01$	$5.03^{e}\pm0.02$	$9.52^{d}\pm0.02$	$1.10^{e}\pm0.02$	$10.63^{d}\pm0.01$
N-Hexane extract	$6.20^{b}\pm0.01$	$2.87^{c}\pm0.01$	$9.08^{b}\pm0.01$	$7.49^{e}\pm0.01$	$1.36^{d}\pm0.01$	$8.86^{e}\pm0.01$
Water extract	$9.24^{a}\pm0.03$	$3.71^{a}\pm0.02$	$12.96^{a}\pm0.01$	$9.69^{c}\pm0.02$	$3.67^{a}\pm0.02$	$13.37^{c}\pm0.03$
LSD	0.08699	0.01000	0.01041	0.0086	0.00707	0.01443

($\pm$) = Mean and standard deviation of triplicate sample, a,b,c,…= mean scores with different superscripts down the same column are significantly different ($p<0.05$). LSD = Least significant difference.

Table 4.3.4: The Saponins content (%) of Liquid Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spice Samples

Spice extract sample	Samples from *X. aethiopica*			Samples from *C. longa*		
	Liquid	Residue	Total	Liquid	Residue	Total
Ethanol (absolute) extract	0.21^{f}±0.0	0.19^{d}±0.03	0.41^{e}±0.01	0.02^{e}±0.02	0.44^{a}±0.04	0.47^{e}±0.01
Ethanol (40%) extract	0.50^{e}±0.02	0.38^{b}±0.03	0.89^{d}±0.01	0.31^{c}±0.01	0.42^{a}±0.01	0.74^{b}±0.03
Methanol extract	3.49^{a}±0.03	0.17^{e}±0.01	3.67^{a}±0.02	0.47^{b}±0.01	0.19^{c}±0.01	0.67^{c}±0.01
Acetone extract	1.35^{c}±0.01	0.15^{e}±0.0	1.51^{c}±0.02	0.0^{e}±0.03	0.21^{c}±0.03	0.21^{f}±0.02
N-Hexane extract	1.47^{b}±0.01	0.20^{c}±0.01	1.68^{b}±0.03	1.0^{a}±0.03	0.30^{b}±0.03	1.31^{a}±0.02
Water extract	0.83^{d}±0.02	0.79^{a}±0.02	1.63^{b}±0.01	0.24^{d}±0.02	0.32^{b}±0.01	0.57^{d}±0.01
LSD	0.00707	0.00707	0.01000	0.00645	0.00707	0.01041

(±) = Mean and standard deviation of triplicate sample, a,b,c,… = mean scores with different superscripts down the same column are significantly different ($p<0.05$). LSD=Least significant difference.

Table 4.3.5: The Tannins content (%) of Liquid Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spice Samples

Spice extract sample	Samples from *X. aethiopica*			Samples from *C. longa*		
	Liquid	**Residue**	**Total**	**Liquid**	**Residue**	**Total**
Ethanol (absolute) extract	$1.19^{b}\pm0.02$	$0.11^{b}\pm0.02$	$1.31^{c}\pm0.01$	$0.63^{d}\pm0.02$	$0.17^{a}\pm0.03$	$0.81^{d}\pm0.01$
Ethanol (40%) extract	$0.97^{d}\pm0.02$	$0.14^{a}\pm0.01$	$1.12^{d}\pm0.01$	$1.46^{a}\pm0.01$	$0.10^{b}\pm0.03$	$1.57^{a}\pm0.03$
Methanol extract	$2.13^{a}\pm0.03$	$0.11^{b}\pm0.02$	$2.25^{a}\pm0.02$	$1.20^{b}\pm0.02$	$0.11^{b}\pm0.01$	$1.32^{b}\pm0.01$
Acetone extract	$0.56^{f}\pm0.04$	$0.11^{b}\pm0.03$	$0.68^{f}\pm0.03$	$0.76^{c}\pm0.01$	$0.12^{b}\pm0.02$	$0.89^{c}\pm0.02$
N-Hexane extract	$0.086^{e}\pm0.03$	$0.13^{ab}\pm0.04$	$1.0^{e}\pm0.02$	$0.19^{f}\pm0.03$	$0.10^{b}\pm0.01$	$0.30^{f}\pm0.01$
Water extract	$1.22^{b}\pm0.01$	$0.15^{a}\pm0.04$	$1.38^{b}\pm0.01$	$0.22^{e}\pm0.01$	$0.12^{b}\pm0.01$	$0.35^{e}\pm0.02$
LSD	0.00707	0.00707	0.01000	0.00707	0.00707	0.01000

($\pm$) = Mean and standard deviation of triplicate sample, a,b,c,… = mean scores with different superscripts down the same column are significantly different ($p<0.05$). LSD = Least significant difference.

Table 4.3.6: The Oxalate content (mg100g^{-1}) of Liquid Extracts and Extraction Residues of *X. aethiopica* and *C. longa* Spice Samples

Spice extract sample	Samples from *X. aethiopica*			Samples from *C. longa*		
	Liquid	Residue	Total	Liquid	Residue	Total
Ethanol (absolute) extract	$0.80^{b}\pm0.02$	$0.98^{a}\pm0.03$	$1.79^{a}\pm0.02$	$0.54^{e}\pm0.01$	$0.36^{d}\pm0.03$	$0.91^{e}\pm0.01$
Ethanol (40%) extract	$0.35^{e}\pm0.01$	$0.44^{c}\pm0.01$	$0.80^{e}\pm0.02$	$1.08^{b}\pm0.02$	$0.26^{e}\pm0.02$	$1.35^{c}\pm0.02$
Methanol extract	$0.44^{d}\pm0.01$	$0.35^{e}\pm0.02$	$0.80^{e}\pm0.01$	$0.81^{c}\pm0.0$	$0.62^{a}\pm0.01$	$1.43^{b}\pm0.01$
Acetone extract	$0.54^{c}\pm0.02$	$0.36^{d}\pm0.01$	$0.91^{d}\pm0.01$	$0.45^{f}\pm0.01$	$0.45^{b}\pm\mathbf{0.0}$	$0.91^{e}\pm0.01$
N-Hexane extract	$1.25^{a}\pm0.01$	$0.27^{f}\pm0.02$	$1.53^{b}\pm0.03$	$1.61^{a}\pm0.03$	$0.47^{b}\pm0.03$	$2.09^{a}\pm0.02$
Water extract	$0.45^{d}\pm0.02$	$0.87^{b}\pm0.01$	$1.33^{c}\pm0.02$	$0.72^{d}\pm0.02$	$0.41^{c}\pm0.02$	$1.13^{d}\pm0.02$
LSD	0.00707	0.00707	0.01291	0.00707	0.00816	0.01041

($\pm$) = Mean and standard deviation of triplicate sample, a,b,c,… = mean scores with different superscripts down the same column are significantly different ($p<0.05$). LSD = Least significant difference.

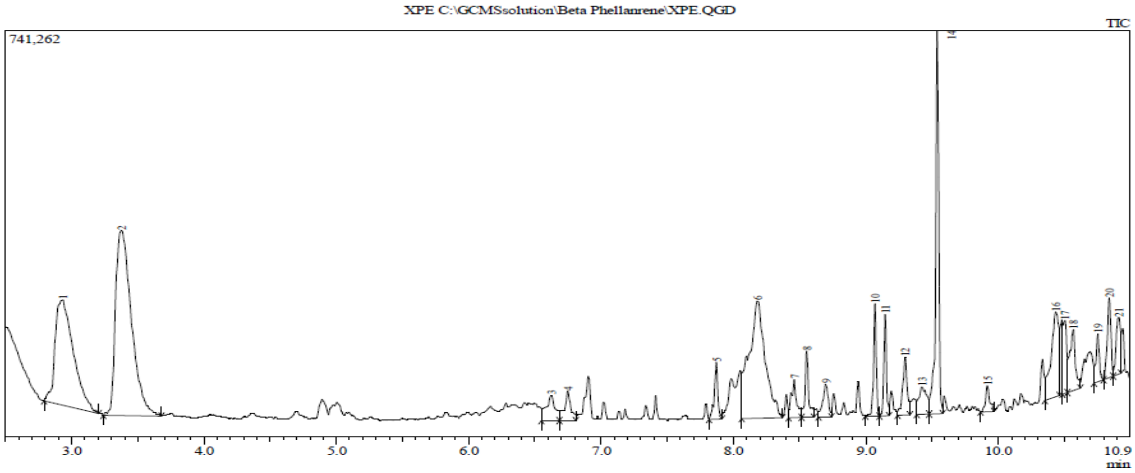


Figure 4.1: Peaks of major flavour compounds in the absolute ethanol extract of *X. aethiopica*

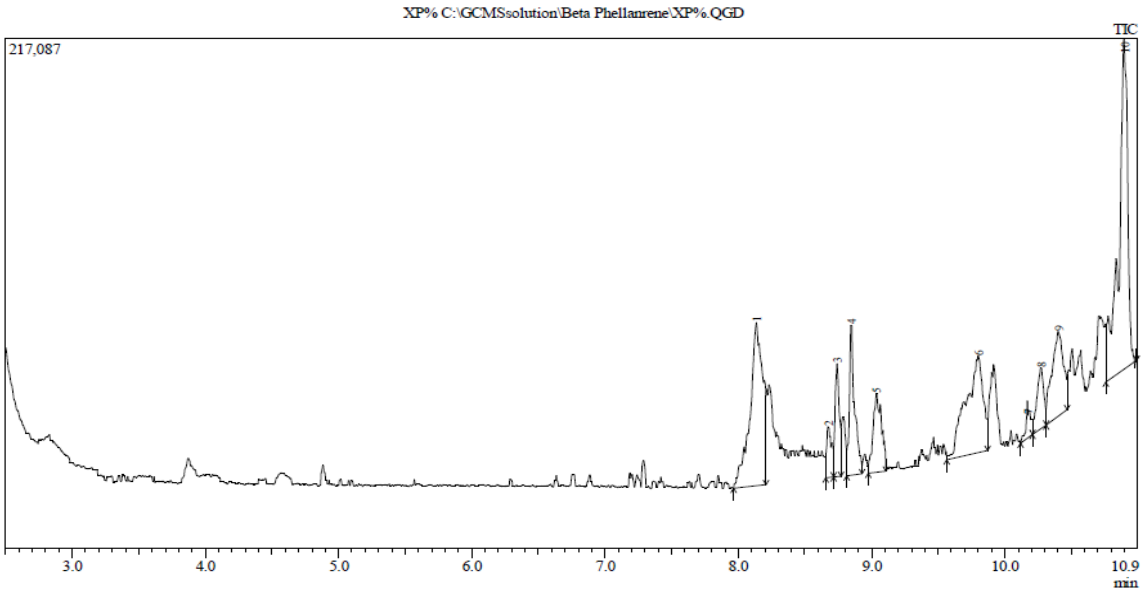


Figure 4.2: Peaks of major flavour compounds in the 40% ethanol extract of *X. aethiopica*

4.1.4: Content (%) of Major Flavour Principles (Compounds) Identified in *X. aethiopica* and *C. longa* Spice Extracts

Table 4.4.1: The Mean Concentration (%) of Major Flavour Principles (Compounds) identified in Ethanol Extracts of *X. aethiopica*

Compounds Identified	Concentration (%) Absolute Ethanol Extract	Concentration (%) 40% Ethanol Extract
Beta-pinene	15.48	20.28
Trans-beta-ocimene	7.59	9.61
Beta-Phellandrene	18.93	23.93
Terpinen-4-ol	4.40	Nil
Kauren-18-ol acetate, (4.beta)	11.33	Nil
(-)- Spathulenol	2.46	Nil
Cholestane,4-5-epoxy-(4-alpha,5-alpha)	Nil	27.88
Pregan -20- one	2.16	Nil
Andrographolide	9.66	8.06
Aromadendrene	5.42	Nil
5-alpha- Ergost -8- (14)- ene	2.24	Nil
Androsta -1,4-dien -3- one	2.75	Nil
1,6- Cyclodecadiene (Germacrene D)	7.40	Nil
Ledene oxide – (11)	3.52	Nil
n-Hexadecanoic acid	2.89	Nil

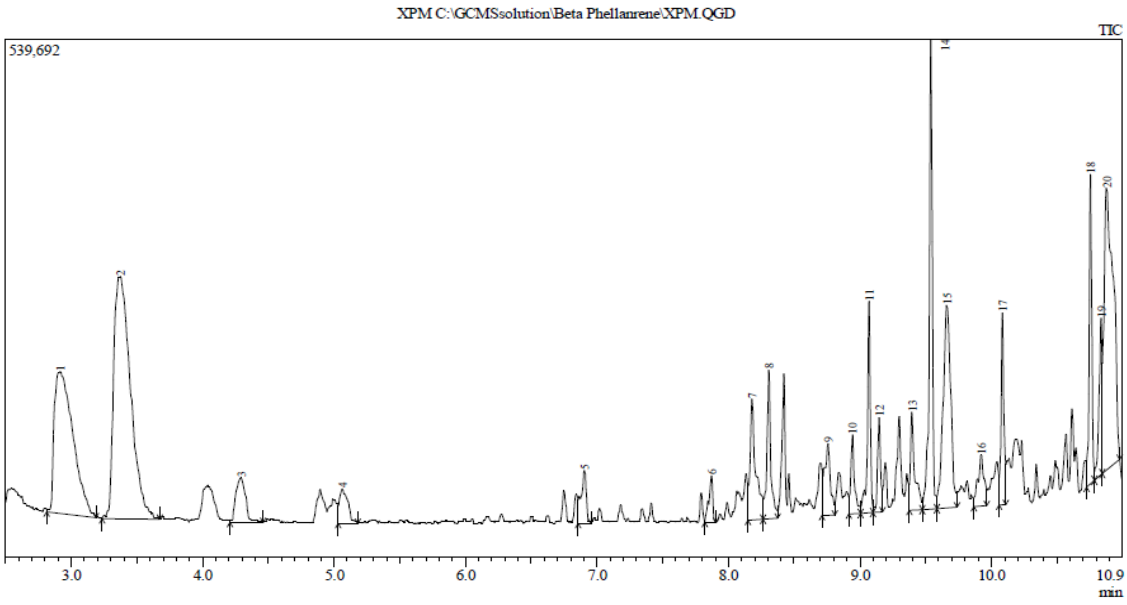


Figure 4.3: Peaks of major flavour compounds in the methanol extract of *X. aethiopica*

Table 4.4.2: The Mean Concentration (%) of Major Flavour Principles (Compounds) identified in Methanol Extract of *X. aethiopica*

Compounds Identified	Concentration (%)
Beta pinene	14.03
Beta-Phellandrene	21.54
(-)- Spathulenol	3.33
Bioallethrin	3.03
Andrographolide	7.67
Ar-turmerone	21.56
Kaur-16-ene	2.38
5-Eicosene-(E)	8.20
Thumbergol	2.08
Cholestane,4-5-epoxy-(4-alpha,5-alpha)	13.25

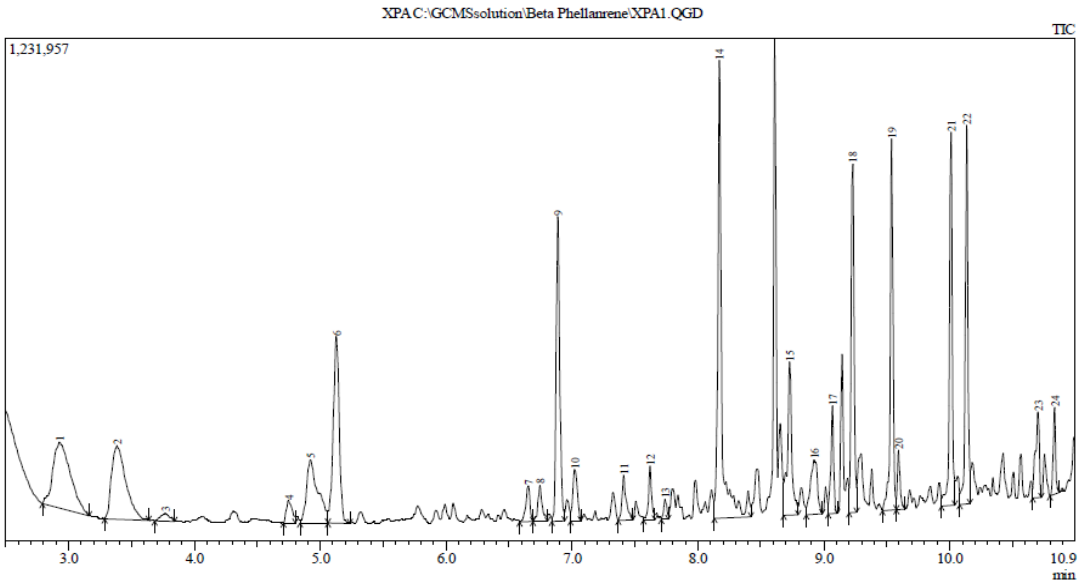


Figure 4.4: Peaks of major flavour compounds in the acetone extract of *X. aethiopica*

Table 4.4.3: The Mean Concentration (%) of Major Flavour Principles (Compounds) identified in Acetone Extract of *X. aethiopica*

Compounds Identified	Concentration (%)
Beta-pinene	8.11
Beta. Phellandrene	7.38
3-Tridecene (z)	5.08
1-Pentadecene	7.85
1-Tridecene	7.78
1-heptadecene	12.76
Aromadendrene	4.63
Pregan -20- one, 2-hydroxy-5,6-expoxy	2.68
(-)- Spathulenol	2.15
Androstan -17-one, 3ethyl-3-hydroxy-5 alpha	7.16
Andrographolide	7.29
1-Nonadecene	7.19
9-Tricosene (z)	6.86
Aromadendrene oxide(2)	2.24

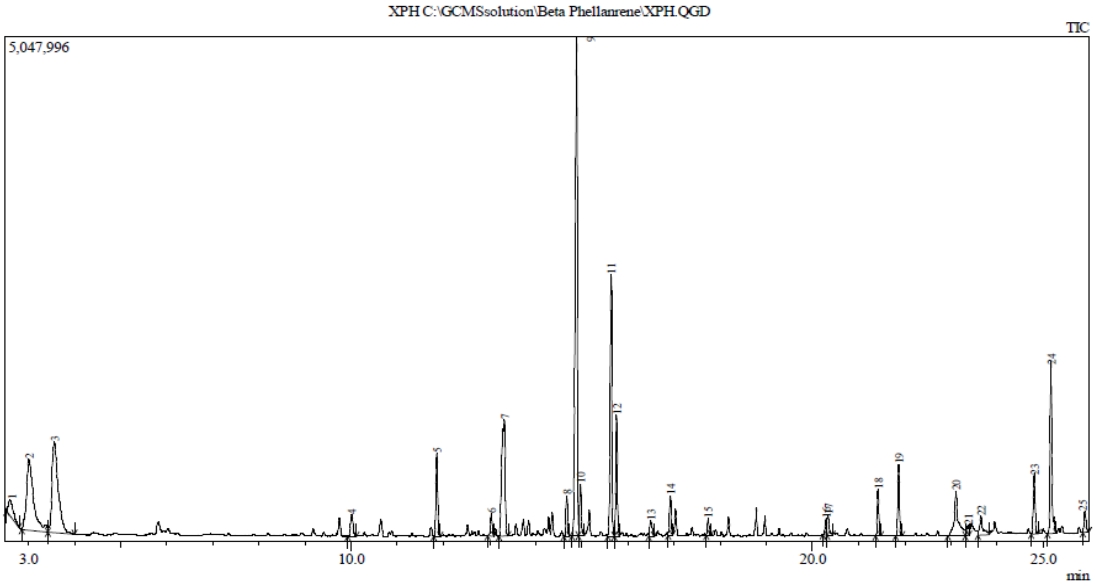


Figure 4.5: Peaks of major flavour compounds in the n-hexane extract of *X. aethiopica*

Table 4.4.4: The Mean Concentration (%) of Major Flavour Principles (Compounds) identified in n-Hexane Extract of *X. aethiopica*

Compounds Identified	Concentration (%)
Bicyclo [3,1,1] heptanes,6,6-dimethyl -2-methylene	10.80
Beta-Phellandrene	12.09
1,6-cyclodecadiene, 1-methyl, 1-methyl -5-methylene	3.06
Diethyl Phthalate	8.83
Ar-Turmerone	21.56
Curlone	9.41
Andrographolide	4.20
Kaur-16-ene	2.35
Oleic acid	3.84
Thumbergol	2.06
Card-20(22)-enolide,3,5,14,19-tetrahydroxy	6.71

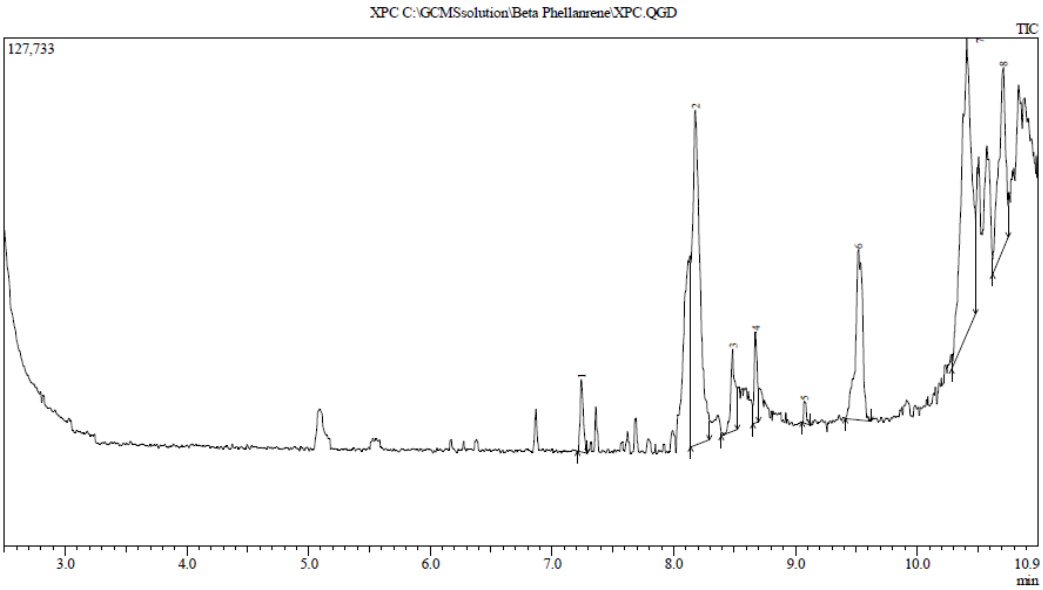


Figure 4.6: Peaks of major flavour compounds in the water (aqueous) extract of *X. aethiopica*

Table 4.4.5: The Mean Concentration (%) of Major Flavour Principles (Compounds) identified in Water (Aqueous) Extract of *X. aethiopica*

Compounds Identified	Concentration (%)
Benzene-ethanol, 4-hydroxy	2.53
Bioallethrin	28.31
Beta-Phellandrene	4.29
Phthalic acid	3.15
Adipic acid	13.67
Pregan-20-one	33.26
Pregan-4-ene -1, 20-dione	14.12

Table 4.4.6: The Contents (%) of Common Flavour Compounds in Solvent Extract Samples of *X. aethiopica*

Chemical compound	Ethanol (absolute) extracts %	Ethanol (40% conc.) %	Methanol Extract %	Acetone Extract %	n-hexane extract %	Aqueous extract %	LSD
Beta-pienene	$15.48^{b}\pm0.02$	$20.28^{a}\pm0.02$	14.03^{c}	$8.11^{d}\pm0.02$	ND	ND	0.01500
Beta-Phellandrene	$18.93^{c}\pm0.02$	$23.93^{a}\pm0.02$	$21.54^{b}\pm0.02$	$7.38^{e}\pm0.02$	$12.09^{d}\pm0.01$	$4.29^{f}\pm0.01$	0.01848
Andrographolide	$9.66^{a}\pm0.01$	$8.06^{b}\pm0.02$	$7.67^{c}\pm0.01$	$7.29^{d}\pm0.01$	$4.20^{e}\pm0.0$	ND	0.01472

ND = Not detected

(±) = Mean and standard deviation of duplicate analysis, a,b,c,… = mean scores with different superscripts across the rows are significantly different ($p<0.05$). LSD = Least significant difference

4.1.5 Retention of Most Predominant Flavour Compound in *X. aethiopica*

Table 4.5.1: The Concentrations (%) of the Most Predominant Flavour Compound (Beta-Phellandrene) in *X. aethiopica* Spice Products during 6 months Storage under Ambient Temperature

Spice product sample	Ambient storage period									
	Zero hr	**2wks**	**4wks**	**6wks**	**2months**	**3months**	**4months**	**5months**	**6months**	**LSD**
Powder	$19.42^{a}\pm0.02$	$19.40^{a}\pm0.1$	$19.16^{b}\pm0.01$	$18.01^{b}\pm0.0$	$17.46^{b}\pm0.02$	$15.90^{e}\pm0.01$	$15.01^{f}\pm0.01$	$14.75^{g}\pm0.03$	$14.22^{h}\pm0.02$	0.0190
40% Ethanol Extract.	$23.93^{a}\pm0.01$	$23.81^{a}\pm0.01$	$23.18^{a}\pm0.01$	$23.01^{a}\pm0.02$	$21.48^{b}\pm0.02$	$21.02^{c}\pm0.02$	$20.11^{cd}\pm0.06$	$19.96^{cd}\pm0.01$	$19.56^{d}\pm0.01$	0.23138
Wheat Grit Encapsulation (1:2 ratio).	$19.66^{a}\pm0.02$	$19.12^{b}\pm0.02$	$19.0^{c}\pm0.0$	$18.33^{d}\pm0.03$	$17.96^{e}\pm0.02$	$17.0^{f}\pm0.02$	$15.83^{g}\pm0.01$	$14.94^{h}\pm0.01$	$13.54^{i}\pm0.01$	0.0190
Wheat Grit Encapsulation (1:3 ratio).	$15.96^{a}\pm0.02$	$15.18^{b}\pm0.01$	$14.06^{c}\pm0.02$	$13.86^{d}\pm0.02$	$13.14^{e}\pm0.01$	$12.83^{f}\pm0.02$	$10.65^{g}\pm0.02$	$9.82^{h}\pm0.01$	$8.96^{i}\pm0.01$	0.01716
Yam Grit Encapsulation (1:2 ratio).	$17.92^{a}\pm0.02$	$17.01^{b}\pm0.01$	$16.64^{c}\pm0.02$	$16.64^{c}\pm0.02$	$14.84^{d}\pm0.01$	$13.74^{e}\pm0.01$	$10.41^{f}\pm0.01$	$9.86^{g}\pm0.01$	$9.77^{h}\pm0.01$	0.01528
Yam Grit Encapsulation (1:3 ratio).	$16.15^{a}\pm0.02$	$15.03^{b}\pm0.01$	$14.01^{c}\pm0.02$	$13.58^{d}\pm0.01$	$12.66^{e}\pm0.01$	$11.28^{f}\pm0.02$	$9.36^{g}\pm0.01$	$8.11^{h}\pm0.03$	$7.06^{i}\pm0.02$	0.02014

(±) = Mean and standard deviation of duplicate analysis, a,b,c,… = mean scores with different superscripts across the rows are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.5.2: Percentage Retention of the Most Predominant Flavour compound (Beta-Phellandrene) in *X. aethiopica* Spice Products during 6 months Storage under Ambient Temperature

Spice product sample	Ambient storage period									
	Zero hr	**2wks**	**4wks**	**6wks**	**2months**	**3months**	**4months**	**5months**	**6months**	**LSD**
Powder	100.00^{a}±0.01	99.90^{a}±0.1	98.66^{b}±0.01	92.74^{b}±0.0	89.91^{b}±0.02	81.87^{e}±0.01	77.29^{f}±0.01	75.95^{g}±0.03	73.22^{h}±0.02	0.0190
40% Ethanol Extract.	100.00^{a}±0.01	99.50^{a}±0.01	96.87^{a}±0.01	96.15^{a}±0.02	89.76^{b}±0.02	87.84^{c}±0.02	84.04^{cd}±0.06	83.41^{cd}±0.01	81.74^{d}±0.01	0.23138
Wheat Grit Encapsulation (1:2 ratio).	100.00^{a}±0.01	97.25^{b}±0.02	96.64^{c}±0.0	93.23^{d}±0.03	91.35^{e}±0.02	86.47^{f}±0.02	80.52^{g}±0.01	75.99^{h}±0.01	68.87^{i}±0.01	0.0190
Wheat Grit Encapsulation (1:3 ratio).	100.00^{a}±0.01	95.11^{b}±0.01	88.10^{c}±0.02	86.84^{d}±0.02	82.33^{e}±0.01	80.39^{f}±0.02	66.73^{g}±0.02	61.53^{h}±0.01	56.14^{i}±0.01	0.01716
Yam Grit Encapsulation (1:2 ratio).	100.00^{a}±0.01	94.92^{b}±0.01	92.86^{c}±0.02	92.86^{c}±0.02	82.81^{d}±0.01	76.67^{e}±0.01	58.09^{f}±0.01	55.02^{g}±0.01	54.52^{h}±0.01	0.01528
Yam Grit Encapsulation (1:3 ratio).	100.00^{a}±0.01	93.06^{b}±0.01	86.75^{c}±0.02	84.09^{d}±0.01	78.39^{e}±0.01	69.85^{f}±0.02	57.96^{g}±0.01	50.22^{h}±0.03	43.72^{i}±0.02	0.02014

(±) = Mean and standard deviation of duplicate analysis, a,b,c,… = mean scores with different superscripts across the rows are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

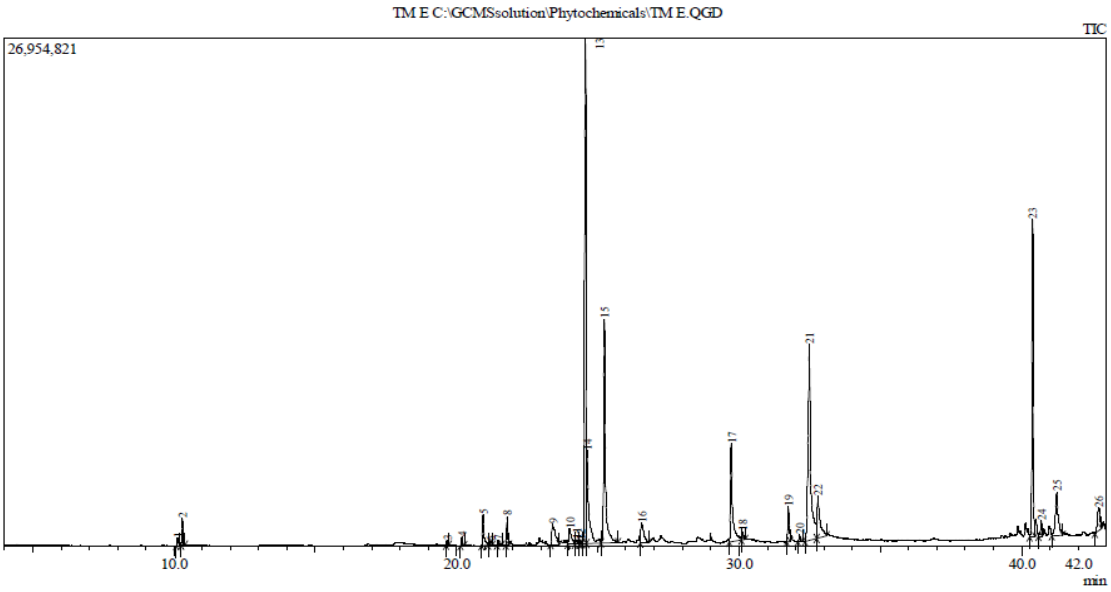


Figure 4.7: Peaks of concentration of major compounds in the 40% ethanol extract of *C. longa*

4.1.6 Profile and Concentrations of Compounds in Ethanol Extract of *C. longa*

Table 4.6: The Profile and Concentrations of Major Compounds in 40% Ethanol Extract of *C. longa*

Chemical compound	Concentration (%)
Ar-turmerone	22.31
Turmerone	6.36
Curlone	11.88
N – hexadecanoinc acid	6.80
Oleic acid	16.65
Octadecanioc acid	3.71
Curcumin	12.02
Stigmasterol	4.35

4.1.7 Curcumin Content of different Solvent Extracts of *C. longa*

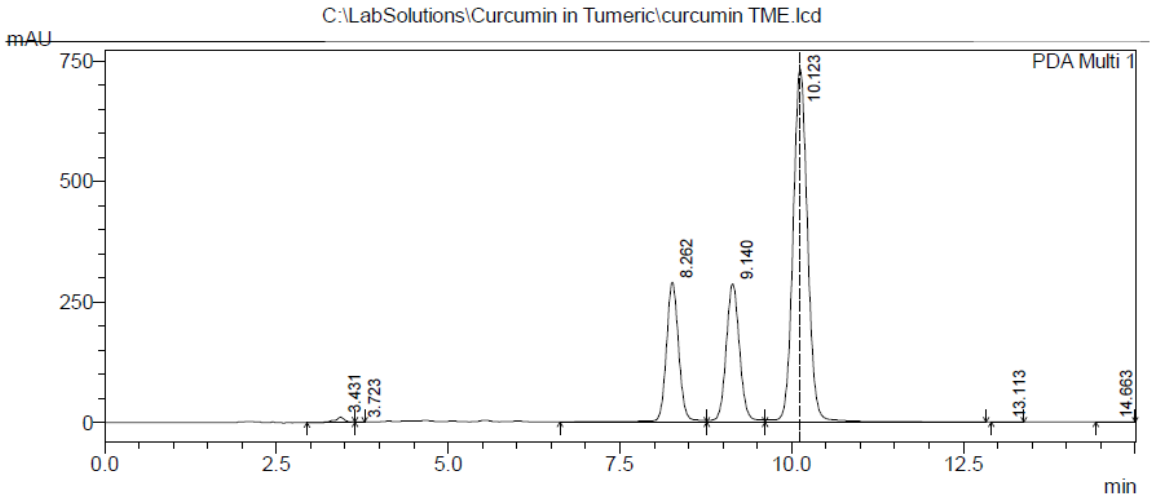


Figure 4.8: Peaks of major compounds in the absolute ethanol extract of *C. longa*

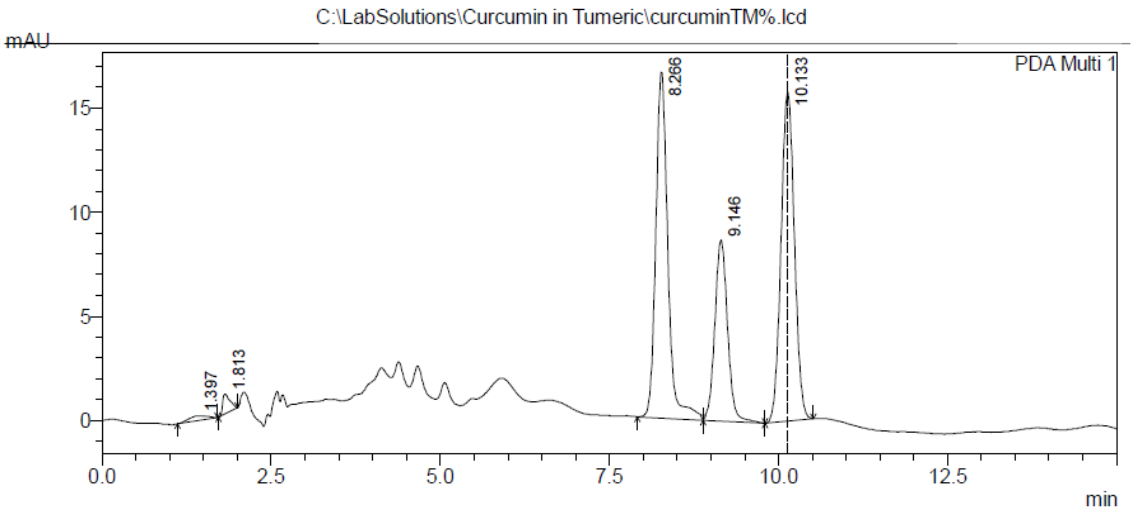


Figure 4.9: Peaks of major compounds in the 40% ethanol extract of *C. longa*

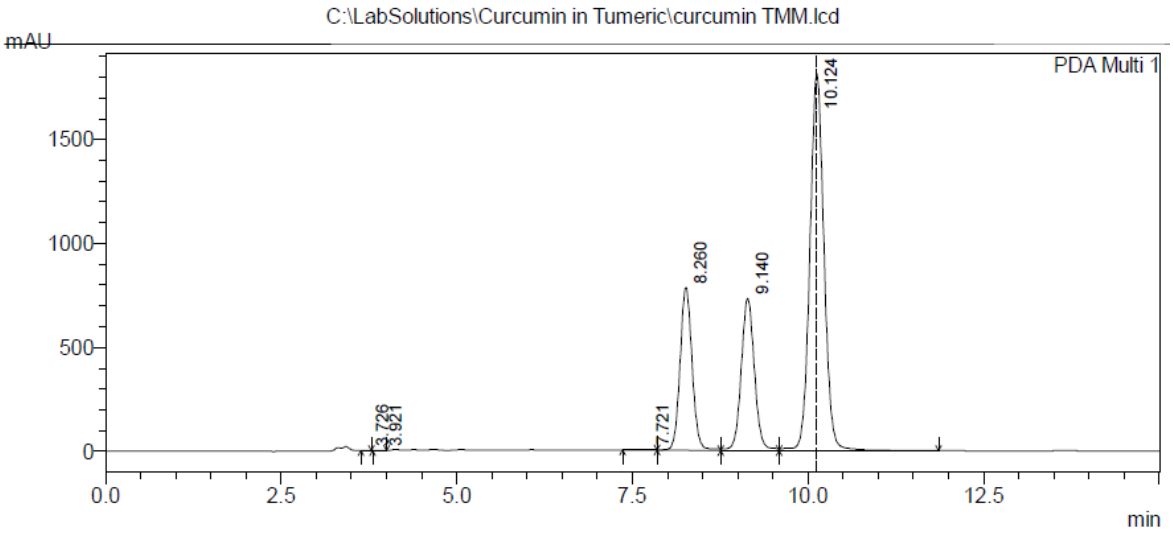


Figure 4.10: Peaks of major compounds in the methanol extract of *C. longa*

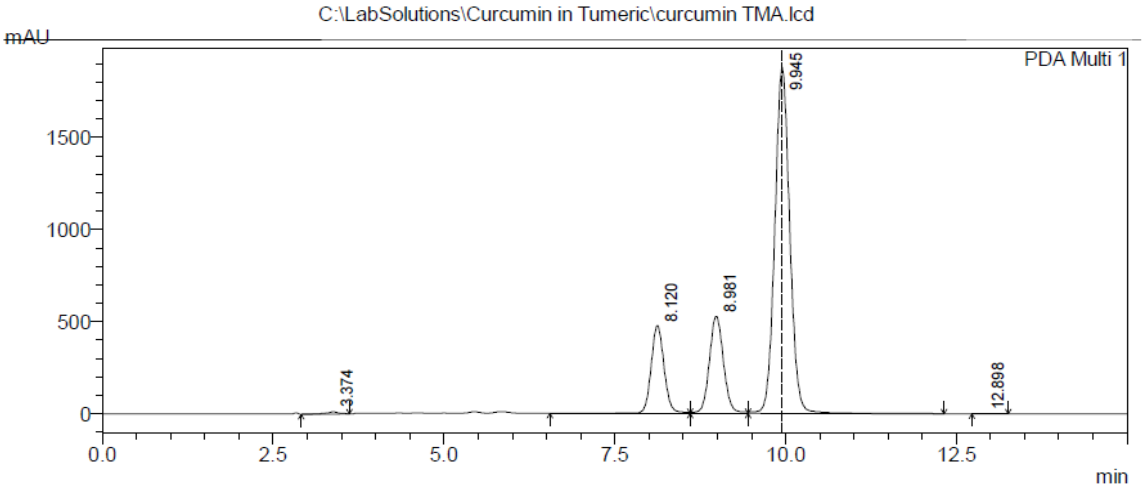


Figure 4.11: Peaks of major compounds in the acetone extract of *C. longa*

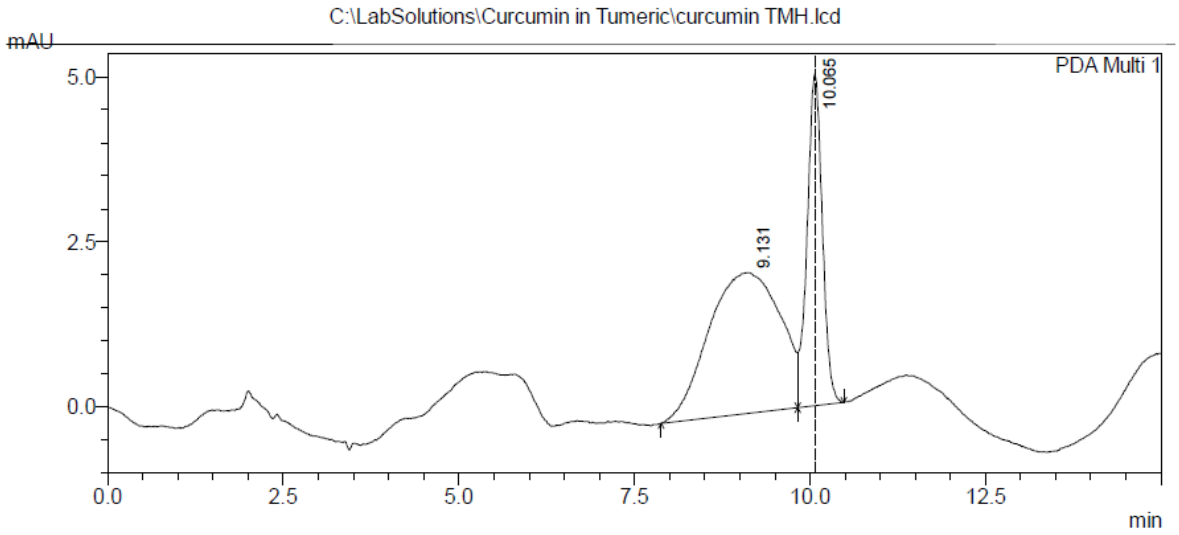


Figure 4.12: Peaks of major compounds in the n-hexane extract of *C. longa*

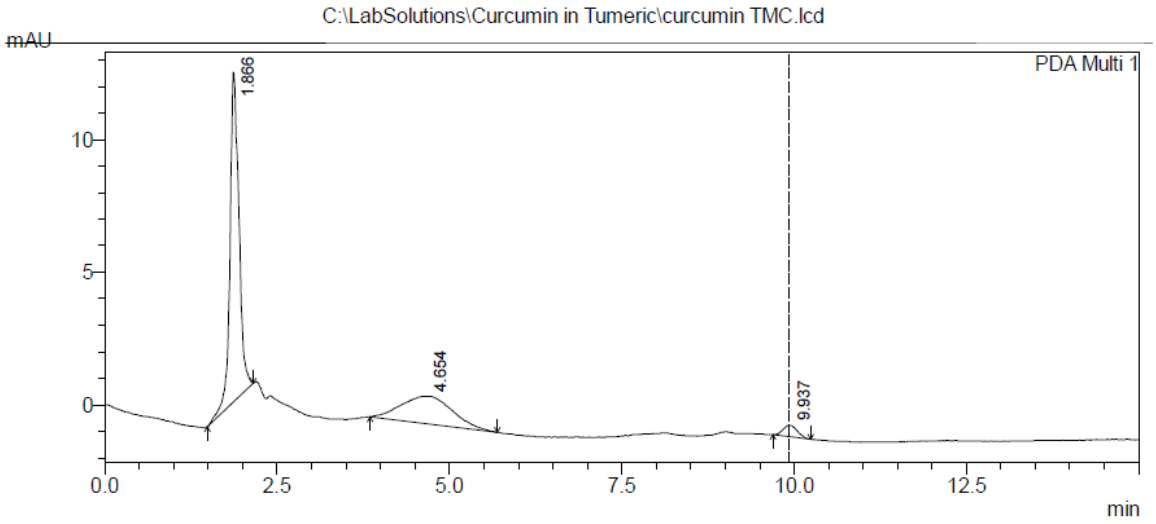


Figure 4.13: Peaks of major compounds in the water (aqueous) extract of *C. longa*

Table 4.7: The Curcumin Content (%) of Different Solvent Extracts of *C. longa*

Sample	*Concentration (%)
Ethanol (absolute) extract	$53.27^{a}\pm0.01$
Ethanol (40%) extract	$10.74^{d}\pm0.02$
Methanol extract	$12.46^{c}\pm0.007$
Acetone extract	$14.15^{b}\pm0.03$
n-Hexane extract	$3.77^{e}\pm0.03$
Aqueous (Water) extract	$2.29^{f}\pm0.0$
LSD	0.01958

($\pm$) = Mean of Curcumin in duplicate sample, a,b,c,… = mean score with different superscripts in the same column are significantly difference ($p<0.05$), LSD = Least significant different.

4.1.8 Retention of Curcumin in *C. longa* during 6 Months Storage at Ambient Temperature

Table 4.8.1: The Curcumin [Concentrations (%)] in *C. longa* Spice Product Samples during 6 Months Storage at Ambient Temperature

Spice product sample	Ambient storage period									
	Zero hr	**2wks**	**4wks**	**6wks**	**2months**	**3months**	**4months**	**5months**	**6months**	**LSD**
Powder	$53.27^{a}\pm0.01$	$53.22^{a}\pm0.01$	$53.09^{b}\pm0.01$	$50.49^{c}\pm0.01$	$50.06^{d}\pm0.01$	$47.18^{e}\pm0.02$	$46.58^{f}\pm0.03$	$42.02^{g}\pm0.01$	$39.67^{h}\pm0.01$	0.01546
40% Ethanol Extract.	$10.74^{a}\pm0.02$	$10.71^{a}\pm0.0$	$10.69^{ab}\pm0.01$	$10.63^{b}\pm0.04$	$9.89^{b}\pm0.01$	$8.47^{d}\pm0.01$	$8.04^{e}\pm0.02$	$6.93^{f}\pm0.0$	$5.65^{g}\pm0.01$	0.01841
Wheat Grit Encapsulation (1:2 ratio).	$5.30^{a}\pm0.01$	$5.05^{b}\pm0.01$	$4.65^{c}\pm0.01$	$4.39^{d}\pm0.02$	$4.30^{e}\pm0.0$	$3.95^{f}\pm0.01$	$3.67^{g}\pm0.02$	$3.18^{h}\pm0.0$	$3.08^{i}\pm0.02$	0.01491
Wheat Grit Encapsulation (1:3 ratio).	$4.39^{a}\pm0.01$	$4.36^{ab}\pm0.0$	$4.31^{b}\pm0.01$	$4.31^{b}\pm0.01$	$4.10^{c}\pm0.03$	$3.67^{d}\pm0.01$	$3.09^{e}\pm0.01$	$2.77^{f}\pm0.0$	$2.03^{g}\pm0.02$	0.01546
Yam Grit Encapsulation (1:2 ratio).	$9.46^{a}\pm0.02$	$9.44^{a}\pm0.0$	$9.41^{a}\pm0.01$	$9.19^{b}\pm0.0$	$9.10^{c}\pm0.01$	$7.68^{d}\pm0.01$	$6.59^{e}\pm0.01$	$6.04^{f}\pm0.0$	$5.46^{g}\pm0.04$	0.01633
Yam Grit Encapsulation (1:3 ratio).	$5.58^{a}\pm0.0$	$5.54^{a}\pm0.01$	$5.27^{b}\pm0.01$	$5.0^{c}\pm0.0$	$5.0^{c}\pm0.0$	$4.12^{d}\pm0.01$	$4.02^{e}\pm0.02$	$3.46^{f}\pm0.01$	$2.57^{g}\pm0.01$	0.01333

(±) = Mean and standard deviation of duplicate analysis, a,b,c,… = mean scores with different superscripts across the rows are significantly different (p=0.05). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.8.2: The Percentage Retention of Curcumin in *C. longa* Spice Product Samples during 6 Months Storage at Ambient Temperature

Spice product sample	Ambient storage period									
	Zero hr	**2wks**	**4wks**	**6wks**	**2months**	**3months**	**4months**	**5months**	**6months**	**LSD**
Powder	$100.00^{a}\pm0.01$	$99.91^{a}\pm0.01$	$99.66^{b}\pm0.01$	$94.78^{c}\pm0.01$	$93.97^{d}\pm0.01$	$88.57^{e}\pm0.02$	$87.44^{f}\pm0.03$	$78.88^{g}\pm0.01$	$74.47^{h}\pm0.01$	0.01546
40% Ethanol Extract.	$100.00^{a}\pm0.01$	$99.72^{a}\pm0.01$	$99.53^{ab}\pm0.01$	$98.98^{b}\pm0.04$	$92.09^{b}\pm0.01$	$78.86^{d}\pm0.01$	$74.86^{e}\pm0.02$	$64.52^{f}\pm0.0$	$52.61^{g}\pm0.01$	0.01841
Wheat Grit Encapsulation (1:2 ratio).	$100.00^{a}\pm0.01$	$95.28^{b}\pm0.01$	$87.74^{c}\pm0.01$	$82.83^{d}\pm0.02$	$81.13^{e}\pm0.0$	$74.53^{f}\pm0.01$	$69.24^{g}\pm0.02$	$60.00^{h}\pm0.0$	$58.11^{i}\pm0.02$	0.01491
Wheat Grit Encapsulation (1:3 ratio).	$100.00^{a}\pm0.01$	$99.32^{ab}\pm0.01$	$98.18^{b}\pm0.01$	$98.18^{b}\pm0.01$	$93.39^{c}\pm0.03$	$83.60^{d}\pm0.01$	$70.39^{e}\pm0.01$	$63.10^{f}\pm0.0$	$46.24^{g}\pm0.02$	0.01546
Yam Grit Encapsulation (1:2 ratio).	$100.00^{a}\pm0.01$	$99.79^{a}\pm0.02$	$99.47^{a}\pm0.01$	$97.15^{b}\pm0.0$	$96.19^{c}\pm0.01$	$81.18^{d}\pm0.01$	$69.66^{e}\pm0.01$	$63.85^{f}\pm0.0$	$57.72^{g}\pm0.04$	0.01633
Yam Grit Encapsulation (1:3 ratio).	$100.00^{a}\pm0.01$	$99.28^{a}\pm0.01$	$94.44^{b}\pm0.01$	$89.61^{c}\pm0.0$	$89.61^{c}\pm0.0$	$73.83^{d}\pm0.01$	$72.04^{e}\pm0.02$	$62.01^{f}\pm0.01$	$46.06^{g}\pm0.01$	0.01333

(±) = Mean and standard deviation of duplicate analysis, a,b,c,… = mean scores with different superscripts across the rows are significantly different (p=0.05). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

4.1.9 Microorganisms in *C. longa* and *X. aethiopica* Spice Samples

Table 4.9: Total Counts and Identity of Fungal Isolates from *C. longa* and *X. aethiopica* Spice Samples

Sample code	Total colony count (cfu/g)	Identity of isolates
Ground *C. longa* (Processed) sample (J)	2.0×10^5	*Saccharomyces cerevisiae* *Mucor spp*.
Raw *C. longa* Rhizomes (F)	3.2×10^8	*Saccharomyces cerevisiae*
Ground *X. aethiopica* (Processed) sample (G)	2.20×10^5	*Mucor spp.* *Penicillium notatum* *Fusarium spp.*
Raw *X. aethiopica* Pods (E)	5.1×10^8	*Saccharomyces cerevisiae* *Penicillium notatum*

Table 4.10: Total Counts and Identity of Bacteria Isolates from Samples of *C. longa* and *X. aethiopica* Spice on Nutrient agar

Sample code	Total counts (cfu/g)	Suspected Microorganism
Ground *C. longa*. (Processed) (J)	4.7×10^8	*Enterococcus faecalis*
Raw *C. longa* Rhizomes (F)	7.1×10^8	*Bacillus subtilis* *Staphylococcus aureus* *Micrococcus luteus* *Enterococcus faecalis* *Bacillus subtilis*
Ground *X. aethiopica* (Processed) (G)	3.5×10^8	*Staphylococcus aureus* *Bacillus cereus* *Enterococcus faecalis*
Raw *X. aethiopica* Pods (E)	3.9×10^8	*Enterococcus faecalis* *Bacillus cereus*

Table 4.11: Total Counts and Identity of Bacteria Isolates from *C. longa* and *X. aethiopica* Spice Samples on MacConkey Agar

Sample code	Total counts (cfu/g)	Suspected Microorganism
Raw *C. longa* Rhizomes (F)	1.1×10^8	*Escherichia coli*
Raw *X. aethiopica* Pods (E)	6.4×10^8	*E. coli*
Ground *C. longa* (processed) (J)	NG	-
Ground *X. aethiopica* (processed) (G)	NG	-

NG = No Growth

Table 4.12: Total Counts and Identity of Bacteria Isolates from *C. longa* and *X. aethiopica* Spice Samples on Brain Heart Infusion Agar (BHIA)

Sample code	Colony Count (cfu/g)	Probable identity
Ground *C. longa* (processed) (J)	2.0×10^3	*Lactobacillus brevis* *Streptococcus anginosus*
Raw *C. longa* Rhizomes (F)	3.0×10^6	*Lactobacillus brevis* *Streptococcus anginosus*
Ground *X. aethiopica* (processed) (G)	1.0×10^3	*Lactobacillus brevis* *Bacillus cereus* *Streptococcus anginosus*
Raw *X. aethiopica* Pods (E)	2.1×10^6	*Lactobacillus brevis* *Bacillus cereus*

4.1.10 Sensory Attribute Score of the Spice Samples

Table 4.13: Mean Scores (Product Oriented test) of the Sensory Attributes of *C. longa* Spice Samples

Spice sample	Colour	Pungency	Sweetness	Mintiness	Bitterness	Harshness	Hotness	After-taste	Overall acceptability
Powder	5.71^{ab}	6.0^{a}	3.57^{a}	2.14^{a}	3.71^{a}	3.14^{a}	2.57^{a}	5.85^{a}	6.28^{a}
40% Ethanol Extract	6.28^{a}	6.0^{a}	4.0^{a}	1.57^{ab}	3.14^{ab}	1.85^{b}	1.71^{ab}	5.85^{a}	6.42^{a}
Wheat Grit Encapsulation (1:3 ratio).	5.0^{b}	5.42^{a}	3.28^{a}	1.57^{ab}	2.57^{bc}	1.57^{b}	1.71^{ab}	5.42^{a}	5.28^{a}
Wheat Grit Encapsulation (1:2 ratio).	5.14^{ab}	5.28^{a}	2.14^{b}	1.85^{b}	1.57^{d}	1.71^{b}	1.14^{b}	5.71^{a}	5.14^{a}
Yam Grit Encapsulation (1:3 ratio).	5.42^{ab}	5.0^{a}	1.28^{b}	1.14^{b}	1.85^{cd}	1.57^{b}	1.57^{ab}	5.0^{a}	6.0^{a}
Yam Grit Encapsulation (1:2 ratio).	5.28^{ab}	5.0^{a}	1.28^{b}	1.14^{b}	1.42^{d}	1.57^{b}	1.71^{ab}	5.85^{a}	5.28^{a}
LSD	0.40686	0.51287	0.29354	0.31944	0.28571	0.40125	0.28966	0.35315	0.47857

($\pm$) = Means and standard deviation of twenty panelists, a,b,c,.. = Mean scores on the same column having different superscripts are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.14: Mean Scores (Product Oriented test) of the Sensory Attributes of *X. aethiopica* Spice Samples

Spice sample	Colour	Pungency	Sweetness	Mintiness	Bitterness	Harshness	Hotness	After-taste	Overall acceptability
Powder	5.28[b]	6.28[b]	1.85b	1.0[a]	5.85[b]	1.00[b]	5.71[b]	6.00[a]	5.85[b]
40% Ethanol Extract	5.42[b]	6.71[a]	1.85[b]	1.85[b]	6.00[a]	1.14[b]	5.85[a]	5.85[b]	6.14[a]
Wheat Grit Encapsulation (1:3 ratio).	5.57[b]	4.71[d]	1.00[c]	1.0[a]	4.42[c]	1.00[b]	3.71[d]	4.28[e]	5.71[c]
Wheat Grit Encapsulation (1:2 ratio).	6.00[a]	5.71[c]	2.00[a]	1.14[a]	4.14[d]	1.14[b]	3.71[d]	5.14[c]	5.28[e]
Yam Grit Encapsulation (1:3 ratio).	6.28[a]	4.42[e]	1.85[b]	1.00[a]	3.57[e]	1.28[b]	3.14[e]	3.57[f]	4.42[f]
Yam Grit Encapsulation (1:2 ratio).	5.85[ab]	5.71c	1.85[b]	1.14[a]	4.00[d]	2.00[a]	4.28[c]	4.71[d]	5.14[d]
LSD	0.282	0.3870	0.154	0.194	0.171	0.225	0.166	0.115	0.257

(±) = Means and standard deviation of twenty panelists, a,b,c,… = Mean scores on the same column having different superscripts are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.15: Mean Scores (Consumer Oriented test) of the Three Major Sensory Attributes of *C. longa* Spice Samples

Spice sample	Colour	Pungency	Aroma	Overall acceptability
Powder	5.35[b]	6.05[a]	6.15[a]	5.60[a]
40% Ethanol Extract	6.25[a]	5.80[a]	6.10[a]	6.05[a]
Wheat Grit Encapsulation (1:3 ratio).	5.25[b]	5.50[a]	5.70[a]	5.70[a]
Wheat Grit Encapsulation (1:2 ratio).	5.65[ab]	6.05[a]	6.05[a]	5.80[a]
Yam Grit Encapsulation (1:3 ratio).	5.80[ab]	5.95[a]	4.75[b]	5.15[a]
Yam Grit Encapsulation (1:2 ratio).	5.65[ab]	5.90[a]	6.05[a]	5.40[a]
LSD	0.278	0.259	0.196	15.962

(±) = Means and standard deviation of twenty panelists, a,b,c,… = Mean scores on the same column having different superscripts are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.16: Mean Scores (Consumer Oriented test) of the Three Major Sensory Attributes of *X. aethiopica* Spice Samples

Spice sample	Colour	Pungency	Aroma	Overall acceptability
Powder	5.10^{a}	5.90^{ab}	6.10^{a}	6.25^{a}
40% Ethanol Extract	5.95^{a}	6.20^{a}	4.80^{c}	6.25^{a}
Wheat Grit Encapsulation (1:3 ratio).	6.15^{a}	4.75^{d}	5.50^{b}	4.55^{c}
Wheat Grit Encapsulation (1:2 ratio).	6.30^{a}	5.50^{bc}	6.0^{ab}	5.65^{b}
Yam Grit Encapsulation (1:3 ratio).	6.05^{a}	4.65^{d}	4.55^{c}	4.60^{c}
Yam Grit Encapsulation (1:2 ratio).	5.90^{a}	5.0^{cd}	5.45^{b}	5.80^{ab}
LSD	0.24107	0.18802	0.19624	0.18964

($\pm$) = Means and standard deviation of twenty panelists, a,b,c,… = Mean scores on the same column having different superscripts are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.17: Percentage Consumer Preference for the Use of *C. longa* Spice Products in different Nigerian Foods

	Consumer preference (%)															
Spice products	**Rice**	**Meat**	**Porridge**	**Yoruba local soup**	**Pepper soup**	**Ugba**	**Stew**	**Igbo local soup**	**Sauce**	**Tea**	**Akamu /pap**	**Hausa local soup**	**Baked products**	**Ice cream**	**Yoghurt**	**Non-alcoholic beverage**	**Alcoholic beverage**
Powder	60	30		0	0	0	0	0	35	25	80	0	45	0	0	0	0
40% Ethanol extract	70	35	0	0	0	0	0	0	50	30	80	0	50	100	100	90	70
Wheat grit encapsulation (1:3 ratio)	45	25	0	0	0	0	0	0	25	0	40	0	40	0	0	0	0
Wheat grit encapsulation (1:2 ratio)	45	25	0	0	0	0	0	0	25	0	40	0	40	0	0	0	0
Yam grit encapsulation (1:3 ratio)	45	25	0	0	0	0	0	0	25	0	40	0	40	0	0	0	0
Yam grit encapsulation (1:2 ratio)	45	25	0	0	0	0	0	0	25	0	40	0	40	0	0	0	0

Ratio refers to Spice extract to Carrier

Table 4.18: Percentage Consumer Preference for the Use of *X. aethiopica* Spice Products in different Nigerian Foods

	Consumer preference (%)																
Spice products	**Rice**	**Meat**	**Porridge**	**Yoruba local soup**	**Pepper soup**	**Ugba**	**Stew**	**Igbo local soup**	**Sauce**	**Tea**	**Akamu /pap**	**Hausa local soup**	**Baked products**	**Ice cream**	**Yoghurt**	**Non-alcoholic beverage**	**Alcoholic beverage**
Powder	0	70	75	80	100	0	0	90	0	50	0	75	0	0	0	0	100
40% Ethanol extract	0	70	80	75	100	0	0	90	0	65	0	75	0	0	0	0	100
Wheat grit encapsulation (1:3 ratio)	0	70	75	80	100	0	0	90	0	0	0	75	0	0	0	0	0
Wheat grit encapsulation (1:2 ratio)	0	70	75	80	100	0	0	90	0	0	0	75	0	0	0	0	0
Yam grit encapsulation (1:3 ratio)	0	70	75	80	100	0	0	90	0	0	0	75	0	0	0	0	0
Yam grit encapsulation (1:2 ratio)	0	70	75	80	100	0	0	90	0	0	0	75	0	0	0	0	0

Ratio refers to Spice extract to Carrier

Table 4.19: Mean Consumer Scores of the Four Major Sensory Attributes of *X. aethiopica* Spiced Pepper Soup

Sensory property	Powdered spice	40% ethanol extracted spice	Wheat grit encapsulated with spice (1:2 ratio)	LSD
Colour	$6.0^{a}\pm0.02$	$6.2^{a}\pm0.22$	$5.3^{c}\pm0.15$	0.366
Pungency	$6.1^{b}\pm0.12$	$6.4^{a}\pm0.10$	$5.9^{c}\pm0.26$	0.182
Taste	$6.5^{a}\pm0.08$	$6.2^{b}\pm0.12$	$5.7^{c}\pm0.02$	0.238
Mouth-feel	$6.4^{a}\pm0.11$	$6.6^{a}\pm0.10$	$5.2^{b}\pm0.13$	0.429
Overall acceptability	$6.3^{a}\pm0.18$	$6.4^{a}\pm0.02$	$6.2^{a}\pm0.01$	0.358

($\pm$) = Mean and standard deviation of twenty panelists, a,b,c = Mean scores across rows having different superscripts are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

Table 4.20: Mean Consumer Scores of the Four Major Sensory Attributes of *C. longa* Spiced Akamu (Pap)

Sensory property	Powdered Spice	40% ethanol Extracted spice	Yam grit encapsulated with spice (1:2 ratio)	LSD
Colour	$6.62^{a}\pm0.10$	$6.18^{b}\pm0.02$	$5.82^{c}\pm0.02$	0.366
Pungency	$6.34^{a}\pm0.11$	$6.31^{a}\pm0.12$	$6.2^{a}\pm0.02$	0.420
Taste	$6.22^{a}\pm0.12$	$6.20^{a}\pm0.10$	$6.0^{a}\pm0.13$	0.538
Mouth-feel	$6.0^{a}\pm0.02$	$6.05^{a}\pm0.10$	$5.98^{a}\pm0.02$	0.755
Overall acceptability	$6.38^{a}\pm0.10$	$6.26^{a}\pm0.11$	$6.0^{a}\pm0.12$	0.423

($\pm$) = Mean and standard deviation of twenty panelists, a,b,c = Mean scores across rows having different superscripts are significantly different ($p<0.05$). LSD = Least significant difference

Ratio refers to Spice extract to Carrier

4.2 DISCUSSION

4.2.1 Moisture contents of wheat and yam grits encapsulated in spice extracts samples at different ratios

The results of moisture contents of powdered and encapsulation products of *X. aethiopica* and *C. longa* are shown in Table 4.1.

The yam grits encapsulation product of *X. aethiopica* at a ratio of 1:2 spice extract to carrier (YGCFC1) had the least moisture content (6.98%) followed by the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier (YGCFC2) with 7.01%, while the powdered sample had the highest moisture content (9.22%). There was no significant difference ($p>0.05$) between the products of yam grits encapsulation at a ratio of 1:2 spice extract to carrier (YGCFC1) and the products of yam grits encapsulation at a ratio of 1:3 spice extract to carrier (YGCFC2). However, there was significant difference ($p<0.05$) between them and the rest of the samples. There was significant difference ($p<0.05$) between the products of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier (WGCFC1) and those of wheat grits encapsulation at a ratio of 1:3 spice extract to carrier (WGCFC2). From these results it is clear that the products of wheat grits encapsulation had higher moisture content than those of yam grits and this inferred that wheat grits retained more of the liquid extracts than the yam grits. It could as well be deduced that with wheat grits encapsulation, there was emulsion stability with the extract that made the surface structure of the wheat starch more absorbable to the extracts at a quicker rate than that of yam grits carrier. According to reports by Naviglio *et al.* (2010) on the determination of moisture in powder and lyophilized saffron (*Crocus sativus*) the average moisture content in dried saffron powder ranged between 10 -12% and within this range, slow degradation of the product was expected. They also stressed that lyophilized saffron had lower moisture content with better stability. Therefore, the findings of Naviglio *et al.* (2010) is in line with the results obtained in this study, as all the encapsulated samples had lower moisture content than the powder. All the samples had moisture content in the range of 7.01 -9.22% indicating that there would be slow product degradation.

The purpose of encapsulation was to allow liquid flavour extracts into free flowing powders thus protecting the flavour principles with possible extension of shelf life. The determination of

moisture contents in products such as *X. aethiopica* and *C. longa* is very crucial as it helps in the prediction of microbial stability and shelf life.

For the *C. longa* samples, the product of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier (WGCFC1) had the least moisture content (6.45%) followed by the product of wheat grits encapsulation at a ratio of 1:3 spice extract to carrier (WGCFC2) with 6.72%, while the powder had the highest moisture content of 10.01%. There were significant differences ($p<0.05$) among all the samples. It was not surprising that the product of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier (WGCFC1) had a moisture content lower than the product of wheat grits encapsulation at a ratio of 1:3 spice extract to carrier (WGCFC2) because at higher level of carrier the proportion of released water would be less. The wheat grits encapsulated spice products had lower moisture content values than their yam grits counterparts (products). It could be inferred that when encapsulated with yam grits, there might have been better emulsion stability with the extract that made the surface structure of the yam starch more absorbable to the extract at a quicker rate than that of wheat grits carrier. According to the grading designations and quality of *C. longa* powder as published in the Gazatte of India (2015), the maximum acceptable moisture content of *C. longa* powder is in the range of 10-12%. All the experimental samples had moisture contents in the range of 6.45 – 10.01% which implied that they all made good grades with respect to the acceptable moisture limit of *C. longa* powder.

4.2.2 Specific gravity of spice extracts

The results of the specific gravity of *X. aethiopica* and *C. longa* solvent extracts are shown in Table 4.2. The specific gravity values of the solvents used for the extraction of the spices ranged from 0.659 (n-Hexane) to 1.000 (water) while values for the solvent extracts of *X. aethiopica* ranged from 0.859 (n-Hexane) to 1.235 (water). These results showed that there was increase in the specific gravity after the extraction operation hence all the extracts were heavier than the extracting solvents. The values of these increases in the specific gravity of the extracting solvents ranged from 0.20 (n-Hexane extract) to 0.426 (40% ethanol extract).

The 40% ethanol extract had the highest increase (0.426) in specific gravity followed by the acetone extract with a value of 0.376 while n-hexane extract had the least increase (0.20) in

specific gravity. There were significant differences in the specific gravity increases ($p<0.05$) of all the solvent extracts. In industries, specific gravity is a simple means of obtaining information about the concentration of solutions of various materials most often regarded as "Brix". For the purpose of this study, the specific gravity gave an idea of the extractable compounds in the extract, and hence the performance of the solvent for the extraction operation. It therefore means that the 40% ethanol solvent extracted the greatest amount of compounds and thus should contain the highest amount of spice compound, while n-hexane should contain the least. This result suggested that *X. aethiopica* have more polar substances than non-polar ones which was the reason why a polar solvent extract such as the 40% ethanol extract had greater increase in specific gravity than the non-polar n-hexane solvent extract. Based on these observations, in the choice of solvent, 40% ethanol was chosen as it dissolved/extracted greater amount of the spice flavour components (as indicated by the highest increase in specific gravity).

In the case of *C. longa*, the 40% ethanol extract also had the highest increase (0.570) in the value of specific gravity followed by acetone with 0.292 while water had the least with 0.20. There were significant differences ($p<0.05$) in the specific gravity increases of all the solvent extracts. With the higher values observed in specific gravity increases for the extracts obtained with polar solvents, it simply means that majority of the substances extracted from *C. longa* were polar compounds. The observation that the increase in specific gravity of n-hexane extract (0.228) was higher than that of water extract (0.20) and absolute ethanol extract (0.212), indicate that n-hexane extract of *C. longa* contained some non-polar compounds. Due to the fact that 40% ethanol produced the highest increase in specific gravity among the extracts, contained the greatest amount of extracted compounds and also acceptable in food, it was chosen for further extraction studies. The Successful detection of biologically active compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure. The choice of solvent is dependent on so many factors which include quantity of phytochemicals expected, rate of extraction, diversity of different compounds expected, diversity of inhibitory compounds expected in the extract, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the solvent (extractants) (Eloff, 1998). The intended use of the extract also plays a key role in the choice of solvent. Since the end product may contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the food and its properties.

4.2.3 Phytochemical content of solvent extracts of *X. aethiopica* and *C. longa* spices

The solvent extracts of *X. aethiopica* and *C. longa* spices contained alkaloids, flavonoids, phytic acids, saponins, tannins and oxalate [Tables 4.3.1 -4.3.6 and Appendix II (figures 1-6)].

Phytochemicals are naturally occurring biochemicals in plants that contribute to their characteristic colour, flavour, smell and texture (Ukoha *et al.,* 2011). They are also non-nutritive plant chemicals that have disease preventive properties. Previous reports on methanolic extracts of rhizomes of *C. longa* by Saxena and Sahu (2012) and Rajesh *et al*. (2013) indicated the isolation of flavonoids, alkaloids, tannins and saponins while Swadhini *et al*. (2011) detected six phytochemicals namely alkaloids, flavonoids, tannins, saponins, cardiac glycosides and oxalates from aqueous extract of *C. longa*.

4.2.3.1 Alkaloids

The 40% ethanol extract of *X. aethiopica* gave the highest concentration (21.5%) of alkaloids followed by the absolute ethanol extract with 20.93% while the water extract had the least with 1.90% (Table 4.3.1 and Appendix II fig.1). The higher content of alkaloids in 40% ethanol than in absolute ethanol extract might be due to its higher polarity. The addition of water to the absolute ethanol up to 60% for preparing the 40% ethanol solvent increased the polarity (Bimakr, 2010). This result is in line with the report by Doughari (2012) that most alkaloids are readily soluble in alcohol and may be sparingly soluble in water, but their salts are usually soluble in water. There were significant differences ($p<0.05$) in the alkaloid values of the different solvent extracts of *X. aethiopica*. Therefore 40% ethanol was the best solvent of choice for extracting alkaloids in *X. aethiopica*. With regards to the alkaloid contents in the liquid extracts of *C. longa,* the n-Hexane extract gave the highest concentration (6.54%) followed by the methanol extract with 6.05% while the water extract had the least with a value of 1.93%. It could be inferred that since n-hexane which is a non-polar solvent gave the highest alkaloid content among the extractions, it could be that most of the extractable alkaloid compounds in *C. longa* are non-polar, though alkaloids comprise both polar and non-polar compounds. There were also significant differences ($p<0.05$) in alkaloid values obtained from the *C. longa* extracts with the different solvents. In choosing the best extracting solvent for alkaloids, n-Hexane should be the

choice, followed by methanol while the least should be water. This finding indicated that n-hexane and methanol could be used for non –food products, however 40% ethanol which extracted more alkaloids (5.76%) than the other remaining solvents is acceptable in food and was most suitable for the study, since edibility and safety were priorities.

4.2.3.2 Flavonoids

Flavonoids are important group of polyphenols widely distributed among the plant flora, and numerous reports support their use as antioxidants or free radical scavengers (Kar, 2007). They play a role in providing colour, fragrance and taste to the fruits, flowers and seeds, and these make them attractive to insects, birds or mammals (Koes and Quattrocchio, 1994). The methanolic extract gave the highest concentration (8.8%) of flavonoids among the liquid extracts of *X. aethiopica* , followed by the acetone extract with 4.11%, while the 40% ethanolic extract had the least value with 0.35% (Table 4.3.2 and Appendix II, fig.2). This result is in contrast with the report of Bimakr (2010) that higher concentrations of flavonoid compounds were detected with 70% ethanol due to its higher polarity than absolute ethanol, but here 40% ethanol with higher polarity than absolute ethanol gave a lower yield (0.35%) compared to absolute ethanol with 3.63%. This contrasting result might be due to the plant variety or species, its growth environment, maturation and post-harvest handling operations. There were significant differences ($p<0.05$) in the flavonoid values of *X. aethiopica* among the different solvent extracts. From the findings in the research work on Oxytocic effect of aqueous, ethanolic, n-hexane and chloroform extracts of *X. aethiopica (Anonaceae)* and *Ocimum gratissium (Labiate)* on guinea pig uterus by Omodamiro *et al*. (2012), pregnant women were advised to avoid the regular consumption of these spices in the first trimester of pregnancy, since their consumption may likely result in uterine contraction and consequently miscarriage. However, the use of these spices in preparing food for pregnant women ready for birth as well as nursing mothers is encouraged. Considering their acceptability in food, the most suitable extracting solvent is absolute ethanol (3.63%).

In considering the percentage yield of flavonoids in the liquid extracts of *C. longa*, n-Hexane extract gave the highest concentration (6.55%) followed by methanol extract with 6.05% while water extract had the least value (1.93%). The finding that n-hexane, a non-polar solvent extracted the highest value of flavonoids in *C. longa* and was closely followed by methanol

(polar solvent) indicated that flavonoids have both polar and non-polar components. There were also significant differences ($p<0.05$) in the flavonoids content obtained from the *C. longa* extracts with the different solvents. Considering the best extracting solvent based on acceptability in food, 40% ethanol was most suitable having shown a concentration yield of 5.05%, but for other industrial uses n-hexane could be used.

4.2.3.3 Phytic acid

Phytic acid (known as inositol hexakisphosphate or phytate when in salt form) is an organic acid extracted from plant seeds and contributing about 70% of total phosphorus in many plant tissues especially bran and seeds (Nielsen *et al*., 2013). From Table 4.3.3 (and Appendix II, fig. 3), water extract gave the highest concentration of phytic acid (9.24%) among the liquid extract of *X. aethiopica* , followed by n-hexane extract with 6.20% while absolute ethanolic extract had the least value (2.90%). It was not surprising that water which is a polar solvent gave the highest yield of phytic acid. This was because phytic acid is an organic substance with high polarity due to many phosphate groups. Thus solvent with high polarity will enhance its extraction (Wu *et al*., 2009). Phytic acid has been known to have strong chelating characteristics that reduce the bioavailability of some essential dietary nutrients such as minerals (e.g. Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, $Fe^{2+/3+}$), proteins and amino acids (Gerke, 2015). Therefore, in considering solvent of choice for food products, absolute ethanol which gave the least yield should be the solvent for extracting *X. aethiopica* so that less phytate will be available and chelating effect will be reduced but for other industrial uses where high phytic acid content is required, water should be the solvent of choice. The result also revealed that n-hexane, a non-polar solvent extracted a reasonable quantity (6.20%) of phytic acid and this indicated that the extracts also had non –polar substances. Thus the solvent extract with the highest phytic acid content was water (highest polarity) and was followed by n-hexane (non-polar solvent) extract. Hence phytates comprise both polar and non-polar substances. There were significant differences ($p<0.05$) in phytic acid values obtained from *X. aethiopica* extracts with the different solvents. For the amount of phytic acid in the liquid extracts of *C. longa*, methanolic extract gave the highest value (14.95%) followed by 40% ethanolic extract with 12.85% while absolute ethanol extract had the least with 6.93%. Duy (2013) stated that phytic acid exhibited antioxidant action and prevention of colour degradation, therefore to prevent colour degradation from *C. longa*, 40% ethanol solvent should be the most

suitable solvent for extracting phytic acid because of its acceptability in food as against methanol, however for other industrial purposes methanol can be used. However when the priority is to reduce the chelating characteristic, absolute ethanol should be the best solvent of choice. There were also significant differences ($p<0.05$) in the phytic acid content obtained from *C. longa* with the different solvent extracts.

The concentration of phytic acid in the liquid extracts was lower in *X. aethiopica* than in *C. longa* in all the solvent extracts.

4.2.3.4 Saponins

The naturally occurring saponins are glycosides of steroids and triterpenoids. They are recognized by their ability to produce a soapy lather when shaken with water and they possess a bitter and acrid taste (Negi *et al.*, 2013). The methanol extract gave the highest concentration of saponins (3.49%) in the liquid extract of *X. aethiopica* , followed by n-hexane extract (1.47%) while the absolute ethanolic extract had the least with 0.21% (Table 4.3.4 (and Appendix II, fig. 4). This result contradicted the findings of Negi *et al.*, (2013) and Doughari (2012) who stated that saponins were soluble in ethanol and water, but insoluble in non-polar solvents like benzene and n-hexane. Negi *et al.* (2013) went further to state that there were presence of lipid-soluble aglycone (glycosyl group on a glycoside being replaced by hydrogen atom) and water soluble sugar chain in the structure of saponin making them amphiphilic in nature (having both hydrophilic and lipophilic properties) however, the lipophilicity (fat friendly) could make saponin to dissolve in non-polar solvents like n-hexane which is also lipophilic. There were significant differences ($p<0.05$) in the saponin values obtained from *X. aethiopica* among the different solvent extracts. For acceptability in food, water should be the solvent of choice for saponins which have been found useful in the brewing and soft drink industries where foaming is one of the sensory characteristics. The concentrations of saponins were relatively low in all the solvent extracts. While considering the percentage of saponins in the liquid extracts of *C. longa*, the n-hexane extract gave the highest value (1.0%) followed by the methanol extract with 0.47% and zero percent for acetone extract. Thus the absence of saponins in the acetone extract of *C. longa* might limit this solvent as choice in the extraction of this bioactive ingredient from *C. longa*. This result also contradicted the findings of Negi *et al.* (2013) and Doughari (2012) who stated that saponins were soluble in ethanol and water, but insoluble in non-polar solvents like

benzene and n-hexane. Negi *et al.* (2013) indicated that organic solvents like chloroform, acetone and ether inhibit the foaming property of saponins and this inhibition might have caused the zero yield on acetone extract. Considering the fact that the saponins content were very low in all the solvent extracts, n-hexane, a non-polar solvent giving the highest yield, suggested that saponins may comprise of both polar and non-polar compounds. In food considerations, when little foaming is required in the food, 40% ethanol will be the best solvent but when foaming is not required, absolute ethanol should be the solvent of choice. There were also significant differences ($p<0.05$) in the saponin values obtained from the different solvent extracts of *C. longa*.

4.2.3.5 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripe fruit (McGee, 2004). Tannins are used in dyeing, photography, refining beer and wine as well as astringency in medicines. Significantly, tannins form a vital element of tea (Praveen and Kumud, 2012). In the food industry tannins are used to clarify wine, beer and fruit juices (Karamali and Teunis, 2001). The methanol extract had the highest percentage of tannin (2.13%) in the liquid extracts of *X. aethiopica* , followed by the water extract with 1.22% while the n-hexane extract had the least with 0.086% (Table 4.3.5 and Appendix II, fig. 5). This indicated that tannins comprise more of polar substances. This result is in conformity with the reports of Doughari (2012) that tannins are soluble in water and alcohol but contradictory to the report of Elgailani and Ishak (2016) who stated that acetone was an efficient solvent for the extraction of tannins. However, since in *X. aethiopica* methanol gave the highest yield of tannins, it suggested that the lower the molecular weight of the used alcohol, the higher the solubility of tannins. In choosing the extracting solvents for *X. aethiopica* flavours for food where a reasonable level of astringency is required water should be the solvent of choice but where low detectable level is needed, 40% ethanol should be chosen and not n-hexane because n-hexane is not acceptable in food. There were significant differences ($p<0.05$) in the tannins content of *X. aethiopica* among the different solvent extracts. Among the liquid extracts of *C. longa*, 40% ethanol extract gave the highest percentage (1.46%) followed by methanol extract with 1.20% while n-hexane extract had the least with 0.19%. The results also confirmed

that tannins comprise of more polar substances. There were also significant differences ($p<0.05$) in the tannins content of the different *C. longa* solvent extracts. In choosing the extracting solvents for tannins in *C. longa*, when astringency is required the 40% ethanol solvent should be the solvent of choice but when low detectable level is needed, water should be chosen and not n-hexane since n-hexane is not acceptable in food.

The observed quantities of tannins in the extracts (2.13% for *X. aethiopica* and 1.46% for *C. longa*) were very small especially if they are to be considered as source of tannins for non-food industries, including pharmaceutical industries. But, for human consumption, excess of tannins could be toxic. This is because tannins are metal ion chelators and tannin-chelated metal ions are not bioavailable hence could decrease the bioavailability of iron leading to aneamia. Besides, El-Waziry *et al.* (2005) had connected esophageal cancer in humans to regular consumption of certain herbs with high tannins concentration. However, the observed concentration of tannins in *X. aethiopica* and *C. longa* may not be enough to induce overt toxicity, hence may be appropriate for use in herbal tea or at best nutraceutical beverage. Idolo and Emeruwa (2011) reported that high level of tannins 7.6% and above could be detrimental if consumed by humans.

4.2.3.6 Oxalates

Oxalate (or Oxalic acid) is a common widespread constituent of plants that occurs as a free acid in the form of soluble salts of potassium and sodium and can also be present in precipitated form, mostly with calcium, magnesium and iron (Yu and Ni, 2005). The n-hexane extract gave the highest concentration of oxalate (1.25%) among the liquid extracts of *X. aethiopica* , followed by absolute ethanol extract with 0.80% while the 40% ethanol extract had the least value with 0.35% (Table 4.3.6 and Appendix II, fig. 6). This result showed that oxalates comprise both polar and non-polar substances. There were significant differences ($p<0.05$) in the oxalate content of *X. aethiopica* among the different solvent extracts. Considering the oxalate content of the liquid extracts of *C. longa*, n-hexane extract gave the highest percentage (1.61%) followed by 40% ethanol extract with 1.08% while the acetone extract had the least with 0.45%. The result also indicated that oxalates comprise of both polar and non-polar compounds. There were also significant differences ($p<0.05$) in the oxalate values of the different solvent extracts of *C. longa*.

4.2.4 The major flavour principles identified in *X. aethiopica*

The compounds identified in the different solvent extracts during the gas chromatography-mass spectrometer are shown in Figs 4.1 – 4.6 and Tables 4.4.1 – 4.4.5. It was observed that the type of flavour compounds extracted depended on the solvent used indicating their different ability to solubilize in the solvents and this might be based on polarity. El-Hadi *et al.* (2013) stated that fruit volatiles that contribute to their distinct flavour include esters, alcohols, aldehydes, ketones, lactones, terpenoids, apocarotenoids and some sulfur compounds. They further stated that many C_{10} monoterpenes and C_{15} sesquiterpenes compose the most abundant group of compounds present in fruit aroma profile. But there are usually the key compounds determining the characteristic aroma. In these results, although numerous chemical compounds were detected as volatile compounds but only a fraction of these compounds contribute to the flavour of the fruit (El-Hadi *et al*., 2013).

4.2.4.1 Absolute ethanol extract

Table 4.4.1 showed the major flavour compounds in the ethanol extract of *X. aethiopica* . The major compounds identified in absolute ethanol extract (Fig. 4.1) were monoterpenes (46.4%) - mainly represented by beta-pinene (15.48%), trans-beta ocimene (7.59%), beta-phellandrene (18.93%) and terpinen-4-ol (4.40%); diterpenes (20.99%) - represented by kauren-18-ol acetate (4beta) (11.33%) and andrographolide (9.66%); sesquiterpenes (18.88%) - mainly represented by spathulenol (2.46%), aromadendrene (5.42%), ledene oxide -(II) and 1,6-cyclodecadiene (germacrene D) (7.40%); steroids (9.92%) - mainly represented by androsta-1,4-diene-3-one (4.89%), pregan-20-one (2.16%) and 5-alpha-ergost-8-(14)-ene (2.24%) and fatty acid (2.89%) - represented by n-Hexadecanoic acid (2.89%).

The results obtained are partly in agreement with the reports of Hassan *et al.* (2016), Karioti *et al.* (2004) and Tairu *et al.* (1999) who found beta-pinene, beta –Phellandrene, 1,6-cyclodecadiene (germacrene D), terpinen- 4 – ol and trans-beta- ocimene as the odour active compounds detected in *X. aethiopica* . The variations in other compounds detected might be as a result of differences arising from geographical location, genetics, climate and post-harvest operations. Tairu *et al.* (1999) further stated that all the flavour active compounds identified were terpenes which are responsible in eliciting the characteristic flowery and terpeny notes of *X. aethiopica*. The aroma principles as stated by Tairu *et al.* (1999) were trans-beta-ocimene

(flowery note), beta-pinene (terpeny odour note) beta-phellandrene (terpeny note, described as peppery-minty and slightly citrusy), terpinen – 4-ol (light minty, terpeny note) and 1, 6-cyclodecadiene (germacrene D) (woody-like note).

Spathulenol was identified by Rubiolo *et al.* (2010) as having earth aromatic odour and bitter spicy taste and so could have contributed on the characteristic odour of *X. aethiopica.* Palmitic acid (n-hexadecanoic acid) is the most common saturated fatty acid found in animals, plants and micro-organisms (Gunstone *et al.*, 2007). It does not contribute to the flavour of *X. aethiopica*. It is a major component of oil from palm trees.

The sesquiterpene ledene oxide (II) has been identified by Sugumarpandian and Nagarajan (2015) as one of the major components in the extract of *Nardostachys jatamansi* and as one of the antioxidants in the herb used in the treatment of nervous disorders and menstruations. Sugumarpandian and Nagarajan (2015) reported that it had a sweet odour. Al-Qudah *et al.* (2012) also reported ledene oxide II as one of the major volatile constituents of the leaf oil of *Gynandriris sisyrinchium,* which gives the characteristic violet like odour of the oil, thus ledene oxide II could have some impact on the flavour profile of *X. aethiopica.*

4.2.4.2 40% ethanol extract

The major constituents/compounds in the 40% ethanol extracts (Fig. 4.2) were found to contain monoterpenes (53.82%) - mainly represented by beta-phellandrene (23.93%), beta-pinene (20.28%) and trans-beta-ocimene (9.61%); diterpenes (8.06%) - solely represented by Andrographolide (8.06%) and steroid (27.88%) - represented by cholestane, 4-5-eppoxy – (4-alpha, 5-alpha) (27.88%). These results are partly in line with the report by Tairu *et al*. (1999) who stated that the most important odourants in volatile oil of the fruit of *X. aethiopica* were linalool, trans-beta-ocimene, α-farnesene, α-pinene, β-pinene, myrtenol, beta-phellandrene and 3-ethylphenol. There are usually variations in the volatile oil composition as it is usually affected by factors such as genetic make-up, degree of maturity, environmental conditions, post- harvest handling and storage. These variations were also observed in the different results obtained by using different solvents for the extraction of the compounds. Degenherdt *et al*. (2009), Koudou *et al.* (2005) and Mosciano (2000) had earlier identified these monoterpenes as flavour compounds

in their respective works. Kligler *et al.* (2006) reported Andrographolide as an extremely bitter substance used in treating liver disorder, colic pain and upper respiratory tract infection, and that it did not contribute to flavour. They further stated that Andrographolide dissolve in methanol, ethanol, acetone and hexane but has limited solubility in water which is in conformity with the results obtained in this study; Andrographolide was absent only in the water extract.

4.2.4.3 Methanol extract

In Fig. 4.3 and Table 4.4.2, diverse chemical compounds were detected from methanol extract of *X. aethiopica*. The percentages of these compounds were; monoterpenes (35.57%), sesquiterpenes (6.38%), aromatics (21.56%), diterpenes (18.25%), steroids (13.25%) and alcohol (2.08%) among others. The major constituents within the monoterpenes were beta-phellandrene (21.54%) and beta-pinene (14.03%); the sesquiterpenes were –(-) Spathulenol (3.33%) and bioallethrin (3.05%); the aromatic hydrocarbon was Ar-turmerone (21.56%); the diterpenes were Andrographolide (7.67%), Kaur-16-ene (2.38%) and 5-Eicosene-(E) (8.20%); the steroid was Cholestane,4-5-epoxy-(4alpha-5alpha) (13.25%%) and a diterpene alcohol thumbergol (2.08%).

Different authors [Hassan *et al.* (2016), Karioti *et al.* (2004) and Tairu *et al.* (1999)] have examined the composition of essential oils of *X. aethiopica* from different countries and identified different compounds with some having similarities. The variations might be due to differences in the geographical locations of the source of *X. aethiopica* fruit and other factors such as genetics, maturity and post-harvest operations. The results in Table 4.4.2 were in line with the findings of other authors from different countries. Karioti *et al.* (2004) analyzed the essential oils of *X. aethiopica* from Ghana and found the presence of germacrene D, beta and alpha pinene, cineole and kaur-16-ene, while Noudjou *et al.* (2007) identified beta phellandrene, 1.8-cineole, beta and alpha pinene and 13-ent-epimanoyl oxide in the essential oils of *X. aethiopica* from Cameroun. When Onayade-Sontan (1991) and Olonisakin *et al.* (2007) studied the essential oils of *X. aethiopica* fruit from Nigeria, they found the predominant compounds to include beta pinene (13.78%), beta phellandrene (12.36%). γ-terpinene (7.66%), eucalyptol (6.9%) and alpha pinene (5.56%). They attributed the variations to geographical location. It was noticed that Ar-turmerone came out at reasonable concentration (21.56%) in the sample studied

and it seemed in this work that this was the first time an investigation of essential oils of *X. aethiopica* detected the presence of Ar-turmerone as different authors have never reported it. Thumbergol is a monocyclic diterpene alcohol which was found to be one of the detected flavour chemical component of *Euphorbia echinus* when extracted with chloroform, ethanol and aqueous solvents (Fatima *et al.*, 2014). El-Hadi *et al.* (2013) also reported 5-Eicosene in an essential oil extracted from *Manglietia glauca* leaves which had strong bactericidal and antifungal properties and so did not contribute to the flavour of *X. aethiopica.*

4.2.4.4 Acetone extract

In Fig. 4.4 and Table 4.4.3, the major chemical compounds from the acetone extract of *X. aethiopica* were monoterpenes (15.49%) - mainly represented by beta pinene (8.11%) and beta phellandrene (7.38%); alkenes (47.52%) - mainly represented by 3-tridecene (z) (5.08%), 1-pentadecene (7.85%) , 1-tridecene (7.78%), 1-heptadecene (12.76%), 1-nonadecene (7.78%) and 9-tricosene (z) (6.86%) ; sesquiterpenes (9.02%) - mainly represented by aromadendrene (4.63%), -spathulenol (2.15%) and aromadendrene oxide (2) (2.24%); steroids (9.84%) - represented by Androstan-17-one, 3-ethyl-3-hydroxyl-5 alpha (7.16%) and pregan-20-one, 2-hydroxy-5,6-epoxy (2.68%), and diterpene (7.29%) - represent by Andrographolide (7.29%). In this study, high percentage of alkenes were identified though beta pinene and beta phellandrene were the major flavour compounds identified and this finding is partially in line with the reports of Olonisakin *et al.* (2007) and Noudjou *et al.* (2007). These alkenes found in reasonable concentration are unsaturated compounds containing at least one carbon-carbon double bond. They are very reactive and can easily engage in addition reactions to form the more stable corresponding alkanes and so might have little or no impact on the flavour contribution of *X. aethiopica.*

Spathulenol, which is a tricyclic sesquiterpene alcoholic has been reported by Rubiolo *et al.* (2010) as a colourless, viscid compound with an earth aromatic odour and bitter spicy taste and therefore could have contributed to the flavour component of *X. aethiopica*. The steroids do not contribute to flavour but are phytochemicals that have clinical uses such as in general anesthetics.

4.2.4.5 n-Hexane extract

Diverse chemical compounds were also detected from the n-hexane flavour extract of *X. aethiopica* (Fig. 4.5 and Table 4.4.4). The compounds identified ranged from monoterpenes (22.97%), sesquiterpenes (12.47%), esters (8.83%), aromatic hydrocarbon (21.56%), diterpenes (6.58%), fatty acid (3.84%), alcohols (2.08%) and steroids (6.73%). The major constituents within the monoterpenes were Bicyclo (3,1,1) heptane (10.88%) and beta-phellandrene (12.09%); the sesquiterpenes were 1,6 cyclodecadiene (germacrene D) (3.06%) and curlone (9.41%); the ester was diethyl phthalate (8.83%); the aromatic hydrocarbon was Ar-turmerone (21.56%); the diterpenes were andrographolide (4.20%) and Kaur-16-ene (2.38%); the fatty acid was oleic acid (3.84%); the alcohol was thumbergol (2.08%) and the steroid was card-20 (22) enolide (6.73%). This result is partly in agreement with the reports of Hassan *et al.* (2016) where bicyclo (3,1,1) heptane, beta-phellandrene, 1,6-cyclodecadiene, 1-metlyl-5-methylene, diethyl phthalate and kaurene were identified in the essential oils of *X. aethiopica* while Ar-turmerone, curlone, andrographolide, oleic acid, thumbergol and card-20 (22) –enolide could not be identified.

Diethyl phthalate, an etlyl ester was identified at a concentration of 8.83%. Abad *et al.* (2005) and USEPA (1992) reported diethyl phthalate as having bitter tastes with slightly aromatic odour but cautioned that due to its ecological potential with high environmental relevance and its low solubility in water, they tend to precipitate and concentrate as sludge and suggested that concentration of phthalic acid esters should be closely monitored. Matsumoto *et al*. (2008) reported that phthalic acid esters and their metabolites produce reproductive and developmental toxicities in laboratory animals. Their findings have raised concern about the possibility of phthalic acid esters as contributors to reproductive and developmental adverse effects in humans. El-Hadi *et al.* (2013) found esters as one of the most abundant aroma compounds in apples, pears and many other fruits. Diethyl phthalate was reported as one of the essential oils of *X. aethiopica* by Hassan *et al.* (2016).

Curlone which belong to the sesquiterpenes and usually a constituent of *C. longa* was identified. Though curlone is found in herbs and spices, it is usually applied as either a nutrient, stabilizer or surfactants and emulsifiers and so does not contribute to flavour in *X. aethiopica*.

Parimalakrishnan *et al.* (2015) identified curlone as one of the phytonutrients during petroleum ether extraction of *Cleome chelidonil*.

Oleic acid which is an unsaturated fatty acid was identified, however it does not contribute to the flavour of *X. aethiopica*. It is said to be an odourless and colourless oil.

4.2.4.6 Aqueous (water) extract

In Fig. 4.6 and Table 4.4.5, the major chemical compounds identified in the aqueous (water) extract of *X. aethiopica* were alcohols - represented by benzene-ethanol, 4-hydroxy (2.53%); sesquiterpenes - represented by bioallethrin (28.31%); monoterpenes - represented by beta-phellandrene (4.29%); esters - represented by phthalic acid (3.15%); organic acid - represented by adipic acid (13.67%) and steroids (47.38%) - mainly represented by pregan-20-one (33.26%) and pregan-4-ene-1,20-dione (14.12%). This result is partially in line with the reports of Hassan *et al.* (2016) who stated that benzene-ethanol, bioallethrin, beta-phellandrene, and phthalic acid were in the essential oils of *X. aethiopica*. Bioallethrin does not contribute to flavour, it is a yellow to brown oil, almost odourless and a synthetic pyrethroids with fast knock-down activity against house hold pest insect and so it is used as an insecticide (WHO, 2002). This justified the reason why *X. aethiopica* was being used in making insecticides. The adipic acid also identified is a dicarboxylic acid and does not contribute to the flavour profile of *X. aethiopica*. Cotton and Wilkenson (1998) stated that adipic acid is odourless but has a tart taste and is used mainly in the production of nylon as an additive and gelling agent in jello or gelatins.

4.2.4.7 Common flavour compounds identified in the solvent extract samples

Table 4.4.6 showed the most common flavour compounds identified in the solvent extract samples of *X. aethiopica*. The three predominant compounds detected in the solvent extracts were beta-pinene, beta-phellandrene and andrographolide. Reports from different authors across African countries like Sudan (Elkamali and Adams, 2009; Itimad *et al.,* (2010), Benin (Poitou *et al.,* 1996), Cameroun (Jirovetz *et al.,* 1997; Tatsadjieu *et al.,* 2003), Nigeria (Asekun and Adeniyi, 2004; Olonisakin *et al.*, 2007), Chad and Cameroun (Issakou *et al.*, 2004, Noudjoui *et*

al., 2007), Togo (Koba and Sanda, 2008), Mali (Kieta *et al.*, 2003), Ghana (Karioti *et al.*, 2004), and Ivory coast (N'dri-Konan, 2009) have identified beta pinene as an important essential oil that contribute to the flavour of *X. aethiopica* since it was present in all the essential oils obtained from *X. aethiopica* from these countries.

Hassan *et al.* (2016), Noudjoui *et al.* (2007), Olonisakin *et al.* (2007), Onayade-Sontan, (1991) and Tairu *et al.* (1999) also identified beta phellandrene as one of the major components of essential oils of *X. aethiopica*.

The Andrographolide detected in all the solvent extracts except the aqueous extract, was reported by Kligler *et al.* (2006) as a bitter substance used in treating liver disorder, Colin pain and upper respiratory tract infection and thus may not contribute to flavour. It therefore means that from the result of this study (Table 4.4.6), the monoterpenes; beta pinene and beta phellandrene are the major flavour compounds of *X. aethiopica* detected in the solvent extracts. Thus the result is in agreement with the reports of many African authors as cited earlier.

Beta-Phellandrene had the highest concentration (23.93%) in 40% ethanol extract which was significantly different ($p<0.05$) from the concentrations in the other solvent extracts, while beta-pinene had the highest concentration (20.28%) in 40% ethanol extract which was also significantly different ($p<0.05$) from the absolute ethanol and methanol extracts. Beta-pinene was not detected in n-hexane and aqueous extracts.

The Andrographolide in this study had the highest concentration (9.66%) in absolute ethanol which was significantly different ($p<0.05$) from 40% ethanol, acetone, methanol and n-hexane extracts. Andrographolide was not detected in aqueous extract which is in line with the reports of Kligler *et al.* (2006) who stated that andrographolide has limited solubility in water. As earlier stated, many authors across African countries had earlier identified beta pinene as an important compound in the essential oils of *X. aethiopica* responsible for it aroma. Therefore in considering the best yield and cost of solvent, 40% ethanol is recommended and should be chosen as the best solvent for extraction of the bioactive flavour compounds beta-Pinene and beta-Phellandrene.

4.2.5 The Concentrations of the most predominant flavour principle (beta-phellandrene) in *X. aethiopica* spice products under ambient storage condition.

Table 4.5.1 shows the concentrations of the most predominant flavour principle (beta-phellandrene) in *X. aethiopica* spice products under ambient storage condition for 6 months.

At zero hour of storage, the 40% ethanol extract had the highest concentration of beta Phellandrene (23.93%) followed by the product of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier, with 19.66% while the product of wheat grits encapsulation at a ratio of 1:3 spice extract to carrier had the least value with 15.18%. There was no significant difference ($p>0.05$) among all the samples. It could be seen from the result that most of the encapsulated products had lower value of beta phellandrene at zero hour compared to that of the spice powder and 40% ethanol extract, except the samples encapsulated with wheat grits at a ratio of 1:2 spice extract to carrier, which had a concentration of 19.66%, a little above the spice powder. The powder had less value than the 40% ethanol extract probably because the powder had more exposure to the atmosphere and so lost some flavour to the surrounding environment more than the 40% ethanol extract. Augustine *et al.* (2001) and Desai and Park, (2005) stated that four criteria were mostly considered in food oils during flavour encapsulation. They include properties of carrier materials, characteristics of the oil or flavour materials, characteristics of the emulsion (specification of the infeed emulsion) and condition of the drying system. Among these factors and in relation to this work, properties of the carrier materials and emulsion characteristics were the two critical factors that affected the encapsulation of the spice samples with respect to their retention of beta phellandrene. The other two factors were presumed constant. The pre-gelatinization treatment given to the wheat and yam grits modified the starches and improved their physico-chemical properties. These starches comprised of amylose and amylopectin as their macromolecules. Wheat also contain gluten.

Singh *et al.* (2003) reported that starches of wheat and rice have higher phospholipids content than starches of potato, yam or corn and that the phospholipids content in starch granules is proportionally related to amylose since the phospholipids tend to form complex with amylose resulting in limited solubility of the starch. Campos *et al.* (2011) and Dhall (2013) also stated that during gelatinization, amylose form excellent films that are very strong which can prevent moisture (or flavour transfer), gas exchange, oxidation and the movement of solids. Dhall, (2013) went further to state that amylopectin molecules cannot adequately form films, the branched structure impacts poor mechanical properties to the film reducing its tensile strength

and elongation. Native starch does not produce films with adequate mechanical properties and requires pre-treatment. Schirmer *et al.* (2013) also reported that cereal starches had higher lipid content than the root and tuber starches. It therefore means that wheat grits could form a better and stronger film than the yam grits and that might be the reason while at zero hour the product of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier had higher value of beta Phellandrene than yam grits counterpart. The product of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier produced an excellent film that had better hold of the flavour compound and so had a higher value.

It could also be deduced that the emulsion complex of spice extract and wheat grits had a higher solid content and higher viscosity than the emulsion complex of spice extract and yam grits. Liu *et al.* (2001) stated that higher solid content and higher viscosity help volatile flavour retention by reducing the required time to form a semi permeable surface and also enable decreased internal circulations in the droplets. Based on this statement, wheat grits as a carrier material is favoured to hold the flavour better.

At two weeks investigation of the effect of storage on *X. aethiopica,* it was observed that there was significant difference ($p<0.05$) between the values obtained at zero hour and those obtained at 2 weeks for the encapsulation products, thus indicating a significant drop in the flavour compounds. However, no significant difference ($p>0.05$) was observed in the spice powder and 40 % ethanol extract.

At the 4^{th} week of storage, a significant difference ($p<0.05$) was observed within the powder samples, showing a drop in the values of the flavour principles but there was no significant difference ($p>0.05$) within the 40% ethanol extract showing that 40% ethanol extract did not lose any significant amount of flavour components from zero hour to 4 weeks of storage.

For all the encapsulated samples (products of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier, wheat grits encapsulation at a ratio of 1:3 spice extract to carrier, yam grits encapsulation at a ratio of 1:2 spice extract to carrier and yam grits encapsulation at a ratio of 1:3 spice extract to carrier) a significant difference ($p<0.05$) was observed within each of the samples between two weeks of storage and 4 weeks of storage showing a continuous decline in flavour retention in the encapsulated samples.

No significant difference ($p>0.05$) was observed in the powder sample and the 40 % ethanol extract between the 4 weeks storage value and the values obtained at 6 weeks. The 40% ethanol extract showed no significant difference from zero hour of storage to 6 weeks of storage, indicating that it was able to retain the flavour compounds for 6 weeks without loss. No significant difference ($p>0.05$) was observed within products of yam grits encapsulation at a ratio of 1:2 spice extract to carrier from 4 weeks of storage but significant differences ($p<0.05$) were observed within products of wheat grits encapsulation at a ratio of 1:2 spice extract to carrier, wheat grits encapsulation at a ratio of 1:3 spice extract to carrier and yam grits encapsulation at a ratio of 1:3 spice extract to carrier.

At 2 months of storage, it was found that no significant difference ($p>0.05$) existed within the spice powder from its 6 weeks of storage. In the 40% ethanol extract, a significant difference ($p<0.05$) was observed for the first time in the sample between 6 weeks of storage and 2 months of storage. This implied that the 40% ethanol extract retained the flavour component for a period of 6 weeks or more before a noticeable drop was observed after 2 months of storage.

A significant difference ($p<0.05$) was observed within all the values obtained from the encapsulated samples from 6 weeks to 2 months of storage.

From 3 months to 6 months of storage, there were significant differences ($p<0.05$) in the values obtained from the spice powder as the month progressed showing a continuous decline in flavour retention. Among the different samples, there were some relative differences in the beta-phellandrene content at all storage periods. In the 40% ethanol extract, from the 3rd month to 6th month of storage time, a significant difference ($p<0.05$) existed between the values on 2 months storage and 3 months storage but no significant difference ($p>0.05$) was observed in the values obtained between 4 months and 5 months of storage while at the sixth month of storage only a relative difference was observed. From 3 months to 6 months storage, there was significant differences ($p<0.05$) within all the encapsulated samples as the months progressed.

Comparing the rate of flavour retention (or loss) of beta-phellandrene, investigated for a 6 months storage time at ambient ($28\pm3\ ^{o}C$) condition, it could be summarized that from Tables 4.5.1 and 4.5.2, the spice powder beta-phellandrene values ranged from 19.42% at zero hour to 14.22% at 6 months storage indicating a percentage retention of 73.22% (26.78% loss). The 40%

ethanol extract beta-phellandrene values ranged from 23.93% at zero hour to 19.56% at 6 months storage indicating a percentage retention of 81.74% (18.26% loss). The wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier beta-phellandrene values ranged from 19.66% at zero hour to 13.54% at 6 months storage indicating a percentage retention of 68.87% (31.13% loss). The wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier beta-phellandrene values ranged from 15.96% at zero hour to 8.96% at 6 months storage representing a percentage retention of 56.14% (43.86% loss). The yam grits encapsulation product at a ratio of 1:2 spice extract to carrier beta-phellandrene values ranged from 17.92% at zero hour to 9.77% at 6 months storage indicating a percentage retention of 54.52% (45.48% loss). The yam grits encapsulation product at a ratio of 1:3 spice extract to carrier beta-phellandrene values ranged from 16.15% at zero hour to 7.06% at 6 months storage indicating a percentage retention of 43.72% (56.28% loss).

From the above, it could be deduced that the 40% ethanol extract had the highest flavour principle retention (81.74%) over a six month storage period with just 18.26% flavour loss, followed by the spice powder, with flavour principle retention of 73.22% (26.78% flavour loss) while the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least retention of flavour (43.72%) with a whopping 56.28% flavour loss. Therefore the best sample with respect to flavour retention at 6 months storage was the 40% ethanol extract. This result is in line with the findings of Tipsrisukond *et al.* (1998) that spice oleoresin extraction was better when compared with freshly ground or prepared spice in terms of shelf life due to a higher rate of evaporation and oxidation of important volatiles in the ground spice. They went further to state that oleoresins are more stable resulting in less net loss of volatiles over time. The report of Shaik *et al.* (2006) also supported this result as they reported about the stability of oleoresins and the degradation of volatiles in oleoresin over time being due to oxidative and polymeric changes which lead to alteration in both the important fatty acids and volatile monoterpene constituents.

For the encapsulated samples, one would have expected them to have excellent retention of flavour compounds but the result obtained was different as they recorded the highest flavour losses and the least flavour retention values, though the encapsulated samples with wheat grits had better flavour retention than their corresponding yam grits flavour encapsulates, at the same ratio of liquid extract to carrier material. It could as well be deduced that when encapsulated in

wheat grits, there was emulsion stability with the extract that made the surface structure of the wheat starch more absorbable to the volatiles at a quicker rate than that of yam grits carrier. Desai and Park (2005) reported that the ideal carrier for a good flavour encapsulation should have emulsifying properties, be a good film former, have low viscosity at high solids level, exhibit low hygroscopicity, release the flavour when reconstituted in a finished food product, be low in cost, bland in taste, stable in supply and afford good protection to the encapsulated flavour or oil. Because almost no carriers can meet all the properties listed above, in practice they are used in combination with each other to give maximum flavour retention. But since this work did not combine different carriers to achieve the best required characteristic of carriers (wall materials), it could be the reason why all the encapsulated samples showed lower flavour retention and high rate of flavour loss than the spice powder and 40% ethanol extract.

Several researchers have suggested the use of combined carbohydrate wall materials for encapsulation efficiencies [Augustin *et al.*, (2001), Desai and Park (2005), Liu *et al.*, (2001)]. Apintanapong and Noomherm (2003) also reported that a combination of gum arabic (GA) and Maltodextrin (MD) was effective for the encapsulation of rice flavour as against the use of only a single carrier. This study also found that as the ratio of flavour carrier (wall material) to extract increased, the percentage of retained flavour compound (beta-phellandrene) decreased. This means that the flavour extract-grits binding capacity was higher with less grits and so yielded a better stable emulsion than when higher quantity of grits were used.

4.2.6 Profile and concentrations of major compounds in 40% ethanol extract of *C. longa*

Figure 4.7 and Table 4.6 show the concentrations of compounds identified by GC-MS from the 40% ethanol extract of *C. longa*. The major compounds identified were sesquiterpenes (40.55%) - mainly represented by Ar-turmerone (22.31%), turmerone (6.36%) and curlone (11.88%); fatty acids (27.16%) - mainly represented by n-hexadecanoic acid (6.8%), oleic acid (16.65%) and octadecanoic acid (stearic acid) (3.71%); diarylheptanoid - solely represented by curcumin (12.02%) and steroid - represented by stigmasterol (4.35%). Curcumin which is a phenolic compound that belongs to a group of diarylheptanoid and which has an aryl-C7-arly skeleton is responsible for the yellow pigment that encouraged its use for food colouring. The Curcumin

concentration was 12.02% which was in agreement with the reports by Li *et al.* (2011) that curcumin presence in *C. longa* rhizome was within the range of 3-15%. The result obtained was also in agreement with the reports of Chempakan and Parthasarathy (2008) that the major part of the oil from *C. longa* rhizome contained majorly sesquiterpenes which are responsible for its aromatic taste and smell. Sharma *et al.* (1997) reported that Ar-turmerone, α-turmerone, β-turmerone and curlone are the major ketonic sesquiterpene of the essential oils of *C. longa* rhizome and might contribute at least 40% of the essential oils and these are in agreement with the results obtained in this study, with sesquiterpenes contributing 40.55% of the essential oils. Chen *et al.* (2010) and Chowdhury *et al.*(2008) identified the presence of four steriods and five long chain fatty acids from *C. longa* rhizome which includes β-sitosterol, stigmasterol, gitoxigenin and 20-oxopregn-16-en-12-yl acetate for the steroids while the fatty acids include linoleic acid, 8,11-octadecadienoic acid, n-hexadecanoic acid, oleic acid and stearic acid. These were in agreement with the compounds identified in the results as shown on Table 4.6.

Zhengzhi *et al.* (2009) and Ramirez-Ahumada *et al.* (2006) also reported turmerone as one of the main compounds found in the rhizome of *C. longa* and stated that it is an intermediate for the formation of *zingiberene* and *sesquiphellandrene* which are two major sesquiterpenes of *C. longa* rhizome. The variations in the composition of essential oils found in this study with that found by other researchers might be due to differences in varieties and geographical locations as well as the analytical methods employed. Oleic acid was identified to be second highest in concentration with 16.65%.

4.2.7 Curcumin content in different solvent extracts of *C. longa*

Figures 4.8 – 4.13 and Table 4.7 (Appendix II, fig. 7) show the result of the ultra-fast liquid chromatography (UFLC) analysis of the *C. longa* extracts using different solvents. The highest concentration of curcumin (53.27%) was achieved with absolute ethanol while the least concentration (2.29%) was observed in aqueous extract (water). These values were not surprising because Li *et al.* (2011) have earlier stated that curcumin was insoluble in water (hence low value) but dissolve well in ethanol, methanol and acetone (hence higher values). There were significant differences ($p<0.05$) among all the sample extracts. Braga *et al.* (2003) remarked that

the extraction rate and composition of curcumin was significantly affected by various extraction techniques. Absolute ethanol extract having the highest concentration of curcumin (53.27%) conformed with the reports of Braga *et al.* (2003) and Chen *et al.* (2008) who that among different extraction solvents, ethanol extraction gave the highest yield of curcumin. The result in this study is also supported by the reports of Han *et al.* (2007) that ethanol extract, particularly high grade ethanol was more stable with improved yield of Curcumin. They reported a trend that 80% ethanol > acetone > 60% ethanol > methanol > 50% ethanol or ethyl ether > ethyl acetate > 40% ethanol > petroleum ether or water, but what determines choice of the solvent is the cost, the yield and compatibility of the extracting solvent with the intended product.

4.2.8 Curcumin concentrations in *C. longa* spice product samples during 6 months storage

The results of Curcumin content in *C. longa* product samples during 6-months storage are shown in Table 4.8.1. At zero hour of storage the spice powder had the highest curcumin concentration (53.27%) followed by the 40% ethanol extract with a value of 10.74% while the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least concentration value of 4.39%. All the encapsulated samples had lower curcumin content than the spice powder and the 40% ethanol extract. At the zero storage hour, the encapsulated samples with yam grits had more curcumin than the wheat grits counterparts at same ratios of extract (spice extract) to carrier. The factors that determine the rate of curcumin adsorption in these encapsulated samples were probably the properties of the carrier and the emulsion characteristic. As earlier discussed and from inference derived from the reports of Campos *et al.* (2011), Dhall (2013) and Singh *et al.* (2003), one would expect that the samples with wheat grits would have had a higher value than samples with yam grits due to better film forming properties of pre-gelatinized wheat grits, but the reverse was the case as the yam grits encapsulated samples had higher curcumin than the wheat grits encapsulates. It may be that yam grits had higher binding capacity with curcumin in the emulsion and the yam grits may also have had a higher content of undamaged starch granules. The higher binding capacity of the yam starch may have caused a stronger and stable emulsion enabling the curcumin to be better adsorbed on the undamaged granules and thus the higher content. Mayaki *et al.* (2003) also stated that an increase in the amount of undamaged starch granules in a carrier results to higher water binding capacity of the carrier. It therefore could be deduced that the flavour- yam grits emulsion complex had a higher viscosity due to its

higher binding capacity and as stated by Liu *et al.* (2001) a higher viscosity helps flavour holding and retention, by reducing the required time to form a semi-permeable surface.

At two weeks storage, no significant difference ($p>0.05$) was observed within all the samples from their zero hour of storage except in the product of wheat grits encapsulation at a ratio of 1:2 (spice extract to carrier). Among the different samples there were some relative differences in the Curcumin content at all storage periods.

At the fourth week of storage, significant difference ($p<0.05$) was observed between the Curcumin content of the *C. longa* powder samples from 2 weeks and 4 weeks period. In the 40% ethanol extract, not much difference was observed which meant that the 40% ethanol extract was able to retain the curcumin with minor differences up to the 4^{th} week. The wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier had significant difference ($p<0.05$) with the rest of the samples as the curcumin content continued to depreciate as the weeks progressed. The wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier had a negligible difference between the two weeks and 4 weeks of storage. The yam grits encapsulation product at a ratio of 1:2 spice extract to carrier had no significant difference in curcumin values from zero hour to 4^{th} week of storage, probably because the semi-permeable membrane formed was strong to protect the curcumin. The yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had significance difference ($p<0.05$) between the curcumin content of two weeks and four weeks storage.

At 6 weeks of storage, significant differences in curcumin content ($p<0.05$) were observed within the spice powder, the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier, the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier and the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier from their values at 4 weeks of storage. It was at this period of storage time that the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier had its first significant change, its content of curcumin remained significantly same until the 6^{th} week. There was no significant change within the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier and the 40% ethanol extract, only negligible changes were observed.

At two months of storage, only the 40% ethanol extract was not significantly different ($p>0.05$) from the 6 weeks storage, all the other samples showed significant difference ($p<0.05$) in curcumin content within themselves when compared to the values at 6 weeks of storage.

From 3 to 6 months of storage, there were significant differences ($p<0.05$) within all the samples as the months progressed. This implied that from 3 months of storage, there was continuous drop in the curcumin content in all the samples.

Comparing the rate of curcumin retention or loss during the 6 months storage time at ambient ($28\pm3^{o}C$) condition, it could be summarized from Tables 4.8.1 and 4.8.2 that, the curcumin content in the spice powder ranged from 53.27% at zero storage hour to 39.67% at 6 months representing, a percentage retention of 74.47% (25.53% loss). The 40% ethanol extract curcumin values ranged from 10.74% at zero storage hour to 5.65% at 6 months representing a percentage retention of 52.61% (47.39% loss). The wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier curcumin content ranged from 5.30% at zero storage hour to 3.08% at 6 months, representing a percentage retention of 58.11% (41.89% loss). The wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier curcumin content ranged from 4.39% at zero storage hour to 2.03% at 6 months, representing a percentage retention of 46.24% (53.76% loss). The yam grits encapsulation product at a ratio of 1:2 spice extract to carrier curcumin content ranged from 9.46% at zero storage hour to 5.46% at 6 months, representing a percentage retention of 57.72% (42.28% loss). The yam grits encapsulation product at a ratio of 1:3 spice extract to carrier curcumin content ranged from 5.58% at zero storage hour to 2.57% at 6 months, representing a percentage retention of 46.06% (53.94% loss).

From the above comparisons, it could be deduced that at the end of the six months storage, the spice powder had the highest concentration of Curcumin (39.67%) with a retention of 74.47% and a loss of 25.53%, followed by the 40% ethanol extract with a Curcumin concentration of 5.65%, it retained only 52.61% of its initial content with a loss of 47.39%. The wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least concentration of Curcumin (2.03%) with retention of 42.28% of its initial content and a loss of 53.76%. One would have thought that the 40% ethanol extract would have had better retention than the spice powder however, the result was in line with the findings of Mondal *et al.* (2016) that curcumin is photodegradable in aqueous solution.

For the encapsulated product samples, when compared based on same ratio of spice extract to carrier (ie wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier vs yam grits encapsulation product at a ratio of 1:2 spice extract to carrier and wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier vs yam grits encapsulation product at a ratio of 1:3 spice extract to carrier), it was observed that the yam grits had higher concentrations of curcumin, specifically, yam grits encapsulation product at a ratio of 1:2 spice extract to carrier had higher curcumin (5.46%) than wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier (3.08%), while yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had higher curcumin (2.57%) than wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier (2.03%). Singh *et al.* (2003) stated that pregelatinized wheat had amylose and phospholipids which tend to form excellent film that were very strong to prevent moisture or gas exchange or penetration. This may be the reason why the wheat grits encapsulated samples had lower concentration of Curcumin than the yam grits counterparts. In addition, the emulsion complex of spice extract and yam grits may have had a higher solid content and higher viscosity than the spice extract and wheat grits complex.

From these results, it showed that the spice powder had the potential to adsorb and retain curcumin better than the other samples. It is not surprising because Mondal *et al.* (2016) have explained the photo degradability of curcumin in aqueous solutions which all the other samples probably experienced.

4.2.9 Total counts and identities of fungi isolates in *C. longa* and *X. aethiopica*

The results of the total fungal counts and the identities of the isolates are presented in Table 4.9. All the samples were observed to be contaminated. Normal microflora has been reported by Oranusi and Braide (2012) to withstand processing activities and are likely to be found in final products. Thus these could be normal flora fungi of the spices. These could also be introduced through processing equipment, handlers etc during processing.

From the results, the raw *C. longa* at harvest (raw) contained *Saccharomyces cerevisiae,* a yeast species while the processed *C. longa* (powder) contained *Saccharomyces cerevisiae* and *Mucor spp*. (a mould). The raw *X. aethiopica* at harvest contained *Saccharomyces cerevisiae* and

Penicillium notatum while the ground (processed) sample contained *Penicillium notatum* and *Fasarium* spp. These are usually associated with plants in the field.

The mean fungal counts ranged from 2.0 X 10^5 cfu/g in the ground (processed) *C. longa* sample to 3.2 X 10^8 cfu/g in the raw sample while the mean fungal count for *X. aethiopica* ranged from 2.20 X 10^5 cfu/g in the ground (processed) sample to 5.1 X 10^8 cfu/g in the raw sample. The International Microbiological Standard (IMS) limits for microbial contaminants in spices are in the range of 10^1 to 10^3 cfu/g for Coliform, 10^1 to 10^5 cfu/g for total plate count, 10^1 to 10^3 cfu/g for yeast and mould, 0 per 20g for *Staphylococcus aureus* and 0 per 20g for *Escherichia coli* (Awe *et al.*, 2009). Considering the results obtained in this study, both the raw and ground (processed) samples of *C. longa* and *X. aethiopica* were above the recommended IMS limits of 10^1 to 10^3 cfu/g for yeast and mould. This poses potential danger as the moulds are common spoilage organisms that can produce mycotoxins in the products. Therefore it is advisable that the spices be fumigated or irradiated to sterilize the moulds. Peter (2006) reported that γ-irradiation is effective for commercial decontamination of spices. A dose of 3-10 kGy can reduce the viable cell count to an acceptable level and does not affect the sensory quality of spices while Sadecka (2007) stated that spice products are fumigated either using liquid fumigants like methyl bromide at a concentration of 32g/m^3 with an exposure time of 24 to 48 hours or using commercially available solid fumigant aluminium phosphide at concentration of 3 to 6 g/m^3 as liberated phosphine with an exposure time of seven days. The ground (processed) samples of *C. longa* and *X. aethiopica* had lower microbial loads than the raw samples. It could be that the drying and milling as processing techniques reduced the microbial loads thus the fungal counts recorded. Though, the drying temperature was not adequate to kill the microorganisms. The microbial contamination of spices can be influenced by the presence of microorganisms in processing plants, air, dust, use of contaminated water, presence of animal / human excreta, pre and post- harvest procedures including processing, storage and distribution operations (Parveen *et al.*, 2014). This could be the reason why some fungi which were not identified in the raw spice were seen in the ground (processed) sample in this study.

Idu *et al.* (2011) identified *Aspergillus niger*, *Penicillium* spp, *Rhizopus stolonifera* and *Mucor* spp.in *C. longa* and *Penicillium* spp. and *Rhizopus stolonifera* in *X. aethiopica* as the predominant fungal species during their work on microbial load of some medicinal plants. These

findings by Idu *et al.* (2011) are in agreement with the results obtained in this study and the variations may be due to the factors explained by Parveen *et al.* (2014).

Though the *Saccharomyces cerevisiae* and *Mucor* spp. loads identified in *C. longa* were higher than the recommended limits set by International Microbiological Standard, there may likely be no health risk in incorporating the spice for culinary purposes as the effect of additional heat during cooking will further destroy these microbes. But for the *X. aethiopica*, the *Fasarium* spp, which is a filamentous fungi could produce mycotoxins that could affect human health if they enter the food chain, therefore, further processing such as fumigation or irradiation is required to bring this organism within the recommended acceptable limits.

4.2.10 Total counts and identities of bacterial isolates of *C. longa* and *X. aethiopica* from Nutrient agar

Table 4.10 shows the total bacterial counts and identities of bacterial isolates from *C. longa* and *X. aethiopica* cultured on Nutrient agar. The bacterial cells identified from the raw *C. longa* included *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus faecalis* while the isolates identified from the ground (processed) *C. longa* sample included *Enterococcus faecalis* and *Bacillus subtilis*. For the *X. aethiopica*, the bacterial isolates identified in the raw spice were *Enterococcus faecalis* and *Bacillus cereus* while *Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus faecalis* were identified in the ground (processed) sample. The bacterial loads in *C. longa* ranged from 4.7 x 10^8 cfu/g in the ground (processed) sample to 7.1 x 10^8 cfu/g in the raw sample while that of *X. aethiopica* ranged from 3.5 X 10^8 cfu/g in the ground (processed) sample to 3.9 x 10^8 cfu/g in the raw sample. When the results obtained were compared with the limits set in the International Microbiological Standard (10^1 to 10^5 cfu/g), they were all above the recommended limit. The higher microbial counts (7.1 x 10^8 cfu/g) obtained in the raw *C. longa* sample could be as a result of poor handling technique. *S. aureus* that was initially identified in the raw *C. longa* but not seen in the ground sample could have been eliminated as a result of the drying processes, while in the *X. aethiopica* the *S. aureus* that was identified in the ground (processed) sample but absent in the raw sample may have been

transmitted by humans during processing since *S. aureus* resides on the skin and mucus membrane of the humans or due to unhygienic environmental condition.

Gallo *et al.* (1992) reported that faulty food handling techniques especially the storage of foods at improper temperatures for long period of time has been identified as a reason for the microbial proliferation in contaminated foods. Staphylococcal food poisoning has been identified by Vora *et al.* (2003) as a persistent cause of gastroenteritis worldwide, especially in developed countries and this explains the reason why the International Microbiological Standard, recommended limit for *S. aureus* was 0 per 20g. Therefore the ground (processed) *X. aethiopica* with traces of *S. aureus* should not be recommended for use in food, unless further treatment or processing such as irradiation is done to totally eliminate the *Staphylococcus*. *Enterococcus faecalis* found in both raw and ground (processed) *C. longa* and *X. aethiopica* spices are facultative anaerobic bacteria capable of cellular respiration in both oxygen-rich and oxygen-poor environment and could tolerate wide range of extreme environment such as temperature and pH (Fisher and Philips, 2009). *Bacillus spp*. also identified in all the samples could be obligate aerobes (oxygen reliant) or facultative anaerobes (having ability to be aerobic or anaerobic) and so, under stressful environmental condition can produce oval endospores and remain in a dormant state for long period of time and later regenerate to cause food poisoning if not eliminated (Ryan and Ray, 2004). Heat treatment at a very high temperature can subdue *Bacillus spp*. The identified bacteria in these spice products were partially in agreement with those reported by Idu *et al.* (2011) who stated that the predominant bacterial species isolated from *C. longa* included *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis* while those of *X. aethiopica* include *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia.* The variations could be as a result of the spice origin and other environmental conditions as well as handling.

4.2.11 Total counts and identities of bacteria isolated from *C. longa* and *X. aethiopica* cultured on MacConkey agar.

The results of the total counts and identities of bacterial isolates from *C. longa* and *X. aethiopica* cultured on MacConkey agar are presented in Table 4.11. The bacterial isolate identified on raw

C. longa and *X. aethiopica* in this medium was *Escherichia coli* with total count of 1.1 x 10^8 cfu/g and 6.4 x 10^8 cfu/g respectively. No coliforms were isolated from the ground (processed) *C. longa* and *X. aethiopica* samples. This implied that drying and milling eliminated all the coliforms found in the raw sample. This may have been achieved by reduction in the available moisture for microbial growth. The absence of coliforms on the ground (processed) samples suggest that they can be safely incorporated in foods while the raw samples with total microbial count ranging from 1.6 x 10^8 cfu/g to 6.4 x 10^8 cfu/g and being higher than the International Microbiological Standard recommended limit (10^1 to 10^5 cfu/g) should be processed before use. The International Microbiological standard recommended limit for *S. aureus* and *E.coli* is 0 per 20g which means it is of zero tolerance in foods. *E. coli* can cause serious food poisoning in the host and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005). *E. coli* is expelled into the environment with fecal matter. They are indicator organisms applied in testing for environmental/sanitation suitability for food product preparation.

4.2.12 Total counts and identities of bacteria isolated from *C. longa* and *X. aethiopica* spice samples cultured on brain heart infusion agar.

Table 4.12 shows the total counts and identities of bacteria from *C. longa* and *X. aethiopica* cultured on brain heart infusion agar. The bacterial isolates identified in the raw and ground (processed) *C. longa* samples included *Lactobacillus brevis* and *Streptococcus anginosus*. The bacterial load decreased from 3.0 x 10^6 cfu/g in the raw *C. longa* to 2.0 x 10^3 cfu/g in the ground (processed) sample.

For *X. aethiopica*, the bacterial isolates identified in the raw sample were *Lactobacillus brevis* and *Bacillus cereus* while those identified in the ground (processed) sample included *Lactobacillus brevis*, *Bacillus cereus* and *Streptococcus anginosus*. The bacterial load decreased from 2.1 x 10^6 cfu/g in the raw sample to 1.0 x 10^3 cfu/g in the processed sample.

The observed microbial load in the ground (processed) spice samples were within the safety limit (10^1 to 10^5 cfu/g) as recommended by the International Microbiological Standard (Awe *et al.*, 2009). However, the microbial loads found in the raw spice samples were higher than the

recommended safety limits. The higher levels of bacteria in the raw spice samples could be attributed to the soil where the plants were grown, the environment and the handling operations.

Some of the bacterial isolates identified such as *Lactobacillus spp*. are facultative anaerobic or micro- aerophilic non-spore forming bacteria that can produce hydrogen peroxide which inhibit the growth and virulence of the pathogen *Candida albicons*. Similarly, *Streptococcus spp*. are gram positive bacteria that can cause meningitis, respiratory infection and urinary tract infections. Because these bacteria are not spore-forming, they can be eliminated by simple heat process during cooking. Therefore it could be stated that higher heat application during cooking can kill the vegetative cells of the bacteria but not low heat as encountered during the drying operation.

4.2.13 SENSORY EVALUATION

4.2.13.1 Mean scores (product oriented test) of the sensory attributes of *C. longa* spice samples.

The results obtained from the mean scores of the sensory quality attributes of the *C. longa* spice products are presented on Table 4.13.

Based on colour, the 40% ethanol extract had the highest score (6.28) while the least score (5.0) was for the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier. There was no significant difference ($p>0.05$) between the score for colour of the 40% ethanol extract and the scores for the rest of the samples with the exception of the wheat grits encapsulation products. In the 7 points hedonic scale, the colour of the 40% ethanol extract and the spice powder were moderately liked (score = 6.0) while the colour of the other samples were just slightly liked (scores = 5.0).

The 40% ethanol extract having the highest score (6.28) was not surprising since the 40% ethanol extract gave the highest concentration of curcumin which is the main colour pigment in *C. longa* and the findings of Braga *et al.* (2003) and Chen *et al.* (2008) confirmed that ethanol extract of *C. longa* gave the highest yield of curcumin. For the pungency attribute, there was no significant difference ($p>0.05$) among all the *C. longa* samples. All the samples were found to be

pungent, though the *C. longa* powder and its 40% ethanol extract were moderately liked (score = 6.0) with respect to the pungency attribute while all other samples were just slightly liked (score = 5.0). For the sweetness, only the 40% ethanol extract was neither liked nor disliked with a score of 4.0 was neutral in likeness (scored as neither liked nor disliked), others were scored below likeness (score < 4.0). Thus all the other production methods except extraction with 40% ethanol did not influence the sweetness of *C. longa*. Sensory qualities based on Mintiness, bitterness, harshness and hotness are not peculiar with *C. longa*. There were no significant differences ($p>0.05$) among the *C. longa* samples with respect to after-taste and overall acceptability. Though for after-taste, the spice powder, the 40% ethanol extract and the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier had the same score of 5.85 (moderately liked = 6.0) as the wheat grits encapsulation product at a ratio of 1:2 which was scored 5.71. The yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least score of 5.0 (slightly liked). In overall acceptability, the 40% ethanol extract had the highest score with 6.42 while the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier had the least score of 5.14. The acceptability score were relatively different but the powdered sample, 40% ethanol extract and yam grits encapsulation product at 1:2 ratio were moderately liked (score = 6.0). Therefore it can be deduced that the most acceptable of the spice products is the 40% ethanol extract.

In the sensory attributes of colour, pungency and overall acceptability, the encapsulated samples had lower scores when compared with the spice powder and the 40% ethanol extract which may imply that the carbohydrate carrier used for encapsulation of samples were not pleasant to the panelists.

4.2.13.2 Mean scores (product oriented test) of the sensory attributes of *X. aethiopica* flavour

The results obtained from the mean scores of the sensory attribute of *X. aethiopica* spice products are presented in Table 4.14.

Based on colour, it was observed that the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the highest score (6.28), followed by the product of wheat grits

encapsulation at a ratio of 1:2 spice extract to carrier with a score of 6.0 while the least was the spice powder (5.28). There was no significant difference ($p>0.05$) between the yam grits encapsulation products at a ratio of 1:3 and 1:2 spice extract to carrier and the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier, but their scores were significantly different ($p<0.05$) from the scores for colour from other samples. There was also significant difference ($p<0.05$) between the spice powder and the rest of the samples. With the exception of the spice powder and the 40% ethanol extracts which were liked slightly (in colour) (score = 5.0), all the other samples were moderately liked (score = 6.0).

In terms of pungency, the 40% ethanol extract had the highest score with 6.71, followed by the spice powder with 6.28 while the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least score (4.42). There was significant difference ($p<0.05$) between all the samples with the exception of the products of encapsulation on wheat and yam grits at a ratio of 1:2 where no significant difference ($p>0.05$) was observed. The 40% ethanol extract would have extracted more of the volatile essential oils responsible for the spice pungency, which might be the reason it had the highest pungent score while the degree of pungency in the yam grits product at 1:3 ratio was neither liked nor disliked (score = 4.0). The pungency of the wheat grits product of 1:3 ratio was only slightly liked (score = 5.0) but the pungency of the rest of the samples were moderately liked (score = 6.0).

Sensory attributes of sweetness, mintiness and harshness were scored below 4.0 which inferred that they have little contribution on the sensory attributes of *X. aethiopica.* Even from the origin of the name Xylopia – a Greek word meaning "bitter wood" associating the spice with bitterness, one may not expect to observe these attributes.

For bitterness, the 40% ethanol extract had the highest score (6.0) while the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier had a score of 4.0. The least score was observed in the yam grits encapsulation at ratio 1:3 spice extract to carrier as it was scored 3.57. There was significant difference ($p<0.05$) between the 40% ethanol extract and the rest of the samples though the powder sample was moderately liked (score = 6.0). It could be that the 40% ethanol extract had greater content of the extracted phytochemicals responsible for the spice bitterness.

All the encapsulated product samples had lower scores than the spice powder and the 40% ethanol extract, thus suggesting that the carbohydrate grits carrier may have decreased the bitterness threshold of the spice, or some of the bitter compounds may have been lost during the encapsulation process.

For the hotness attribute, the 40% ethanol extract was also scored the highest with a score of 5.85 followed by the spice powder with 5.71 while the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier had the least score (4.28). The hotness was slightly felt in the wheat grits encapsulation product at ratios 1:3 and 1:2 spice extract to carrier, and the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier. There was significant difference ($p<0.05$) between the score for 40% ethanol extract and the rest of the samples.

For the after-taste, the powdered spice had the highest score (6.0) followed by the 40% ethanol extract with 5.85 while the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier had a score of 4.28 and the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier the least score (3.57). There was significant difference ($p<0.05$) among all the samples.

In the overall acceptability, the 40% ethanol extract had the highest score with 6.14, followed by the spice powder while the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least acceptability with a score of 4.42. There was significant difference ($p<0.05$) in the scores among all the samples. Therefore it can be deduced that the most acceptable of the spice products is 40% ethanol extract. Comparing the encapsulated samples, it could be deduced that the sample encapsulated with wheat grits were more acceptable than those encapsulated with yam grits.

4.2.13.3 Mean scores (consumer oriented test) of the sensory attributes of *C. longa* spice samples

The results obtained from the mean consumer preference scores for the three major sensory attributes of *C. longa* spice samples (colour, pungency and aroma) are presented in Table 4.15. For the colour attribute, the 40% ethanol extract had the highest score (6.25) while the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier, had the least score of 5.25. There was a significant difference ($p<0.05$) between the 40% ethanol extract and the rest of the

other samples. There was no significant difference ($p>0.05$) among the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier, the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier and the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier. No significant difference existed between the spice powder and the encapsulation products. The carbohydrate carrier used in the encapsulation (wheat grits) at ratios 1:2 and 1:3 spice extract to carrier, might have reduced the colour intensity in those samples. The 40% ethanol extract was adjudged the best in colour impact.

For aroma, the spice powder had the highest preference score (6.15) while the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least aroma preference rating score (4.75). There was no significant difference ($p>0.05$) in aroma among the spice powder, the 40% ethanol extract, the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier, the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier and the yam grits encapsulation product at a ratio of 1:2 spice extract to carrier but a significant difference ($p<0.05$) existed between the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier and the rest of the samples with regards to aroma, and with the exception of the yam grits encapsulation product (which was merely slightly preferred, score = 4.75), all the product samples were moderately preferred (scores = 6). The spice powder had the highest score possibly because its surface area were more exposed and chances of quick evaporation of the volatile aroma compounds became higher and this could be the reason why the panelist were able to perceive more smell in the spice powder than in the other samples. There were no significant difference ($p>0.05$) among the *C. longa* spice product samples with respect to pungency and overall acceptability. With regards to pungency, the spice powder and the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier had the same score of 6.05 as the most pungent samples while the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least score (5.50). Also with regards to pungency, all the samples were moderately preferred (score = 6.0).

In overall acceptability, the 40% ethanol extract had the highest score with 6.05 while the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least score with 5.15. Therefore, from the results obtained, the most preferred sample was the 40% ethanol extract. Specifically, the spice powder, the 40% ethanol extract, the wheat grits encapsulation product at

both 1:2 and 1:3 ratios were all moderately preferred (score = 6.0) while the yam grits encapsulation products at 1:2 and 1:3 ratios were both slightly preferred (score = 5.0) by the panelists.

4.2.13.4 Mean scores (consumer oriented test) for the three major sensory attributes of *X. aethiopica* spice samples

The results obtained on the mean consumer preference of the three major sensory attributes (colour, pungency and aroma) of *X. aethiopica* are presented in Table 4.16. For the colour, the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier was most preferred with a score of 6.30 among the samples while the spice powder had the least colour score of 5.1. There were no significant differences ($p>0.05$) among all the samples and with the exception of the powder sample whose colour was only slightly preferred, all the colours of all other products were moderately preferred (score = 6.0). In terms of pungency, the most pungent sample was the 40% ethanol extract with a score of 6.20 while the least was the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier with a score of 4.65. The 40% ethanol extract was expected to be more pungent because it extracted more of the essential oils which are known to be responsible for the pungent nature of the spice. All the encapsulated samples had lower scores than the spice powder and the 40% ethanol extract because the carbohydrate carriers (wheat and yam grits) protected most of the pungent responsible compounds coupled with controlled release of the compounds in solution while some were lost during the encapsulation process. There was significant difference ($p<0.05$) between each of the samples. The spice powder had the highest aroma rating with a score of 6.10 while the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least aroma rating with a score of 4.55. The spice powder had the highest score because the particles are more exposed to evaporation of the essential volatile aroma compounds which were easily perceived by the panelists. A more interesting aspect here was that of the wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier. It was earlier established from this study that wheat grits had better encapsulating property than yam grits because of the phospholipid-amylose complex that gave rise to a strong film during the gelatinization process with its capacity to form a semi permeable surface at a quicker time during encapsulation and thus prevent volatile flavour escape. This was the reason why the wheat grits

encapsulation at ratio 1:2 spice extract to carrier was able to retain most of the volatile aroma compounds that were detected by the panelist during the test. There was significant difference ($p<0.05$) between the spice powder, the yam grits encapsulation product and the rest of other samples. No significant difference ($p>0.05$) was observed between the 40% ethanol extract and the yam grits encapsulation product at a ratio of 1:3 spice extract to carrier and also between the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier and the yam grits encapsulation at ratio 1:2 spice extract to carrier. The aroma of the powder and yam grits encapsulation product had clear moderate preference among the panelist. Regarding the overall acceptability, the spice powder and the 40% ethanol extract had the same score of 6.25 as the most preferred while the wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier had the least score in overall preference with 4.55. No significant difference ($p>0.05$) existed between the spice powder, the 40% ethanol extract and the yam grits encapsulation product of 1:2 ratio but they were significantly different from the rest of the samples. No significant difference ($p>0.05$) also existed between the wheat grits encapsulation product at ratio 1:3 spice extract to carrier and the yam grits encapsulation product at ratio 1:3 spice extract to carrier but they were significantly different ($p<0.05$) from the other samples. From the results obtained, the spice powder and the 40% ethanol extract were jointly selected as the most preferred samples.

4.2.13.5 Recommended Foods/Drinks for the spices as ingredient

The results of percentage of interested consumers in the use of the *C. longa* product samples in different Nigerian food products is shown in Table 4.17.

The spice powder was recommended for rice, meat, sauce, tea, akamu/pap (custard) and baked products. The highest recommendation for the spice powder was for akamu/pap (custard) where 80% of the panelists recommended it while 25% of the panelists recommended it for tea.

The 40% ethanol extract was recommended for a greater number of different foods and drinks which includes rice, meat, sauce, tea, akamu/pap (custard), baked products, ice cream, yoghurt, non-alcoholic beverages and alcoholic beverages. The highest recommendation for the 40%

ethanol extract was for akamu/pap (custard), ice cream, yoghurt, non-alcoholic beverages and alcoholic beverages where 80-100% of the panelists accented to its use on those food/drinks.

All the encapsulated samples (wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier, wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier, yam grits encapsulation product at a ratio of 1:2 spice extract to carrier and yam grits encapsulation product at a ratio of 1:3 spice extract to carrier) were recommended by between 25-45% panelists for rice, meat, sauce and akamu/pap. The panelists` recommendations correlated with the reports of Manoharan *et al.* (2012) who recommended *C. longa* oleoresin as a colouring for dairy products, meat, ice cream, yoghurt and frozen desserts. Han (2016) reported seven ways to eat and drink *C. longa* to include: adding it to scrambles and frittatas – "a pinch of *C. longa* in scrambled eggs gives it a familiar colour and subtle flavour; tossing it with roasted vegetables; adding it to rice; trying it with greens; using it in soups; blending it into a smoothie and using it to make tea".

Table 4.18 shows the results of percentage of interested consumers in the use of the *X. aethiopica* product samples in different Nigerian food products. The results showed that the spice powder and the 40% ethanol extract were recommended for greater number of different food and drinks.

The spice powder and the 40% ethanol extract were recommended for meat, porridge, Yoruba local soup, pepper soup, Igbo local soup, tea (herbal), Hausa local soup and alcoholic beverages.

All the encapsulated samples (wheat grits encapsulation product at a ratio of 1:2 spice extract to carrier, wheat grits encapsulation product at a ratio of 1:3 spice extract to carrier, yam grits encapsulation product at a ratio of 1:2 spice extract to carrier and yam grits encapsulation product at a ratio of 1:3 spice extract to carrier) were recommended for meat, porridge, Yoruba local soup, pepper soup, Igbo local soup and Hausa local soup.

All the panelists recommended all the encapsulated samples for pepper soup while the spice powder and 40% ethanol extract were recommended by all the panelists for pepper soup and alcoholic beverages.

4.2.13.6 Mean consumer scores for the four major sensory attributes of *X. aethiopica* spiced (flavoured) pepper soup samples

The results obtained from the mean consumer scores for the four major sensory attributes (colour, pungency, taste and mouth feel) of *X. aethiopica* spiced pepper soup are presented in Table 4.19.

With regards to colour, the pepper soup samples spiced with either *X. aethiopica* powder or 40% ethanol extract of the spice were moderately liked (score ≈ 6.0), but the pepper soup sample spiced with the wheat grits encapsulation product was just slightly liked (score = 5.3). In the pungency of pepper soup samples, though the score for the pepper soup samples were significantly different ($p<0.05$), the panelists had moderate likeness (score ≈ 6.0) for the three spiced pepper soups. The consumer panel liked the taste of pepper soup spiced with *X. aethiopica* powder very much (score ≈ 7) while the other two pepper soup samples were moderately liked (score ≈ 6.0) though the pepper soup spiced with 40% ethanol extract had higher score (6.2) compared to the pepper soup spiced with wheat grits encapsulated product which had a score of 5.7. With regard to the mouth feel of the pepper soup samples, the pepper soup spiced with 40% ethanol extract was very much liked (score ≈ 7) while the sample spiced with *X. aethiopica* powder as usually done, was moderately liked (score = 6.4). The least liked among the pepper soup samples was the one spiced with the wheat grits encapsulation product which had a score of 5.2.

Specifically, there was no significant difference ($p>0.05$) between the pepper soup sample spiced with the powdered form and the pepper soup spiced with the 40% ethanol extract, though there was significant difference ($p<0.05$) between them and the sample spiced with wheat grits encapsulation product. Interestingly, there was no significant difference ($p>0.05$) among the pepper soup samples with regards to overall acceptability where all were moderately accepted.

4.2.13.7 Mean consumer scores for the four major sensory attributes of *C. longa* spiced *akamu* (pap) samples

The results obtained from the mean consumer scores for the four major sensory attributes (colour, pungency, taste and mouth feel) of *C. longa* spiced *akamu* (pap) are presented in Table 4.20.

With regards to colour, the *akamu* (pap) samples spiced with either 40% ethanol extract of the spice or yam grits encapsulation product were moderately liked (score ≈ 6.0), but the *akamu* (pap) sample spiced with the *C. longa* powder was very much liked (score ≈ 7.0). In the pungency of *akamu* (pap) samples, though the score for the *akamu* (pap) samples were not significantly different ($p>0.05$), the panelists had moderate likeness (score ≈ 6.0) for the three spiced *akamu* (pap) samples. The consumer panel moderately liked the taste of the three spiced *akamu* (pap) samples (score ≈ 6) though the *akamu* (pap) spiced with *C. longa* powder had the highest score (6.22) followed by the pap spiced with 40% ethanol extract with a score of 6.2 while the yam grits encapsulated product had the least score (6.0). There was no significant difference ($p>0.05$) among the *akamu* (pap) samples. With regard to the mouth feel of the *akamu* (pap) samples, though the score for the *akamu* (pap) samples were not significantly different ($p>0.05$), the panelists had moderate likeness (score ≈ 6.0) for the three spiced *akamu* (pap) samples. The *akamu* (pap) spiced with 40% ethanol extract had the highest score (6.05) followed by the *akamu* (pap) spiced with *C. longa* powder with a score of 6.0 while the yam grits encapsulated product had the least score (5.98).

Interestingly, there was no significant difference ($p>0.05$) among the *akamu* (pap) samples in the overall acceptability score, with all being moderately accepted.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The moisture contents of the spice samples ranged from 7.01 – 9.22% for *X. aethiopica* and 6.45 – 10.01% for *C. longa* which were all within the 10 – 12% acceptable limits for spice products (Gazette of India, 2015; Naviglio *et al.*, 2010).

The 40% ethanol extract had the highest increase in specific gravity, above the original solvent`s specific gravity among all the other solvents (absolute ethanol, methanol, acetone, n-hexane and water) at 1:5 spice loading which showed that it extracted more of the bioactive compounds than the other solvents. The 40% ethanol also gave the highest concentrations of the major flavour principles (beta-phellandrene and beta-pinene) in *X. aethiopica* and was chosen as the best solvent for extraction among the others. In *C. longa*, the absolute ethanol gave the highest concentration of curcumin however, the 40% ethanol extract had the highest increase in specific gravity which indicated that it extracted more of the bioactive compounds than the other solvent and was cheaper in cost than the absolute ethanol thus was chosen as the best solvent for extraction among the others.

Alkaloids, flavonoids, phytic acid, saponins, tannins and oxalates were detected in the liquid extracts and extraction residues of *X. aethiopica* and *C. longa*.

During the 6 months storage of *X. aethiopica* spice products, the 40% ethanol extract had the highest retention level of the flavour principles and was more effective than the powder, while among the encapsulated samples, the wheat grits were better and more effective than the yam grits. But in the 6 months storage of *C. longa* spice products, the powder retained more of the flavour compounds than the 40% ethanol extract while among the encapsulated samples, the yam grits were better and more effective than the wheat grits. The ratio 1:2 spice extract to carrier was more effective than the ratio1:3 spice extract to carrier in retaining the flavour compounds in both *C. longa* and *X. aethiopica* spice encapsulated product samples.

The identified microbes in the raw *C. longa* spice samples were *Saccharomyces cerevisiae*, *Enterococcus faecalis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia*

coli, Lactobacillus brevis and Streptococcus anginosus while the ground (processed) *C. longa* has *Saccharomyces cerevisiae, Mucor spp, Enterococcus faecalis, Bacillus subtilis, Lactobacillus brevis and Streptococcus anginosus.* The identified microbes in the raw *X. aethiopica* spice samples were *Saccharomyces cerevisiae*, *Penicillium notatum*, *Enterococcus faecalis, Bacillus cereus, E. coli and Lactobacillus brevis* while the ground (processed) *X. aethiopica* has *Mucor spp, Penicillium notatum, Fasarium spp., Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, Lactobacillus brevis and Streptococcus anginosus.*

The sensory evaluation results for the *C. longa* spice products indicated that the 40% ethanol extract was the most highly accepted products and the most commonly recommended for incorporation in foods such as rice, meat, sauce, tea, akamu/pap (custard), baked products, ice cream, yoghurt, non-alcoholic beverages and alcoholic beverages.

Similarly in the *X. aethiopica* flavour products, the results also indicated that the 40% ethanol extract was the most highly acceptable product and the most commonly recommended for incorporation into local foods such as meat, porridge, Yoruba local soup, pepper soup, Igbo local soup, tea (herbal), Hausa local soup and alcoholic beverages.

5.2 RECOMMENDATIONS

Based on the findings, the following recommendations are made:

1. More studies should be conducted using different blends or mixtures of different gelatinized carbohydrates or carriers during encapsulation of spice flavours for better retention. Other types of carriers should be investigated for greater effectiveness.
2. There should be extensive study on the effect of other packaging materials on retention of flavour compound of spices.
3. Different storage temperatures should be investigated for the stability or retention of the flavour compounds over time, possibly over a one year storage time.
4. Different strengths of ethanol should be investigated for optimum extraction of chemicals to establish minimum strength for extraction of major essential oils in the spices.

5.3 CONTRIBUTION TO KNOWLEDGE

This study has revealed that 40% ethanol was the best solvent for the extraction of the flavour compounds from *X. aethiopica* and *C. longa* with regard to compatibility with food.

It has also enlisted different novel foods (ice cream, akamu, yoghurt, alcoholic and non alcoholic beverages, pepper soup, meat, porridge, Yoruba local soup, Igbo local soup and Hausa local soup) where the spice products can be added to create varieties in the different cuisines.

For the first time, Ar-turmerone, a major flavour compound has been identified in *X. aethiopica*. Other authors have only reported the presence of beta-pinene, alpha-pinene, cineole, kaur-16-ene, beta-phellandrene, 13-ent-epimanoyl oxide, γ-terpinene, eucalyptol, 1-6-cyclodecadiene (germacrene D), terpinen-4-ol, trans-beta-ocimene, linalool, α-farnesene, myrtenol and 3-ethylphenol in *X. aethiopica*.

The study has also revealed the presence of beta-pinene (20.28%) and beta-phellandrene (23.93%) in the 40% ethanol extract of *X. aethiopica* which has never been established before.

The study has provided information on the sensory characteristics that are peculiar to the spice flavour samples of *X. aethiopica* (colour, pungency, bitterness, hotness and after taste) and *C. longa* (colour, pungency and after taste) that have never been revealed by previous researchers in the powder, liquid extract and encapsulated samples of *X. aethiopica* and *C. longa*.

REFERENCES

Abad, E., Martinez, K., Planas, C., Palacios, O., Caixach, J. and Rivera, J. (2005). 'Priority organic pollutant assessment of sludges for agricultural purposes', *Chemosphere*, 61, pp. 1358–1369.

Achinewhu, S. C, Aniena, M. I. and Obomanu, F. G. (1995). 'Studies on spices of food value in the South Eastern states of Nigeria: antioxidants properties', *Journal of African Medicinal Plants*, 18, pp. 135-139.

Adams, R.P. (2001). Identification of essential oil components by gas chromatography/mass spectrometry. Illinois: Allured Publishing Corp.

Adelaja, B. A., Adebayo, O. S., Adejoro, M. A. and Nwanguma, E. I. (2008). First stakeholders workshop on spices, Ibadan, Nigeria: NIHORT.

Aguoru, C. U., Pilla, C. and Olasan, J. O. (2016). 'Phytochemical screening of *Xylopia aethiopica* with emphasis on its medicinally active principles', *Journal of Medicinal Plants Research*, 10(22), pp. 306 – 309.

Ajaiyeoba, E. O., Sama, W., Essien, E. E., Olayemi, J. O., Ekundayo, O., Walker, T. M. and Setzer,
W. N. (2008). 'Larvicidal activity of turmerone-rich essential oils of *Curcuma longa*. Leaf and rhizome from Nigeria on *Anopheles gambiae'*, *Pharmaceutical Biology*, *46*, pp. 279-282.

Ajiwe, E. I. V., Okeke, A. C., Ogbuagu, O. J., Ojukwu, U. and Onwukeme, I. V. (1998).
'Characterisation and applications of oils extracted from *Canerium schweinfurttii, Vitex doniana* and *Xylopia aethiopica* fruits/seeds', *Bioresource Technol*ogy, 64, pp. 249-252.

Al-Qudah, M. A., Muhaidat, R., Trawenh, I. N., Al-Jaber, H. I., Abu-Zarga, M. H. and Abu-Orabi, S. T. (2012). 'Volatile constituents of leaves and bulbs of *Gynandriris sisyrinchium* and their antioxidant activities', *Jordan Journal of Chemistry*, 7(3), pp. 287-295.

Anand, P., Kunnumakkara, A. B., Newman, R. A. and Aggarwal, B. B. (2007). 'Bioavailability of

curcumin: problems and promises', *Molecular Pharm*acology, 4(6), pp. 807-818.

Anandaraj, M., Devasahayam, S., Zachariah, T. J., Eapen, S. J., Sasikumar, B. and Thankamani, C. K. (2001). Turmeric (Extension Pamphlet). J. Rema and M.S. Madan (edns.).

Anderson, R. A. (1982). 'Water absorption solubility and amylograph characteristics of roll-cooked small grain products', *Cereal Chemistry*, 59(4), pp. 265-269.

AOAC. (2010). Official methods of analysis. Association of Official Analytical Chemists (18th edn.). Washington D.C (USA).

Apintanapong, M. and Noomhorm, A. (2003). 'The use of spray drying to microencapsulate 2-acetyl-1-pyrroline, a major flavour component of aromatic rice', *International Journal of Food Science and Technology*, 38(2), pp. 95–102.

Arshady, R. (1993). 'Microcapsules for food', *Journal of Microencapsulation*, 10(4), pp. 413–435.

Arun, N. and Nalini, N. (2002). 'Efficacy of turmeric on blood su gar and polyol pathway in diabetic albino rats', *Plant Foods for Human Nutrition*, 57, pp. 41-68.

Asawalam, E. F., Emosairue, S. O. and Hassanali, A. (2006). 'Bioactivity of *Xylopia aethiopica* (Dunal) - a rich essential oil constituents on maize weevil *Sitophilus zeamais* Mot Schulsky (*Coleoptera: Curculionidae*)', *Electronic Journal of Environmental Agriculture and Food Chemistry*, 5(1), pp. 1195-1204.

Ascheri, D. P. R., Marquez, M. O. M. and Martucci, E. T. (2003). 'Microencapsulation of orange essential oil: wall material selection', *Ciencia e Technologia de Alimentos*, 23, pp. 1-6.

Asekun, O. T. and Adeniyi, B. A. (2004). 'Antimicrobial and cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria', *Fitoterapia*, 75, pp. 368-370.

Asghari, G., Mostajeran, A. and Shebli, M. (2009). 'Curcuminoid and essential oil components of

turmeric at different stages of growth cultivated in Iran', *Research in Pharmaceutical*

Sciences, 4(1), pp. 55 – 61.

ASTA. (2000). The American Spice Trade Association Report, Spice Statistics.

ASTA. (2002). A concise guide to spices, herbs, seeds, and extractives. *American Spice Trade Association*. pp. 48-50.

Augustin, M. A., Sanguansri, L., Margetts, C. and Young, B. (2001). 'Microencapsulation of food ingredients', *Food Australia*, 53(6), pp. 220–223.

Augusto, F., Leite e Lopes, A. and Zini, C. A. (2003). 'Sampling and sample preparation for analysis of aromas and fragrances', *Trends in Analytical Chemistry*, 22, pp. 160-169.

Awe, S., Sani, A. and Ojo, F. T. (2009). 'Microbiological quality of some selected spices (*Thymus vulgaris*, *Murraya koenigi* and *Piper nigrium*)', *Nigerian Journal of Microbiology*, 23(1), pp. 1876 – 1881.

Ayedoun, A. M., Adeoti, B. S., Sossou, P. V. and Leclerq, P. A. (1996). 'Influence of fruit conservation methods on the essential oil composition of *Xylopia aethiopica* (Dunal). A. Rich from Benin', *Flavour and Fragrance Journal*, 11(4), pp. 245-250.

Babu, V. A. (2013). 'A clinical study on allergic contact dermatitis to turmeric', *Journal of Evolution of Medical and Dental Sciences*, 2 (17), pp. 3388 – 3404.

Badee, A. Z. M., Amal, E., El-kader, A.B.D. and Hanan, M. A. (2012). 'Microencapsulation of peppermint oil by spray drying', *Australian Journal of Basic and Applied Sciences*, 6 (12), pp. 499 – 504.

Baik, M. Y., Suhendro, E. L., Nawar, W. W., McClements, D. J., Decker, E. A. and Chinachoti, P. (2004). 'Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil', *Journal of the American Oil Chemists Society*, 81 (4), pp. 355–360.

Bambirra, M. L. A., Hunqueira, R. G. and Gloria, M. B. A. (2002). 'Influence of post-harvest processing conditions on yield and quality of ground turmeric (*Curcuma longa* L)', *Brazilian Archives of Biology and Technology*, 45, pp. 423-429.

Bansal, S. S., Vadhanam, M. V. and Gupta, R. C. (2001). 'Development and *in vitro-in vivo* evaluation of polymeric implants for continuous systemic delivery of curcumin', *Pharmaceutical Research, 28*, pp. 1121-1130.

Baranauskiene, R., Venskutonis, P. R., Dewettinck, K. and Verhe, R. (2006). 'Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavours encapsulated into milk protein-based matrices', *Food Research International*, 39 (4), pp. 413–425.

Barrett, D. M., Beaulieu, J. C. and Shewfelt, R. (2010). 'Colour, flavour, texture and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement and the effects of processing', *Critical Reviews in Food Science and Nutrition*, 50, pp. 369 -389.

Basear, K. H. C. and Demirci, F. (2007). Chemistry of essential oil. In flavours and fragrances (Berger, R. G. edn.). Heidelberg: Spring. pp. 43 -86.

Behar, N., Tiwari, K. L. and Jadhav, S. K. (2016). 'Semi-quantitative expression studies of genes involved in biosynthesis of curcuminoid in *Curcuma caesia* Roxl', *Indian Journal of Biotechnology*, 15, pp. 491 – 494.

Benson, H .J. (1998). Microbiological applications: laboratory manual in general microbiology.

Boston: WBC McGrand-Hill.

Bhaskaram, P. (2001). 'Immunobiology of mild micronutrient deficiencies', *Journal of Nutrition*, 85, pp. 75-80.

Bimakr, M. (2010). 'Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves', *Food and Bioproducts Processing*, pp. 1-6.

Borges, P. and Pino, J. (1993). 'Preparation of black pepper oleoresin by alcohol extraction', *Nahrung-Food*, 37, pp. 127-130.

Braga, M. E. M., Leal, P. F., Carvalho, J. E. and Meireles, M. A. A. (2003). 'Comparison of yield, composition, and antioxidant activity of turmeric (*Curcuma longa* L.) extracts obtained using various techniques', *Journal of Agricultural and Food Chemistry*, 51, pp. 6604-6611.

Brazel, C. S. (1999). 'Microencapsulation: offering solutions for the food industry', *Cereal Foods World*, 44(6), pp. 388–392.

Buchannan, R. E. and Gibbon, N. E. (1994). Bergey's manual of determinative bacteriology. Baltimore, USA: Williams and Wilkins Co.

Buescher, R. and Yang, L. (2000). Turmeric. In: Natural food colourants, Science and Technology. G.L. Lauro, and F.J. Fancis (edns.). New York: Marcel Dekker, pp. 205-226.

Campos, C. A., Gerschenson, L. N. and Flores, S. K. (2011). 'Development of edible films and coatings with antimicrobial activity', *Food and Bioprocess Technology*, 4(6), pp. 849-875.

Chainani-Wu, N. (2003). 'Safety and anti-inflammatory activity of curcumin: a component of *C. longa*', *Journal of Alternative and Complementary Medicine,* 9, pp. 161-168.

Chan, E. W. C. (2009). 'Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species', *Food Chemistry,* 113(1), pp. 166-172.

Charve, J. and Reineccius, G. A. (2009). 'Encapsulation performance of proteins and traditional materials for spray dried flavours', *Journal of Agricultural and Food Chemistry,* 57 (6), pp. 2486 – 2492.

Cheesbrough, M. (2000). District laboratory practices in tropical countries, Part 2, UK: Cambridge University Press. pp. 35-38, 62-69.

Chempakam, B. and Parthasarathy, V. A. (2008). Turmeric. In: Chemistry of Spice, Parthasarathy, V. A., Chempakam, B., Zachariah, T. J., (edns.). Cambridge: CABI. pp. 97-123.

Chen, J. J., Tsai, C. S., Hwang, T. L., Shieh, P. C., Chen, J. F. and Sung, P. J. (2010). 'Sesquiterpenes from the rhizome of *Curcuma longa* with inhibitory activity on superoxide generation and elastase release by neutrophils' *Food Chemistry,* 119, pp. 974-980.

Chen, Y. H., Qin, B., Zhang, Y. Y. and Cheng, W. (2008). 'Comparative study on the process of extraction of curcumin from *Curcuma longa* L. with different methods', *Zhongguo Zhongyi Xinxi Zazhi,* 15, pp. 55-56.

Cheng, A. L., Hsu, C. H., Lin, J. K., Hsu, M. M., Ho, Y. F., Shen, T. S., Ko, J. Y., Lin, J. T., Lin, B. R., Ming-Shiang, W., Yu, H. S., Jee, S. H., Chen, T. M., Chen, C. A., Lai, M. K., Pu, Y. S., Pan, M. H., Wang, Y. J., Tsai, C. C. and Hsieh, C. Y. *(*2001). 'Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions', *Anticancer Research,* 21, pp. 2895-2900.

Chinwe, N. D., Nwafor, A. and Danladi, N. B. (2012). 'A comparative study of nutritional and electrolyte qualities of *Xylopia aethiopica* in novel hydro-alcoholic formulations', *American Journal of PharmTech Research,* pp. 342-352.

Chowdhury, J. U., Nandi, N. C., Bhuiyan, M. N. I. and Mobarok, M. H. (2008). 'Essential oil constituents of the rhizomes of two types of *Curcuma longa* of Bangladesh', *Bangladesh Journal of Scientific and Industrial Research,* 43, pp. 259-266.

Christmann, M. (2010). 'Otto Wallach: founder of terpene chemistry and noble laureate 1910', *Angewandte Chemistry International*, 49, pp. 9580-9586.

Close, D. C. and McArthur, C. (2002). 'Rethinking the role of many plant phenolics protection from photo damage', *Journal of Medicinal plants,* 99, pp. 166-172.

Cotton, F. A. and Wilkenson, G. (1998). Advanced inorganic chemistry (5th edn.). ISBN 0-471-84997-9. Wiley, New York, USA.

Dahal, K. R. and Idris, S. (1999). *Curcuma longa* L. In : de Guzman, C.C. and Siemonsma, J.S. (edns.). Plant Resources of South-East Asia No 13. Spices. Netherlands, Backhuys Publishers, Leiden. pp. 111-116.

Dalgleish, D. G. (2006). 'Food emulsion – their structures and structural forming properties', Food Hydrocoll. 20, pp. 415 – 422.

Degenhardt, J., Kollner, T. G. and Gershenzon, J. (2009). 'Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants', *Phytochemistry,* 70(15–16), pp. 1621–1637.

Del-Rio, A., Obdulio, B. G., Castillo, J., Main, F. R. and Ortuno, A. (1997). 'Uses and properties of citrus flavonoids', *Journal of Agricultural and Food Chemistry,* 45, pp. 4505-4520.

Desai, K. G. H. and Park, H. J. (2005). 'Recent developments in microencapsulation of food ingredients. *Drying Technology'*, 23(7), pp. 1361–1394.

Dhall, R. K. (2013). 'Advances in edible coatings for fresh fruits and vegetables: a review', *Critical Reviews in Food Science and Nutrition*, 53(5), pp. 435-450.

Doughari, J. H. (2012). Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents: phytochemicals - a global perspective of their role in nutrition and health, Dr Venketeshwer Rao (edn.), ISBN: 978-953-51-0296-0, InTech, Available from: http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-andhealth/ phytochemicals-extraction-methods-basic-structures-and-mode-of-action-as-potentialchemotherapeutic. Accessed 12th February, 2016.

Duy, N. Q. (2013). 'Different extraction methods to obtain the highest phytic acid yield from peanut seeds', *International Journal of Biology, Pharmacy and Allied Sciences*, 2(12), pp. 2379- 2399.

Eigner, D. and Scholz, D. (1999). '*Ferula asa-foetida* and *Curcuma longa* in traditional medical treatment and diet in Nepal', *Journal of Ethnopharmacology,* 67, pp. 1-6.

Elgailani, I. E. H. and Ishak, C.Y. (2016). 'Methods for extraction and characterization of tannins from some *Acacia species* of Sudan', *Pakistan Journal of Analytical and Environmental Chemistry*, 17(1), pp. 43 – 49.

El-Hadi, M. A. M, Zhang, F., Wu, F., Zhou, C. and Tao, J. (2013). 'Advances in fruit aroma volatile research: a review', *Molecules*, 18, pp. 8200 – 8229. Available at: www.mdpi.com/journal/molecules. doi:10.3390/molecules18078200. Accessed 4th February, 2016.

El-Kamali, H. H. and Adam, H. O. (2009). 'Aromatic plants from the Sudan: part II. chemical composition of the essential oil of *Xylopia aethiopica* (Dunal) A. Rich. existence of chemotype species'., *Advances in Natural and Applied Sciences*, 3(2), pp. 166-169.

Eloff, J. N. (1998). 'Which extractant should be used for the screening and isolation of antimicrobial components from plants', *Journal of Ethnopharmacology*, 60, pp. 1–8.

El-Waziry, A. M., Nasser, M. E. A. and Sallam, S. M. A. (2005). 'Processing methods of soybean meal. Effect of roasting and tannic acid treated soybean meal on gas production and rumen fermentation *in vitro'*, *Journal of Applied Sciences*, 1, pp.313-320.

Ewa, D. and Iwona, W. (2008). 'Microencapsulation of rapeseed oil based on the spray drying method', *Polish Journal of Food and Nutrition Sciences*, 58 (4), pp. 477 -483.

FAO. (2004). Food and Agriculture Organization Statistics.

Fatima, A. L., Fouzia, H., Nadia, T. J., Fatima, B., Mohammed, L. and Noureddine, B. (2014). 'Phytochemical screening chemical composition and toxicity of *Euphorbia echinus'*, *Journal of Biological and Chemical Research*, 31(1), pp. 21 – 38.

Fisher, C. and Scott, T. R. (1997). Food flavours, biology and chemistry. Cambridge, UK: The Royal Society of Chemistry. pp. 13 -170.

Fisher, K. and Philips, C. (2009). 'The ecology, epidemiology and virulence of Enterococcus', *Microbiology*, 155(6), pp. 1749 – 1757. doi10.1099/mic.0.026385-0.

Gallo, G., Berzero, R., Caltai, N., Recchia, S. and Orefici, G. (1992). 'An outbreak of a group of food-borne Streptococcal pharyngitis', *European Journal of Epidemiology*, 8(2), pp. 292-297.

Gazette of India. (2005). Spices grading and marking rules: Grading designations and quality of turmeric powder. Schedule II, Rules 3 and 4. p. 1-30.

Gerke, J. (2015). 'Phytate (Inositol Hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants - a review'. *Plants*, 4, pp. 253 – 266.

Gibbs, B. F., Kermasha, S., Alli, I. and Mulligan, C. N. (1999). 'Encapsulation in the food industry: a review', *International Journal of Food Sciences and Nutrition*, 50, pp. 213–224.

Goubet, I., Le-Quere, J. L. and Voilley, A. J. (1998). 'Retention of aroma compounds by carbohydrates: influence of their physiochemical characteristics and of their physical state- a review', *Journal of Agriculture and Food Chemistry*, 46, pp. 1981–1990.

Green, C., Steele, J. and Willemse, J. (1999). 'The development and prospects for minor industrial crops in selected Southern African countries', *World Bank Technical Paper*, 424, pp. 139-166.

Gregory, P. J., Sperry, M. and Wilson, A. F. (2008). 'Dietary supplements for osteoarthritis', *American Family Physician*, 77(2), pp. 177-194.

Grob, R. L. and Barry, E. F. (2004). Modern practice of gas chromatography. Wiley-Interscience.

Gunstone, F. D., Harwood, J. L. and Dijkstra, D. J. (2007). The lipid handbook. Boca Raton, Florida, USA: CRC Press.

Gupta, S., Sung, B., Kim, J., Prasad, S. and Li, S. (2013). 'Multitargeting by turmeric, the golden spice: from kitchen to clinic', *Molecular Nutrition and Food Research*, *57*(9), pp. 1510-1528.

Han, E. (2016). Spice market intelligence: tips from the kitchen. www.thekitchn.com. Accessed 3rd March, 2016.

Han, G., Liu, Z. M., Wang, X. Y. and Wang, Y. N. (2007). 'Study on effect of different extracts in *Curcuma longa* on stability of curcumin', *Journal of Chinese Medicine and Traditional Chinese Medicine, 32*, pp. 915-917.

Harrigan, G. G., Bolzani, V. S., Gunatilaka, L. A. A. and Kingston, I. G. D. (1994). 'Kaurane and tetrachylobanediterpenes from *Xylopia aethiopica'*, *Phytochemistry*, 36(1), pp. 109-113.

Harrigan, W. F. and Mclance, M. E. (1990). Laboratory methods in food and dairy microbiology (8^{th} edn.). London: Academic Press Inc. pp. 7-23, 286-303.

Hassan, A. M., Almagboul, A. Z. and Kabbashi, A. S. (2016). 'Chemical composition and cytotoxic activity of fruits essential oil from *Xylopia aethiopica* (Dunal) A. Rich', *International Journal of Multidisciplinary Research and Development*, 3(1), pp. 394-399.

Hauschild, A. H. W. (1989). *Clostridium botulinum*. In foodborne bacterial pathogens. Doyle, M.P. (edn.). NY: Marcel Dekker, Inc.

Hossain, A. H. and Ishimine, Y. (2005). 'Growth, yield and quality of turmeric (*Curcuma longa* L.) cultivated on dark-red soil, gray and red soil in Okinawa, Japan', *Plant Production Science*, 8, pp. 482-486.

Husnu-Can-Baser, K. and Buchbauer, G. (2010). Handbook of essential oils: science, technology and applications. Boca Raton, London, New York: CRC press. pp. 3-185.

ICMSF. (International Commission on Microbiological Specification for Food), (2002). Microorganisms in food, Book 7 Microbiological testing in food safety management. NY: Kluver academic/ Plenum ISBN 0306472627.

Idolo, I. and Emeuwa, C. H. (2011). Nutritional and antinutritional characteristics of the larva of *Oryctes monoceros. Agriculture and Biology Journal of North America*, 2(1), pp. 42 – 46.

Idu, M., Erhabor, J. O. and Idele, S. O. (2011). 'Microbial load of some medicinal plants sold in local markets of Benin City, Nigeria', *International Journal of Medicinal and Aromatic Plants*, 1(3), pp. 272-277.

Ilusanya, O. A. F, Odunbaku, O. A., Adesetan, T. O. and Amosun, O. T. (2012). 'Antimicrobial activity of fruit extracts of *Xylopia aethiopica* and its combination with antibiotics against clinical bacteria pathogens', *Journal of Biology, Agriculturen and Healthcare*, 2(9), pp. 1-43.

Indiramma, A. R. (1995). Packaging of spices and spice products. In: profile on food packaging. Mysore and IIP Mumbai: CFTRI. pp. 77 – 99.

Inyang, E. (2003). Ethnobotany: conventional and traditional uses of plants. Vol. A. Akwa Ibom State: The Verdict Press.

Issakou, B., Jean, B., Patrick, H., Valere, F., Lauve, R. T. and Magali, G. (2014). 'Composition and cytotoxic activity of essential oils from *Xylopia aethiopica* (Dunal) A. Rich, *Xylopia parviflora* (A. Rich) Benth. and *Monodora myristica* (Gaertn) growing in Chad and Cameroun', *BMC Complementary and Alternative Medicine,* 14, pp. 125.

Itmad, A. E., Elmubarak, E. E. and Saad, M. H. A. (2010). 'Chemical composition of essential oil in dried fruits of *Xylopia aethiopica* from Sudan', *Open Access Journal of Medicinal and Aromatic Plants*, 1(1), pp. 24-28.

Iwe, M. O. (2002). Handbook of sensory methods and analysis. Uwani Enugu: Rejoint Communication Services Ltd. pp. 40 – 83.

Iwu, M. (1993). Handbook of African medicinal plants. Boca Raton, FL.: CRC Press. pp. 435.

Jayandran, M., Muhamed, H. M. and Balasubramanian, V. (2015). 'Synthesis characterization and

comparative studies of turmeric oleoresin derived from selected turmeric plants', *Asian Journal of Pharmaceutical Science and Technology*, 5 (1), pp. 18 – 21.

Jillavenkatesa, A., Dapkunas, S. J. and Lum, L. (2001). Particle size characterization, Washington: NIST Special Publication. pp. 960 – 961.

Jirovetz, L., Buchbauer, G. and Ngassoum, M. B. (1997). 'Investigation of the essential oils from

the dried fruits of *Xylopia aethiopica* and *Xylopia parviflora* from Cameroun', *Ernahrung*, 21(7-8), pp. 324-325.

Joshi, J., Ghaisas, S., Vaidya, A., Vaidya, R., Kamat, D. V., Bhagwat, A. N. and Bhide, S. (2003).
'Early human safety study of turmeric oil (*Curcuma longa* oil) administered orally in healthy volunteers', *Journal of the Association of Physicians of India,* 51, pp. 1055-1060.

Jovanovic, S. V., Steenken, S., Boone, C. W. and Simic, M. G. (1999). 'H-atom transfer is a preferred antioxidant mechanism of curcumin', *Journal of the American Chemical Society,* 121, pp. 9677-9681.

Jung, J. M. and Sung, T. K. (2000). 'A new method for analysis of capsaicinoids content in microcapsule', *Korean Journal of Food Science and Technology*, 32, pp. 42–49.

Kar, A. (2007). *Pharmaocgnosy and Pharmacobiotechnology (*Revised-expanded 2nd edn.*)*. New Delhi: New Age International Limted Publishres. pp. 332-600.

Karamali, K. and Teunis, V. R. (2001). 'Tannins: classification and definition', *Journal of Natural Product Reports*, 18, pp. 641 – 649.

Karioti, A., Hadjipavolou-Litinna, D., Merlin, L. K., Mensah, P., Theophilus, C., Fleischer, A. and Skaltsa, H. (2004). 'Composition and antioxidant activity of essential oils of *Xylopia aethiopica* (Dunal) A. Rich. (Annonacae) leaves, stems, bark, root, fresh and dried fruits growing in Ghana', *Journal of Agricultural and Food Chemistry*, 52(26), pp. 8094- 8095.

Karthikeyan, P. K., Ravichandran, M., Imas, P. and Assaraf, M. (2009). The effect of potassium on the yield and quality of *C. longa*. . *E-ifc*, no. 21, September 2009.

Kataoka, H., Lord, H. L. and Pawliszyn, J. (2000). 'Applications of solid-phase microextraction in food analysis', *Journal of Chromatography*, 880, pp. 35-62.

Keita, B. L., Sidibe, G., Figueredo, J. C. and Chalchat, J. C. (2003). 'Chemical composition of the essential oil of. *Xylopia aethiopica* (Dunal) A. Rich. from Mali', *Journal of Essential Oil Research*, 15(4), pp. 267-269.

King, A. H. (1995). 'Encapsulation of food ingredients – a review of available technology, focusing on hydrocolloids', In: S. J. Risch and G. A. Reineccius (edns.). *Encapsulation and Controlled Release of Food Ingredients*, ACS Symposium Series, 590, Washington, DC: American Chemical Society. pp. 26–39.

Kligler, B., Ulbricht, C., Basch, E., Kirkwood, C. D., Abrams, T. R., Miranda, M., Singh-Khalsa, K. P., Giles, M., Boon, H. and Woods, J. (2006). '*Andrographis paniculata* for the treatment of upper respiratory infection: a systematic review by the natural standard research collaboration', *Explore*, 2(1), pp. 25-29.

Koba, K. and Sanda, K. (2008). 'Chemical composition and *in vitro* cytotoxic activity of *Xylopia aethiopica* (Dun.) A. Rich (Annonaceae) fruits essential oil from Togo', *Journal of Essential Oil Research*, 20(4), pp. 354-357.

Kobayashi, T., Miyazaki, A., Matsuzawa, A., Kuroki, Y., Shimamura, T., Yoshida, T. and Yamamoto, Y. (2010). 'Change in curcumin content of rhizome in turmeric and yellow
Zedoary', *Nippon Sakumotsu Gakkai Kiji, 79* (1), pp. 10-15.

Koes, R. E. and Quattrocchio, F. (1994). 'The flavonoid biosynthetic pathway in plants: function and evolution', *BioEssays*, *16*, pp. 123–132.

Kolanowski, W. and Laufenberg, G. (2006). 'Enrichment of food products with polyunsaturated fatty acids by fish oil addition', *European Food Research and Technology*, 222(3–4), pp. 472–477.

Kolanowski, W., Swiderski, F. and Berger, S. (1999). 'Possibilities of fish oil application for food products enrichment with omega-3 PUFA', *International Journal of Food Sciences and Nutrition*, 50 (1), pp. 39–49.

Koudou, J., Abena, A. A., Ngaissona, P. and Bessiere, J. M. (2005). 'Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*', *Fitoterapia*, *76*, pp. 700–713.

Kouninki, H., Haubruge, E., Noudjou, F. E., Lognay, G., Malaisse, F., Ngassoum, M. B., Goudoum, A., Mapongmetsem, P. M., Ngamo, L. S. and Hance, T. (2005). 'Potential use of essential oils from Cameroun applied as fumigant or contact insecticides against *Sitophilus zeamais* Motsch. (Coleoptera; curculionidae). Commun', *Agricultural and Applied Biological Sciences*, 70(4), pp. 787-792.

Kramer, J. M. and Gilbert, R. J. (1989). *Bacillus cereus* and other *Bacillus species*. In foodborne bacterial pathogens. Doyle, M.P. (edn.). NY: Marcel Dekker, Inc.

Kumar, K., Asish, G., Sabu, M. and Balachandran, I. (2013). 'Significance of gingers (zingiberaceae) in Indian system of medicine - ayurveda: an overview', *Ancient Science of Life*, *32*(4), pp. 253-505.

Kurien, B. T. and Scofield, R. H. (2009). 'Oral administration of heat-solubilized curcumin for potentially increasing curcumin bioavailability in experimental animals', *International Journal of Cancer,* 125, pp. 1992-1993.

Lahlou, M. (2004). 'Methods to study the phytochemistry and bioactivity of essential oil', *Phytotherapy Research*, 18, pp. 435-448.

Laing, D. G. and Jinks, A. (1996). 'Flavour perception mechanisms', *Trends in Food Science and*
Technology, 7, pp. 387-389.

Lajide, L., Escoubas, P. and Mitzutani, J. (1995). 'Termite antifeedant activity in *Xylopia aethiopica'*, *Phytochemistry*, 40(4), pp. 1105-1112.

Leela, N. K., Tava, A., Shaf, P. M., John, S. P. and Chempakam, B. (2002). 'Chemical composition of essential oils of turmeric (*Curcuma longa* L.)', *Acta Pharmaceutica*, 52, pp. 137-141.

Legrand, P., Barratt, G., Mosqueira, V., Fessi, H. and Devissaguet, J. P. (1999). 'Polymeric nanocapsules as drug delivery systems – a review', *Pharma Sciences*, 9 (5), pp. 411–418.

Li, S., Yuan, W., Deng, G., Wang, P., Yang**,** P. and Aggarwal, B. B. (2011). 'Chemical composition and product quality control of turmeric (*Curcuma longa* L.)', *Pharmaceutical Crops*, 2, pp. 28-54.

Liu, X. D., Atarashi, T., Furuta, T., Yoshii, H., Aishima, S., Ohkawara, M. and Linko, P. (2001). Microencapsulation of emulsified hydrophobic flavours by spray drying. *Drying Technology*, 19(7), pp. 1361–1374.

Lopez, R., Cuca, L. and Delgado, G. (2009). 'Antileishmanial and immunomodulatory activity of *Xylopia discrete*', *Parasite Immunology*, 31, pp. 623-630.

Lv, H. and She, G. (2010). 'Naturally occurring diarylheptanoids', *Pharm. Crops*, 5, pp. 1687-1708.

Madene, A., Jacquot, M., Scher, J. and Desobry, S. (2006). 'Flavour encapsulation and controlled release – a review', *International Journal of Food Science and Technology*, 41(1), pp. 1–21.

Manoharan, A., Ramasamy, D., Dhanalashmi, B., Gnanalashmi, K. S. and Thyagarajan, D. (2012). 'Studies on sensory evaluation of curcumin powder as natural colour for butterscotch flavour ice cream', *Indian Journal of Drugs and Diseases*, 1(1), pp. 43-46.

Matsumoto, M., Hirata-Koizumi, M. and Ema, M. (2008). Potential adverse effects of phthalic acid esters on human health: a review of recent studies on reproduction. *Regulatory Toxicology and Pharmacology Journal*, 50(1), pp. 37 – 49.

Mayaki, O. M., Akingbala, J. O., Baccus-Taylor, G. S. H. and Thomas, S. (2003). 'Evaluation of breadfruit (*Artocarpus communis*) in traditional stiff porridge foods', *Journal of Food Agriculture and Environment*, 1(2), pp. 54-59.

McGee, H. (2004). Food and Cooking, New York: Simon and Schuster. pp. 714.

Merken, H. M. and Beecher, G. R. (2000). 'Measurement of food flavonoids by high-performance

liquid chromatography: a review', *Journal of Agricultural and Food Chemistry,* 48, pp. 577-599.

Michael, C. (1995). The healing herbs. A Bantam book/published by arrangement with Rodale Press. New York: Bantam Books.

Mohamed, E. I. (2016). 'Essential oils isolated from leaves of Egyptian *Verbena triphylla* L. herb using different extraction methods', *International Journal of PharmTech Research*, 9(4), pp. 1 – 7.

Mondal, S., Ghosh, S. and Moulik, S. P. (2016). 'Stability of curcumin in different solvent and solution media: UV-visible and steady-state fluorescence spectral study', *J. Photochem. Photobiol. B.*, 158, pp. 212-218. doi:10.1016/j.jphotobiol.2016.03.004.

Mosciano, G. (2000). 'Organoleptic characteristics of flavour materials', *Perfumer and Flavourist*, 25(6), pp. 26.

Muanya, C. (2016). 'Natural Health: Africa Guinea pepper stops prostate, breast cancer', *The Guardian, Thursday, August 25*, p.31.

Murray, H. (2009). Essential oils: arts, agriculture, science, industry and entrepreneurship. A focus on the Asia-pacific region. New York: Nova science publishers, inc. pp. 41-517.

Murthy, C., Rani, M. and Rao, P. S. (1999). 'Optimal grinding characteristics of black pepper for essential oil yield', *Journal of Food Process Engineering,* 22, pp. 161-173.

N`driKonan, B., Bosson, A. K., Mamyrbekova-Bekro, J. A., Jean, N. and Bekro-Yves, A. (2009). 'Chemical composition and antioxidant activities of essential oil of *Xylopia aethiopica* (Dunal) A. Rich', *European Journal of Scientific research*, 2, pp. 311-318.

Nahar, L. and Sarker, S. D. (2007). Phytochemistry of the genus *Curcuma*. In turmeric: the genus *Curcuma*, Ravindran, P. N., Nirmal Babu, K., and Sivaraman, K. (edns.). Boca Raton: CRC Press. pp. 71-106.

National Cancer Institute. (1996). 'Clinical development plan: curcumin', *Journal of Cellular Biochemistry Supplement*, 26, pp. 72-85.

National Institute of Health. (2012). Herbs at a glance: turmeric, science and safety. National

Centre for Complementary and Alternative Medicine (NCCAM).

Naviglio, D., Conti, S., Ferrara, L. and Santini, A. (2010). 'Determination of moisture in powder and lyophilised saffron (*Crocus sativus* L.) by Karl Fischer method', *The Open Food Science Journal*, 4, pp. 1-6.

Negi, J. S., Negi, P. S., Pant, G. J., Rawat, M. S. M. and Negi, S. K. (2013). 'Naturally occurring saponins: chemistry and biology', *Journal of Poisonous and Medicinal Plant Research*, 1(1), pp. 1-6. Available online at: http://www.apexjournal.org. Accessed 16th February, 2016.

Ngamo, L. S., Ngassoum, M. B., Jirovetz, L., Ousman, Nukenine, E. C. and Mukala, O. E. (2001). 'Protection of stored maize against *Sitophilus zeamais* (motsch.) by use of essential oil of spices from Cameroun', Proceedings of the 53rd International Symposium on Crop Protection, Gent Belgium, 8 May, 2001. Meded. Fac. Landbouwkd. *Toegepaste Biologische Wetenschappen*, 66(2a), pp. 473-478.

Nielsen, A. V. F., Tetens, I. and Meyer, A. S. (2013). 'Potential of phytase-mediated iron release from cereal-based foods: a quantitative view', *Nutrients*, 5, pp. 3074 – 3098.

Noudjou, F., Kouninki, H., Hance, T., Haubruge, E., Leonard, S.T., Ngamo, Pierre, M. M., Ngassoum, M., Malaisse, F., Marlier, M. and Lognay, G. (2007). 'Composition of *Xylopia aethiopica* (Dun) A. Rich. essential oils from Cameroon and identification of a minor diterpene: *ent*-13-epimanoyl oxide', *Biotechnology, Agronomy, Society and Environment*, 11(3), pp. 193-199.

Okonkwo, E. U. and Okoye, W. I. (1996). 'The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: curculionidae) in Nigeria', *International Journal of Pest Management*, 42(3), pp. 143-146.

Okwu, D. E. (2001). 'Improving the nutritive value of cassava tapioca meal with local spices', *Journal of Nutraceuticals, Functional and Medical Food*, 3(4) pp. 43-51.

Okwu D. E. (2003). 'The potential of *Ocimum gratissimum*, *Pergturia extersa* and *Tetrapleura tetraptera* as spice and flavouring agent', *Nigeria Agricultural Journal*, 34, pp. 45 148.

Olojede, A. O., Nwokocha, C. C., Akinpelu, A. O. and Dalyop, T. (2009). 'Effect of variety, rhizome and seed bed types on yield of turmeric (*Curcuma longa* L) under a humid tropical agro-ecology', *Advanced Biomedical Research, 3*, pp. 40-42.

Olonisakin, A. M., Oladimeji, O. and Lajide, L. (2007). 'Composition and antibacterial activity of steam distilled oils of *Xylopia aethiopica* and *Syzigium aromaticum', Journal of Engineering and Applied Sciences*, 2, pp. 236-240.

Oluwatosin, A., Osume, O., Okiti, E. and Farombi, O. (2010). 'Dried fruit extract from *Xylopia aethiopica* (Annonaceae) protects wistar albino rats from adverse effects of whole body radiation', *Experimental and Toxicologic Pathology*, 2, pp. 14-34. doi:10. 1016/ j.etp. 2010.05.005.

Oluwole, O. B., Olapade, A. A., Awonorin, S. O. and Henshaw, F.O. (2013). 'Physicochemical properties of extrudates from white yam and bambara nut blends', *International Agrophysics*, 27, pp. 69-74. doi:10.2478/v10247-012-0069-8.

Omodamiro, O. D., Ohaeri, O. C. and Nweke, I. N. (2012). 'Oxytocic effect of aquous, ethanolic, n-hexane and chloroform extracts of *Xylopia aethiopica (Anonaceae)* and *Ocimum gratissium(Labiate)* on guinea pig uterus', *Asian Journal of Plant Science and Research*, 2 (1), pp. 73-78. Available online at: www.pelagiaresearchlibrary.com. Accessed 28th January, 2016.

Onayade-Sontan, A. O. (1991). Analysis of essential oils of some plants used in traditional medicine in Nigeria. PhD thesis. State University of Leiden.

Oranusi, U. S. and Braide, W. (2012). 'Microbiological safety assessment of Apple fruits (*Malus domestica* Borkh) sold in Owerri Imo State Nigeria', *Advance Journal of Food Science and Technology*, 4 (2), pp. 97-102.

Owokotomo, I. A. and Ekundayo, O. (2012). 'Comparative study of the essential oils of *Monodoura myristica* from Nigeria', *European Chemical Bulletin*, 1(7), pp. 263-265.

Padmakumari, K. P., Sreekumar, M. M. and Sankarikutty, B. (2009). 'Composition of volatile oil of ginger (*Z.officinale Roscoe*) varieties from India', *Indian Perfum*e, 53 (4), pp. 16 – 20.

Parimalakrishnan, S., Akalanka, D., Rajeswari, J. and Ravikumar, K. (2015). 'Extraction and characterization of phytoconstituents of *Cleome chelidonii', International Journal of Characterization and Pharmaceutical Sciences*, 6(1), pp. 63 – 66.

Parthasarathy, V. A., Chempakam, B. and Zachariah, T. J. (2008). Chemistry of spices. Wallingford: Cabi.

Parveen, S., Das, S., Begum, A., Sultana, N., Hoque, M. M. and Ahmad, I. (2014). 'Microbiological quality assessment of three selected spices in Bangladesh', *International Food Research Journal*, 21(4), pp. 1327-1330.

Pelezar, M. J. (Jr.), Chan, E .C .S. and Krieg, N. R. (1993). Microbilogy concepts and applications (1st edn.). New York, USA: McGraw-Hill Inc. pp. 80-100, 158-161, 370.

Peter, K. V. (2006). Handbook of herbs and spices, volume 3. Boca Raton, Boston, New York, Washington DC: CRC Press. pp. 20 – 506.

Pfeiffer, E., Hhle, S., Solyom, A. S. and Metzler, M. (2003). 'Studies on the stability of turmeric Constituents', *Journal of Food Engineering, 56*, pp. 257-259.

Poitou, F., Masotti, V., Guigues de Souza, S., Viano, J. and Gaydou, M. (1996). 'Composition of the essential oil of *Xylopia aethiopica* dried fruits from Benin', *Journal of Essential Oil Res*earch, 8(3), pp. 320-330.

Praveen, K. A. and Kumud, U. (2012). 'Tannins are astringent', *Journal of Pharmacognosy and Phytochemistry*, 1(3), pp. 45-50. Available online at: www.phytojournal.com. Accessed 17th January, 2016.

Pura-Naik, J., Nagalakhmi, S., Bala-Subrahmanyam, N., Dhanaraj, S. and Shankarchraya, N. B. (2001). 'Packaging and storage studies on commercial varieties of Indian chillies (*Capsum annim L.*)', *Journal of Food Science and Technology*, 38 (3), pp. 227 – 230.

Rajesh, H., Rao, S. N., Megha-Rani, N., Prathima, K. S., Rejeesh, E. P. and Chandrashekar, R. (2013). 'Phytochemical analysis of methanolic extract of *curcuma longa linn* rhizome', *International Journal of Universal Pharmacy and Bio Sciences*, 2(2), pp. 39-45.

Rakesh, K. S., Kanti, B. P. and Syed, I. R. (2012). 'Medicinal properties of some Indian spices', *Annals of Phytomedicine*, 1(1), pp. 29- 33.

Ramirez-Ahumada, M. C., Timmermann, B. N. and Gang, D. R. (2006). 'Biosynthesis of curcuminoids and gingerols in *Curcuma longa* and ginger (Zingiber officinale) identification of curcuminoid synthase and hydroxycinnamoyl-CoA thioesterases', *Phytochemistry*, 67, pp. 2017-2029.

Re, M. I. (1998). 'Microencapsulation by spray drying', *Drying Technology*, 16(6), pp. 1195–1236.

Reddi, P. (2013). 'A touch of turmeric: examining an ayurvedic treasure', *Advances in Anthropology*, *3*(2), pp. 91-95.

Reineccius, G. A. (1988). Spray-drying of food flavours. ACS Symposium Series, 370, 55–66.

Reineccius, G. A. (1994). Flavour encapsulation. In Source Book of Flavours, (2nd edn.). Reineccius, G. A. (ed.). New York: Chapman and Hall. pp. 605–625.

Reineccius, G. A. (1994). Flavour manufacturing. In Source Book of Flavours, (2nd ed.). Reineccius, G. A. (edn.). New York: Chapman and Hall. pp. 572–576.

Reineccius, G. A. (1995). Controlled-release techniques in the food-industry. In: *Encapsulation and Controlled Release of Food Ingredients*, ACS Symposium Series, 590, Washington, DC: American Chemical Society. pp. 8–25.

Reineccius, G. A. (2001). Multiple-core encapsulation: The spray drying of food ingredients. In Microencapsulation of food ingredients. Vilstrup, P. (edn.). Leatherhead Food RA Publishing.

Reineccius, G. A. (2004). 'The spray drying of food flavours', *Drying Technology*, 22(6), pp. 1289–1324.

RMRDC. (2013). Studies on some Nigerian indigenous spices for domestic/export markets. RMRDC publication series 024. Onwualu, A. P., Jolaoso, M. A., Uchegbu, K. I. and Olugbemi, B. O. (edns.).

Royal Botanical Gardens. (2012). Turmeric– history, plant cultures. UK: Royal Botanical Gardens.

Rubiolo, P., Sgorbini, B., Liberto, E., Cordero, C. and Bicchi, C. (2010). 'Essential oils and volatiles, sample preparation and analysis', *Flavour and Fragrance Journal*, 25, pp. 282-90.

Ryan, K. J. and Ray, G. G. (2004). Sherries medical microbiology (4th edn.). McGraw Hill.

Sadecka, J. (2007). 'Irradiation of spices – a review', *Czech Journal of Food Science*, 25 (5), pp. 231 -242.

Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P. and Riceevans, C. (1995). 'Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants', *Archives of Biochemistry and Biophysics*, 322(2), pp. 339-46.

Sangwan, N. S., Farooqi, A. H. A., Shabih, F. and Sangwan, R. S. (2001). 'Regulation of essential oil production in plants', *Plant Growth Regulation*, 34, pp. 3-21.

Sasidharan, I. A. N. (2010). 'Comparative chemical composition and antimicrobial activity of fresh and dry ginger oils (*Zingiber officinale Roscoe*)', *International Journal of Current Pharmaceutical Research*, 2 (4), pp. 40 – 45.

Sasikumar, B. (2015). 'Genetic resources of curcuma: diversity, characterization and utilization', *Plant Genetic Resources*, 3, pp. 230-251.

Sawant, R. S. and Godghate, A. G. (2013). 'Qualitative phytochemical screening of rhizomes of *Curcuma longa linn*', *International Journal of Science, Environment and Technology*, 2(4), pp. 634 – 641.

Saxena, J. and Sahu, R. (2012). 'Evaluation of phytochemical constituents in conventional and non-conventional species of *Curcuma'*, *International Research Journal of Pharmacy*, 3(8), pp. 203- 204.

Schirmer, M., Hochstotter, A., Jekle, M., Arendt, E. and Becker, T. (2013). 'Physicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio', *Food Hydrocolloids*, 32, pp. 52-63.

Schwab, W., Davidovich-Rikanati, R. and Lewinsohn, E. (2008). 'Biosynthesis of plant-derived flavour compounds: harnessing plant biomass for biofuels and biomaterials', *The Plant Journal*, 54, pp. 712- 732. doi:10.1111/j.1365-313x2008.03446.x

Seid, M. F., Elham, A., Yinghe, H. and Bhesh, B. (2008). 'Encapsulation efficiency of food flavours and oils during spray drying', *Drying Technology*, 26, pp. 816 – 835.

Shahidi, F. and Han, X. Q. (1993). 'Encapsulation of food ingredients', *Critical Reviews in Food Science and Nutrition*, 33(6), pp. 501–547.

Shaikh, J., Bhosale, R. and Singhal, R. (2006). 'Microencapsulation of black pepper oleoresin',

Food Chemistry, 94, pp. 105-110.

Shanmugam, R., Gowthamarajan, K., Priyanka, D., Madhuri, K., Lokesh, M. and Elango, K. (2014). 'Development and validation of simultaneous estimation method for curcumin and piperine by RP-UFLC', *Pakistan Journal of Pharmaceutical Sciences*, 4, pp. 901-906.

Shapiro, Y. E. (2004). Nanoencapsulation of bioactive substances. In Dekker encyclopedia of nanoscience and nanotechnology. Schwarz, J.A., Contescu, C.I., Putyera, K., (edns.). Dekker. pp. 2339–2352.

Sharma, R. K., Misra, B. P., Sarma, T. C., Bordoloi, A. K., Pathak, M. G. and Leclercq, P. A. (1997). 'Essential oils of *Curcuma longa* L. from *Bhutan*', *Journal of Essential Oil Research*, 9, pp. 589-592.

Sharma, S., Kulkarni, S. K. and Chopra, K. (2006). 'Curcumin, the active principle of turmeric (*Curcuma longa*), ameliorates diabetic nephropathy in rats', *Clinical and Experimental Pharmacology and Physiology*, 33(10), pp. 940-945.

Sharma, V., Arora, S. and Sindhu, J. S. (1999). 'Flavour encapsulation applications', *Indian Food Industry*, 18(1), pp. 39–48.

Singh, N., Singh, J., Kaur, L., Sodhi, N. S. and Gill, B. S. (2003). 'Morphological, thermal and rheological properties of starches from different botanical sources', *Food Chemistry*, 81(2), pp. 219-231.

Singleton, V. L. and Rossi, J. A. (1999). 'Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents', *American Journal of Enology and Viticulture*, 16, pp. 144-158.

Small, D. M. and Prescott, J. (2005). 'Odour/taste integration and the perception of flavour', *Experimental Brain Research*, 166, pp. 345-357.

Smith, G. M., Clegg, C. K., Keen C. and Grivetti L. (1996). 'Mineral values of selected plant foods
common to southern Burkina Faso and to Niamey, Niger, West Africa', *International Journal of Food Sciences and Nutrition*, 47, pp. 41-53.

Soh, D., Nkwengoua, E., Ngantchou, I., Nyasse, B., Denier, C., Hannaert, V., Shaker, K. H. and Schneider, B. (2013). 'Xylopioxyde and other bioactive kaurane-diterpenes from *Xylopia aethiopica* Dunal (Annonaceae)', *Journal of Applied Pharmaceutical Science*, 3(12), pp. 13- 19.

Sotanaphun, U., Phattanawasin, P. and Sriphong, L. (2009). 'Application of Scion image software to the simultaneous determination of curcuminoids in turmeric (*Curcuma longa*)', *Phytochemical Analysis* 20 (1), pp. 19–23. doi:10.1002/pca.1086. PMID 18683171.

Sparg, S. G., Light, M. E. and Vanstaden, J. (2004). Biological activities and distribution of plants saponins. *Journal of Ethnopharmacology*, 94(2-3), pp. 219-243.

Spice market. (2013). Spice market by type, application and region-global trends and forecast to 2019. Food Market Research Report. 322898.

Sugmarpandian, D. and Nagarajan, N. S. (2015). 'Comparison of chemical composition and antioxidant potential of supercritical fluid CO_2 extracts and hydro distilled oil of *Nardostachys jatamansi* DC', *International Journal of Innovative Science, Engineering & Technology*, 2(9), pp. 1- 8.

Suresh, D., Manjunatha, H. and Srinivasan, K. (2007). 'Effect of heat processing of spices on the concentrations of their bioactive principles: *C. longa*, red pepper (*Capsicum annuum*) and black pepper (*Piper nigrum*)', *Journal of Food Composition and Analysis, 20,* pp. 346-351.

Surwase, V. S., Laddha, K. S., Kale, R. V., Syed, I. H. and Lokhande, S. M. (2011). 'Extaction and isolation of turmerone from turmeric', *Electronic journal of Environmental, Agricultural and Food Chemistry,* 10(5), pp. 2173 -2179.

Swadhini, S. P., Santosh, R., Uma, C., Mythili, S. and Sathiavelu, A. (2011). 'Phytochemical screening and antimicrobial activity of five medicinal plants against *Myrothecium sp*.', *International Journal of Pharma and Biosciences*, 2(1), pp. 272-279.

Tahira, J. J. (2010). 'Weed flora of *Curcuma longa'*, *Pakistan Journal of Weed Science Research*, 16(2), pp. 241-246.

Tainter, D. R. and Grenis, A. T. (2001). Spices and seasonings. A Food Technology Handbook (2nd edn.). New York: Wiley-VCH.

Tairu, A. O., Hofmann, T. and Schieberle, P. (1999). Identification of the key aroma compounds in dried fruits of *Xylopia aethiopica*. Perspectives on new crops and new uses. J. Janick (edn.), Alexandria, VA: ASHS Press. pp. 474-478.

Tatsadjieu, L. N., Ngang, J. J. E, Ngassoum, M. B. and Etoa, F. X. (2003). 'Antibacterial and antifungal activity of *Xylopia aethiopica*, *Monodoura myristica*, *Zanthoxylum xanthoxyloides* and *Zonthoxylum leprieuri* from Cameroun', *Fitoterapia*, 74, pp. 469-472.

Tayyem, R. F., Health, D. D., Al-Delaimy, W. K. and Rock, C. L. (2006). 'Curcumin content of turmeric and curry powders,. *Nutrition and Cancer*, 55(2), pp. 126 – 131.

Thomas-Eapen, N. (2009). 'Turmeric: the intriguing yellow spice with medicinal properties', *Explore-the Journal of Science and Healing*, *5*(2), pp. 114-115.

Thompson, D. A. and Tan, B. B. (2006). 'Tetrahydrocurcumin-related allergic contact dermatitis',
Contact Dermatitis, *55*, pp. 254-255.

Tipsrisukond, N., Fernando, L. N. and Clarke, A. D. (1998). 'Antioxidant effects of essential oil and oleoresin of black pepper from supercritical carbon dioxide extractions in ground pork', *Journal of Agricultural and Food Chemistry*, 46, pp. 4329-4333.

Tomi, F., Casanova, J. and Nianga, M. (1996). 'Identification of the seed oil of *Xylopia aethiopica*

from Guinea using 13C-NMR Spectroscopy', *Journal of Essential Oil Research*, 8, pp. 429-431.

Tripathi, M., Chawla, P., Upadhyay, R. and Trivedi, S. (2013). 'Essential oils from family Zingiberaceae for antimicrobial activity – a review', *International Journal of Pharma and Bio Sceience*, 4(4), pp. 149 -162.

Ukoha, P. O., Egbuonu, A. C. C., Obasi, L., Nnamdi, C. and Ejikeme, P. M. (2011). 'Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities', *African Journal of Pure and Applied Chemistry*, 5(8), pp. 237-244.

USEPA. (1992). Code of federal regulation, 40 CFR Part 136.

Usman, L. A., Hamid, A. A., George, O. C., Ameen, O. M., Muhammad, N. O., Zabair, M. F. and
Lawal, A. (2009). 'Chemical composition of rhizome essential oil of *Curcuma longa* L.
growing in North Central Nigeria', *World Journal of Chemistry*, 4 (2), pp. 178 – 181.

Uyovwiesevwa, A. J., Aloamaka, P. C. and Avwioro, O. G. (2011). 'Effect of *Xylopia aethiopica* plant extract on semen quality of the Sprague dawley rats', *International Journal of Recent Scientific Research*, 2 (6), pp. 179 – 181.

Uzodike, E. B. and Onuoha, I. N. (2010). 'The effect of X*ylopia aethiopica* (uda) on intraocular pressure', *Journal of the Nigerian Optometric Association*, 16, pp. 21-25.

Verghese, J. (1993). 'Isolation of curcumin from *Curcuma longa* L. rhizome', *Flavour and Fragrance Journal*, 8(6), pp. 315-319.

Versini, G., Dalla-Serra, A., Orriols, I., Inama, S. and Marchio, M. (1995). 'Bartlett pear unsaturated ethyl decanoates and C_9 compounds among components characterizing Cv, catalan roxo grape marc distillates', *Vitis*, 34 (1), pp. 57 – 62.

Vidita, V. B., Shashank, C. P. and Kruti, S. D. (2013). 'Importance of terpenoids and essential oils in chemotaxonomic approach'. *International Journal of Herbal Medicine*, 1(2), pp. 14- 21.

Vipul, U., Neeru, S., Himanshu, M. J., Amreesh, M., Manoj, M., Singh, B.P. and Sanjeev, T. (2013). 'Development and validation of rapid RPHPLC method for estimation of piperine in *Piper nigrum* L.*', International Journal of Herbal Medicine*, 1(4), pp. 6-9

Vogt, R. L. and Dippold, L. (2005). '*Escherichia coli*: outbreak associated with consumption of ground beef', *Public Health Reports*, 120(2), pp. 174-178.

Voilley, A.J. (1995). Flavour encapsulation – influence of encapsulation media on aroma retention during drying. In: *Encapsulation and Controlled Release of Food Ingredients*, ACS Symposium Series, 590, Washington, DC: American Chemical Society. pp. 169–179.

Vora, P., Senecal, A. and Schaffner, D. W. (2003). 'Survival of *Staphylococcus aureus* ATCC 13565 in intermediate moisture foods is highly variable', *Risk Analysis,* 23(1), pp. 229-236.

Weisberg, S. P., Leibel, R. and Tortoriello, D. V. (2008). 'Dietary curcumin significantly improves obesity – associated inflammation and diabetes in mouse models of diabesity', *Endocrinology*, 149(7), pp. 3549-3558.

Weiss, E. A. (2002). Spice Crops. Oxon, UK: CAB International publishing.

WHO. (2002). The WHO recommended classification of pesticides by hazard and guidelines to classification. WHO, Geneva.

Wu, P., Tian, J. C., Walker, C. E. and Wang, F. C. (2009). 'Determination of phytic acid in cereals–a brief review', *International Journal of Food Science and Technology*, 44, pp. 1671-1676.

Wuthi-Udolmlert, M., Grisanapam, W., Luanratana, O. and Caichompoo, W. (2000). 'Antifungal activity of *Curcuma longa* grown in Thailand`. *Southeast Asia Journal of Tropical Medicine and Public Health*, 3(1), pp. 178-182.

Yu, L. and Ni, Y. (2005). 'Oxalate formation during peroxide bleaching of mechanical pulps', *Journal of Appita*, 58(2), pp. 138- 143.

Zebib, B., Mouloungui, Z. and Noirot, V. (2010). 'Stabilization of curcumin by complexation with
divalent cations in glycerol/water system', *Bioinorganic Chemistry and Applications*, 3(5), pp. 23 -36. doi:10.1155/2010/292760.

Zhengzhi, X., Xiaoqiang, M. and David, R. G. (2009). 'Modules of co-regulated metabolites in *C. longa* rhizome suggest the existence of biosynthetic modules in plant specialized metabolism', *Journal of Experimental Botany*, 60, pp. 87-97.

APPENDICES

APPENDIX I

QUESTIONNAIRE FOR SENSORY EVALUATION

FEDERAL UNIVERSITY OF TECHNOLOGY
DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
SCORING SHEET FOR SENSORY EVALUATION (PRODUCT TEST)

Name of the Panelist: ……

Address of panelist: ……..

Product/Sample: ……

Instruction(s):

Dear panelist, you're provided with six coded samples of spice extract/product. Please, you're required to indicate the degree of your perception of each sample for the under listed organoleptic attributes using assigned numerical values ranging from 7 –points (for **Very Much Liked)** to 1 (**Very Much Disliked)** for your judgment.

Degree	**point**
Very much Liked	7
Moderately Liked	6
Slightly Liked	5
Neither Liked nor Disliked	4
Slightly Disliked	3
Moderately Disliked	2
Very Much Disliked	1

Sensory property	**NJC**	**441**	**102**	**HMT**	**FRS**	**6k8**
Colour/Appearance						
Pungent						
Sweetness						
Minty						
Bitterness						
Harshness						
Hotness						
After-Taste						
Overall acceptability						

Comment:……
……
……
……..

FEDERAL UNIVERSITY OF TECHNOLOGY

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SCORING SHEET FOR SENSORY EVALUATION (CONSUMER PREFERENCE TEST)

Name of the Evaluator: ……

Address of panelist: ……..

Product/Sample: ……

Instruction(s):

Dear panelist, you're provided with six coded samples of spice extract/product/sample. Please, you're required to indicate the degree of your likeness/dislike for each sample for the under listed organoleptic attributes using assigned numerical values ranging from 7 –points (for **Very Much Liked)** to 1 (**Very Much Disliked)** for your judgment.

Degree	**Point**
Very much Liked	7
Moderately Liked	6
Slightly Liked	5
Neither Liked nor Disliked	4
Slightly Disliked	3
Moderately Disliked	2
Very Much Disliked	1

Sensory property	**NJC**	**441**	**102**	**HMT**	**FRS**	**6k8**
Colour						
Pungent flavour						
Aroma						
Overall acceptability						

Recommended Food:

Rice ☐ Meat ☐ Porridge ☐ Yoruba local soup ☐

Peppe-soup ☐ Ugba ☐ Stew ☐ Igbo local soup ☐

SAUCE ☐ Tea ☐ Akamu/pap ☐ Hausa local soup ☐

Baked products ☐ Ice cream ☐ oghurt drink ☐ n-alcoholic beverage ☐

Alcoholic beverage ☐

Comment:……

APPENDIX II

PHYTO-CHEMICAL CONTENTS OF SOLVENTS EXTRACT AND EXTRACTION RESIDUES OF *XYLOPIA AETHIOPICA* AND TURMERIC SPICES

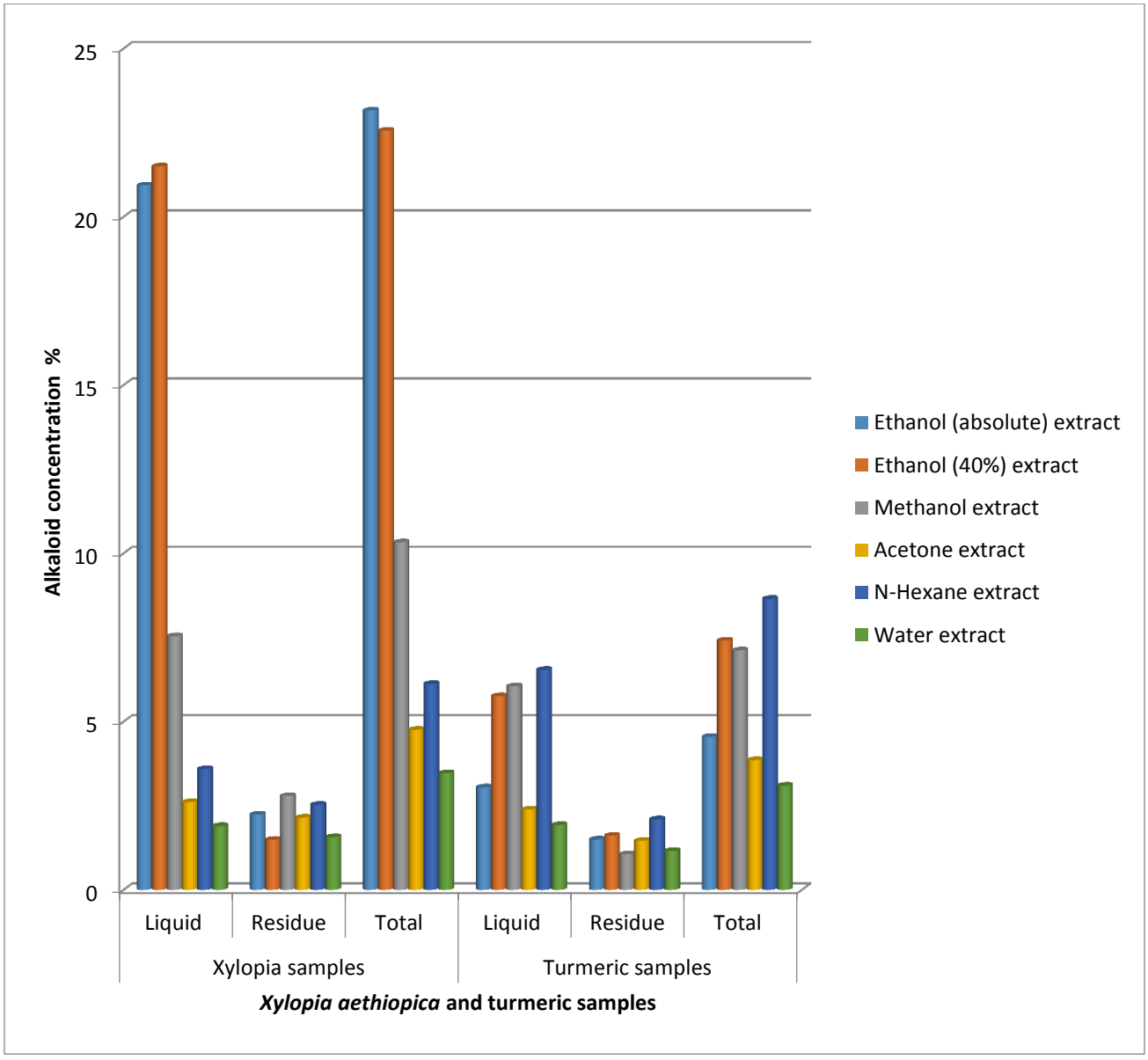


Figure 1: Alkaloid concentrations (%) in extracts and extraction residues content of *Xylopia aethiopica* and turmeric samples.

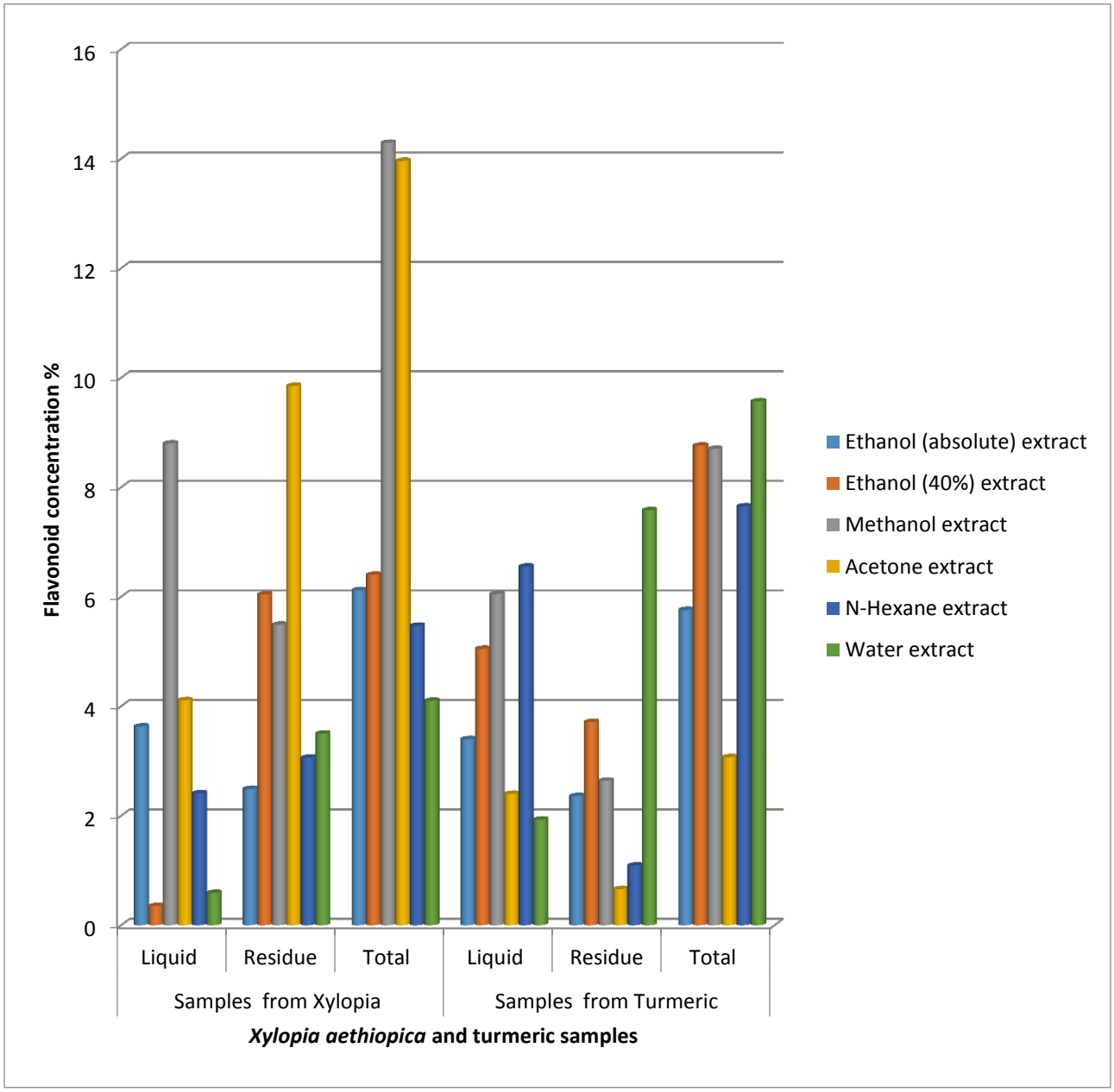


Figure 2: Flavonoid concentrations (%) in extracts and extraction residues of *Xylopia aethiopica* and Turmeric samples

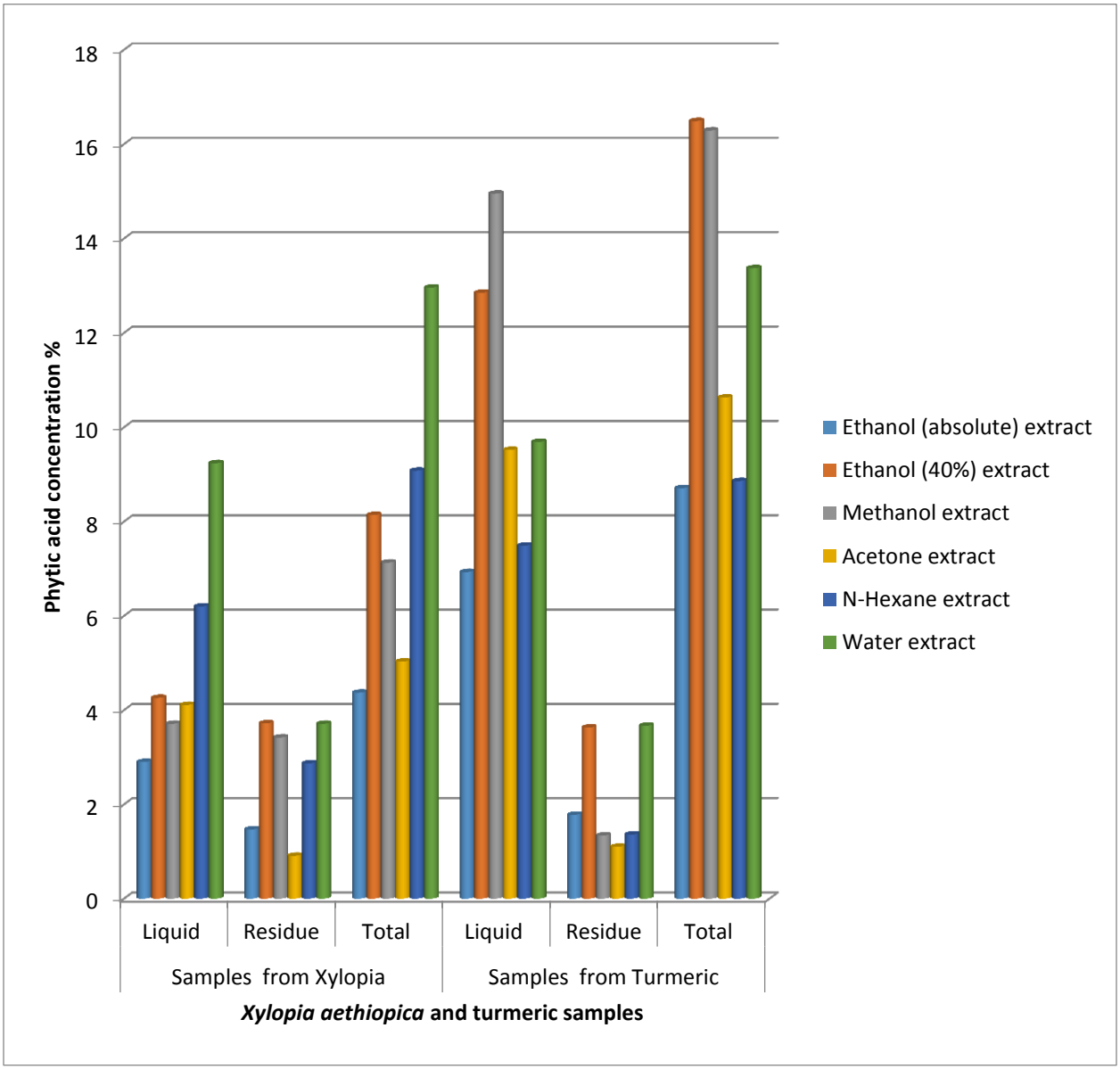


Figure 3: Phytic acid concentrations (%) of liquid extract and extraction residues of *Xylopia aethiopica* and Turmeric samples.

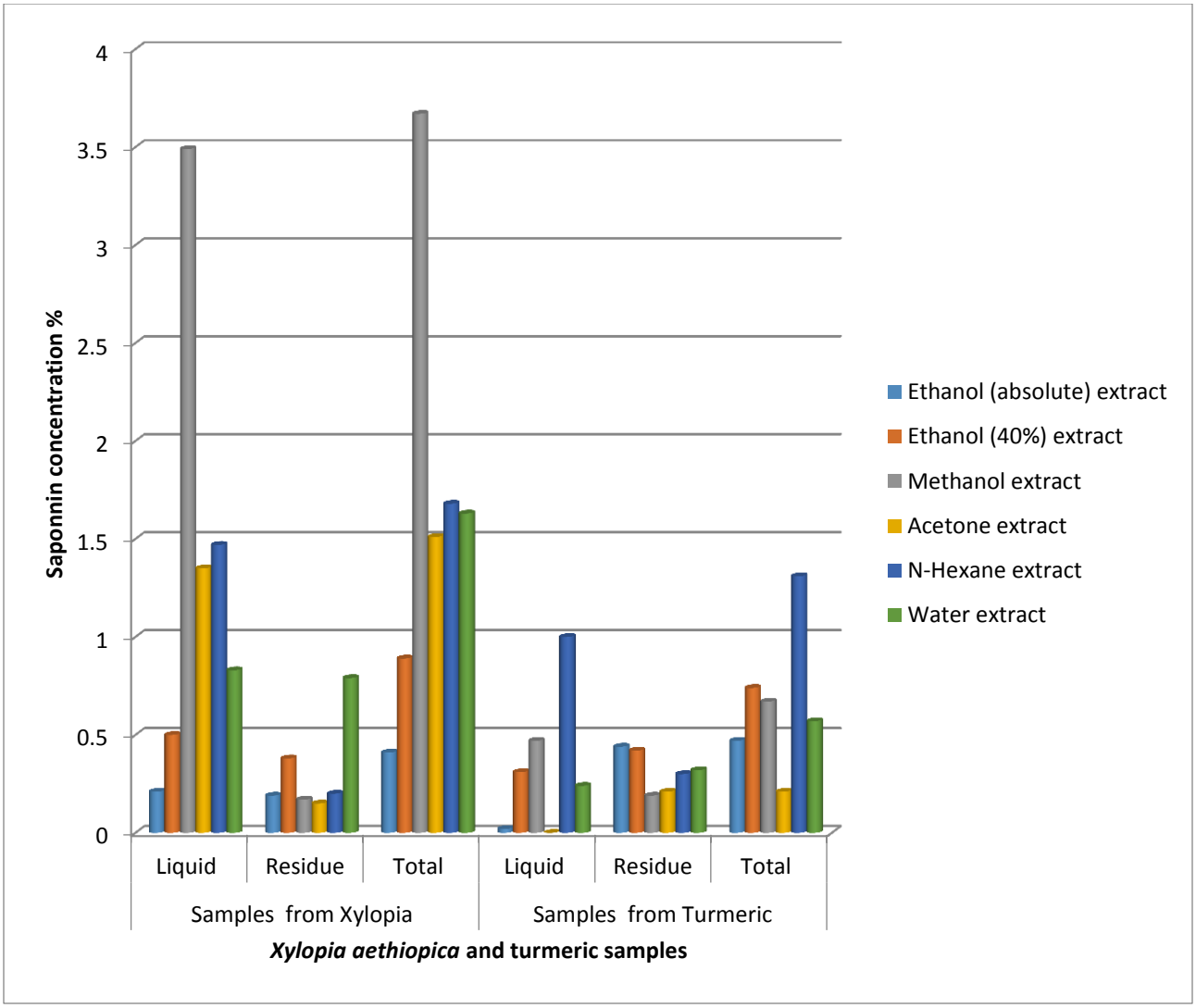


Figure 4: The Saponin concentrations (%) of Liquid extracts and extraction residues of *Xylopia aethiopica* and turmeric samples.

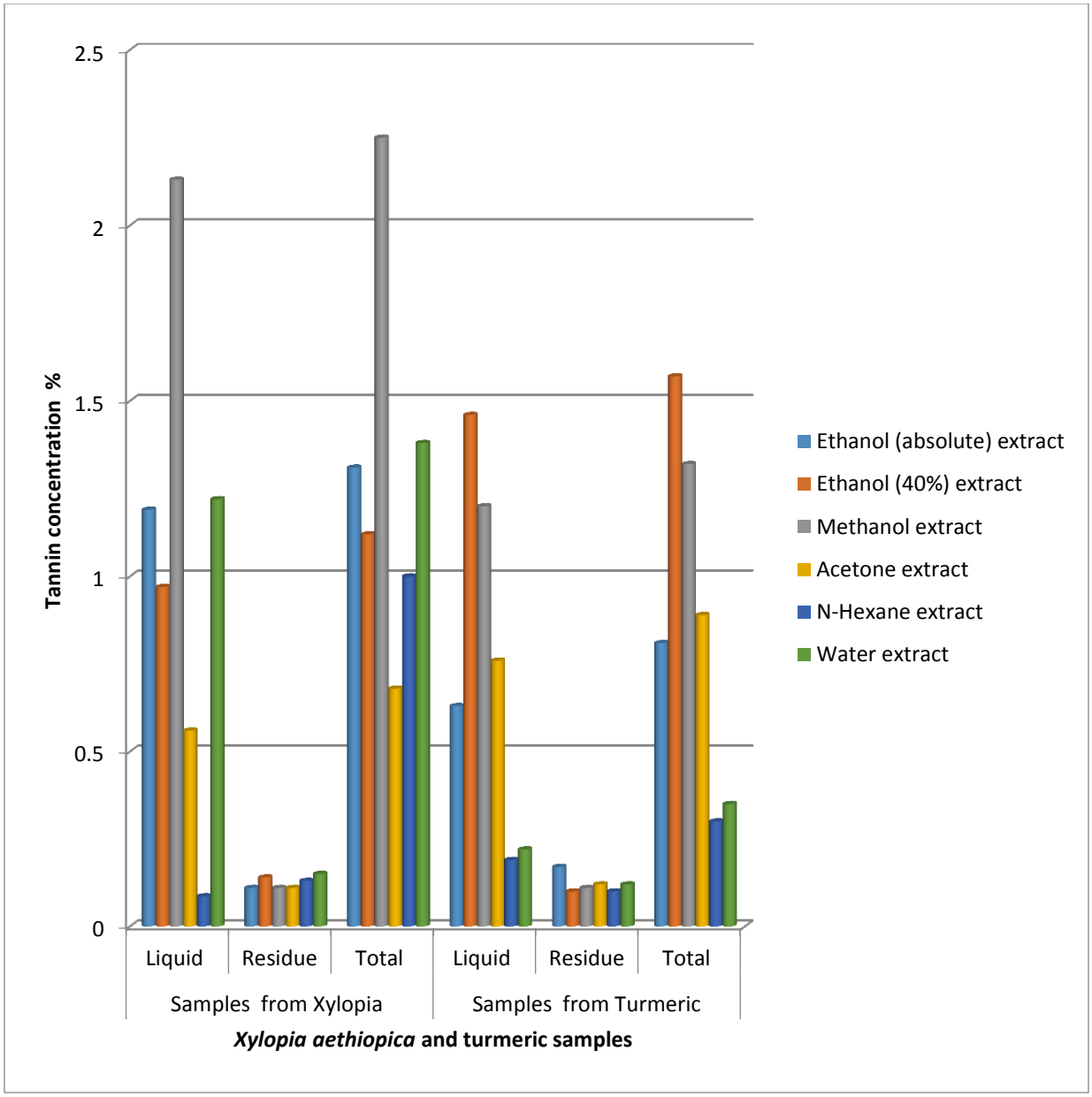


Figure 5: Tannin concentrations (%) of liquid extracts and extraction residues of *Xylopia aethiopica* and Turmeric samples.

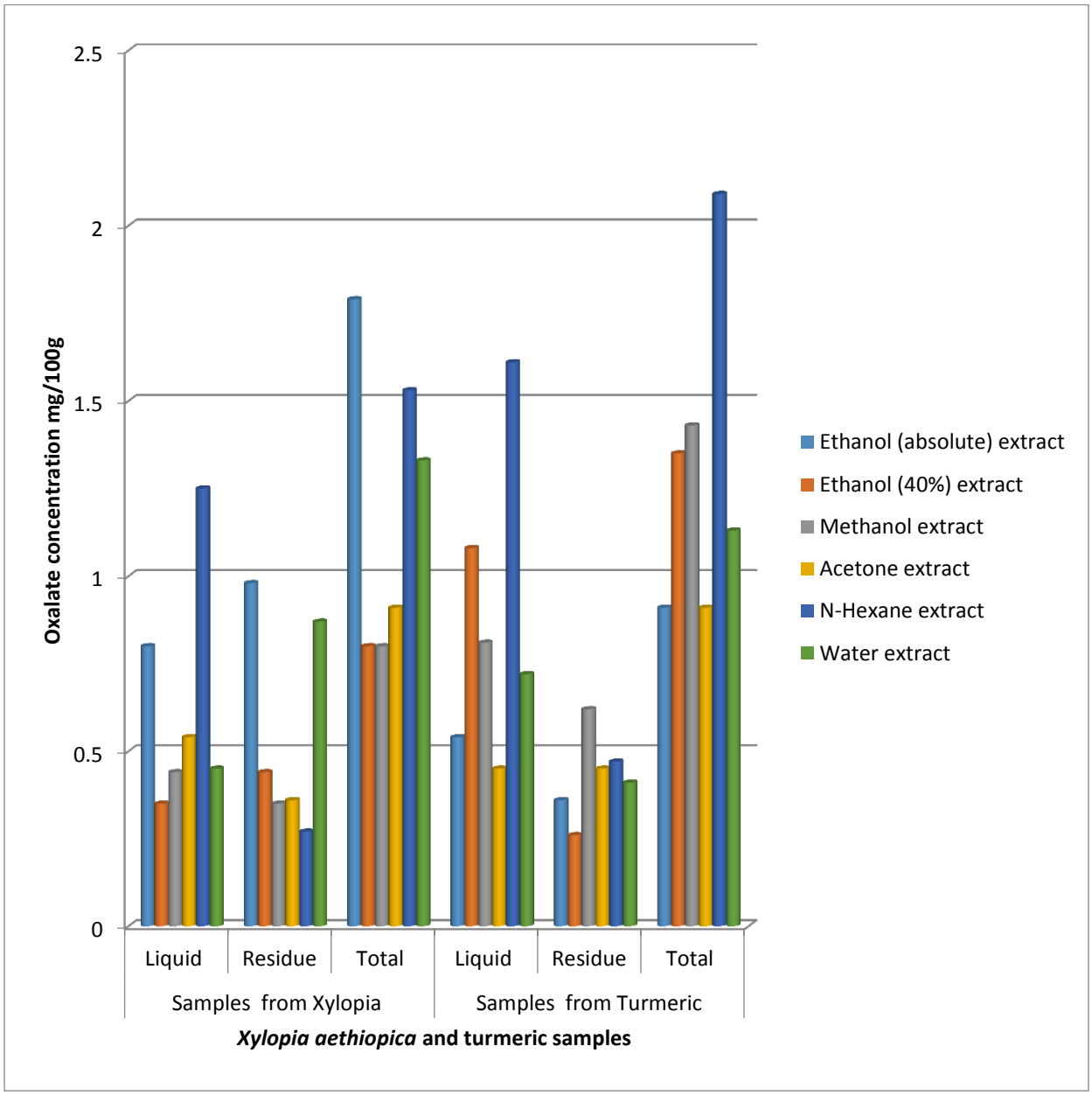


Figure 6: The Oxalate concentration ($mg100g^{-1}$) of liquid extract and extraction residues of *Xylopia aethiopica* and Turmeric samples.

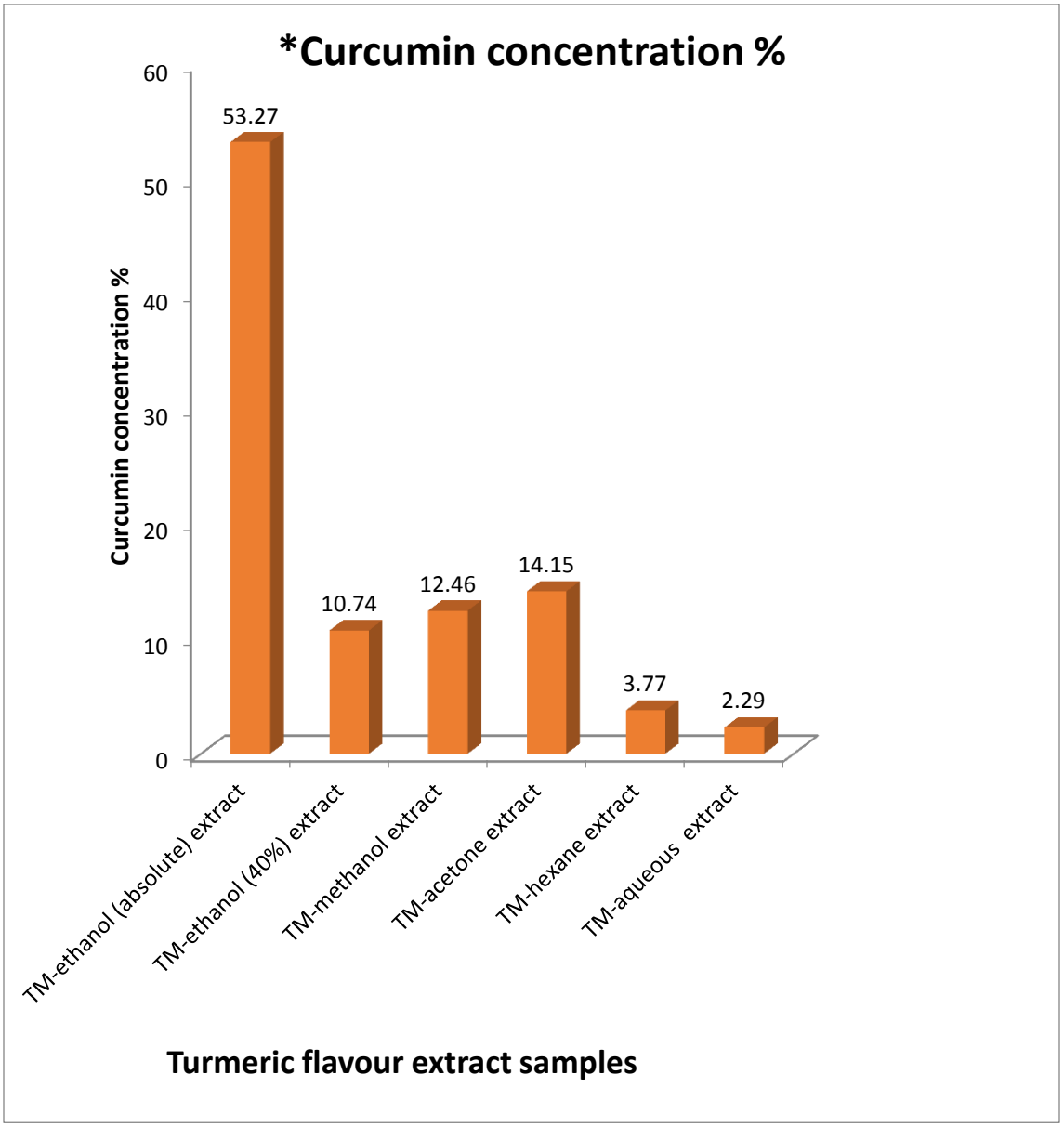


Figure 7: Curcumin concentrations (%) in turmeric spice extract samples

APPENDIX III

CALIBRATION CURVE FOR CURCUMIN STANDARD

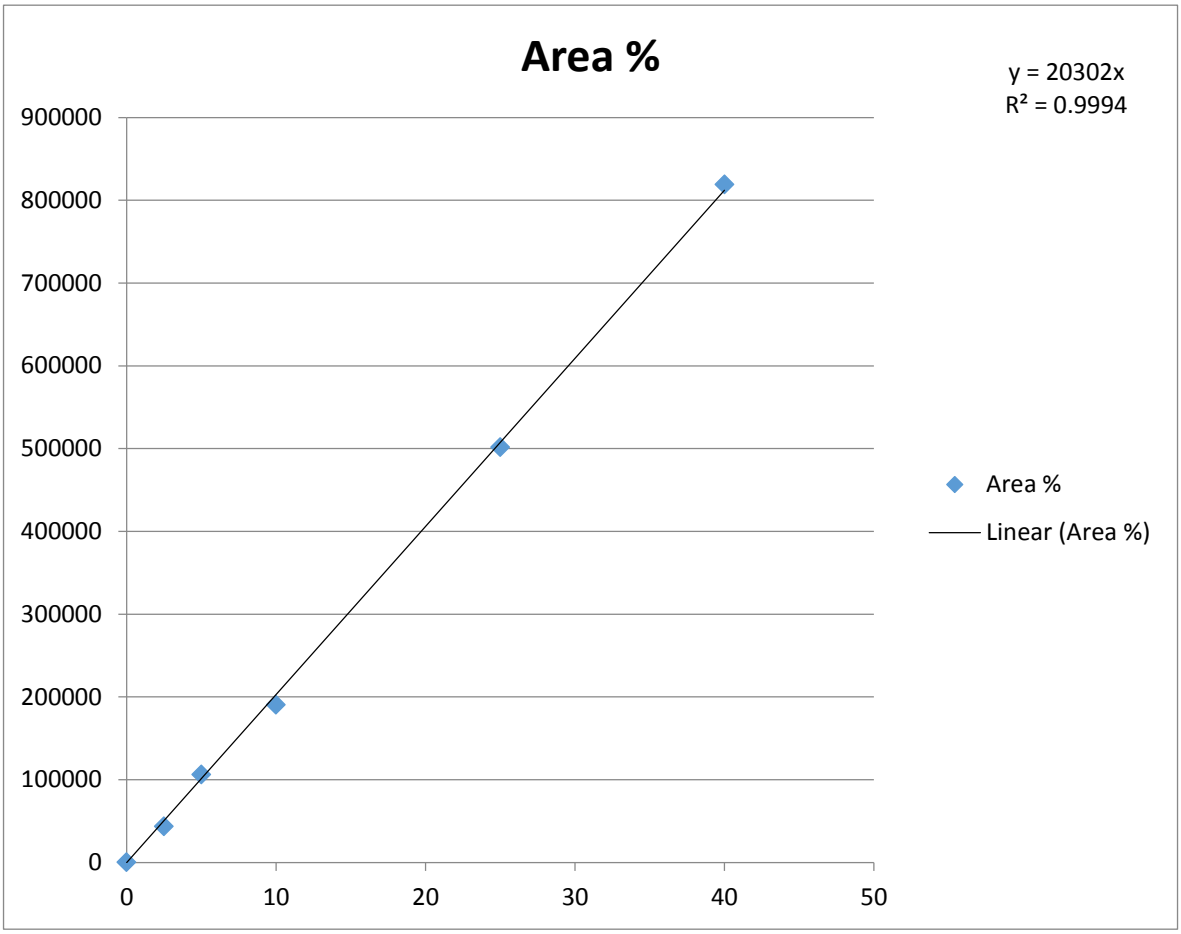


Figure 8: Calibration curve for Curcumin standard

APPENDIX IV

MICROORGANISMS IN *C.LONGA* AND *X. AETHIOPICA* SPICE SAMPLES

Table 1: Total Counts and Identity of Fungal Isolates from *C.longa* and *X. aethiopica* Spice Samples

Sample code	Total colony count (cfu/g)	Colony code	Colonial characteristics	Microscopic characteristics	Identity of isolates
Ground *C.longa* (Processed) sample (J)	2.0×10^5	J1	Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
		J2	White short hyphae	Non septate hyphae, sporangiophore septate	*Mucor spp.*
Raw *C.longa* Rhizomes (F)	3.2×10^8		Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
Ground *X. aethiopica* (Processed) sample (G)	2.20×10^5	G1	White short hyphae	Non septate hyphae, sporangiophore septate	*Mucor spp.*
		G2	Dirty green spores enclosed in short hyphae		*Penicillium notatum*
		G3	White cotton wool like hyphae	Conidia sickle shaped	*Fusarium spp.*
Raw *X. aethiopica* Pods (E)	5.1×10^8	E1	Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
		E2	Dirty green spores enclosed in short hyphae		*Penicillium notatum*

Colony code: J1 and J2 are duplicate samples of fungal isolates from ground *C.longa* sample; G1, G2 and G3 are triplicate samples of fungal isolates from ground *X. aethiopica* sample; E1 and E2 are duplicate samples of fungal isolates from ground *X. aethiopica* sample

Table 2: Total Counts and Identity of Bacteria Isolates from Samples of *C.longa* and *X. aethiopica* Spice on nutrient agar

Sample code	Total counts (cfu/g)	Colony code	Colonial characteristics	Microscopic characteristics	Suspected Microorganism
Ground *C.longa*. (Processed) (J)	4.7×10^8	JX		Gram positive cocci in chains	*Enterococcus* spp.
		JY	Gram positive short rods with central spores Small circular yellow colonies	Gram positive short rods with central spores	*Bacillus* spp.
			Dull and dry flat serrated cream colonies		
Raw *C.longa* Rhizomes (F)	7.1×10^8	FX	Moist and shiny smooth golden yellow colonies	Moist and shiny smooth golden yellow colonies	*Staphylococcus* spp
		FY			*Enterococcus* spp.
			Gram positive short rods with central spores	Gram positive cocci in clusters, few in pairs and tetrads	
		FZ			*Bacillus* spp.
			Dull and dry flat serrated cream colonies	Gram positive cocci in chains	
				Gram positive short rods with central spores	
Ground *X. aethiopica* (Processed) (G)	3.5×10^8	GX	Moist and shiny smooth golden yellow colonies	Moist and shiny smooth golden yellow colonies	*Staphylococcus* spp
		GY			*Bacillus* spp.
		GZ	Dull and dry flat serrated cream colonies	Gram positive short rods with central spores	*Enterococcus* spp.
			Gram positive short rods with central spores	Gram positive cocci in chains	
Raw *X. aethiopica* Pods (E)	3.9×10^8	EX	Gram positive short rods with central spores	Gram positive cocci in chains	*Enterococcus* spp.
		EY	Dull and dry flat serrated cream colonies	Gram positive short rods with central spores	*Bacillus* spp.

Colony code: JX and JY are duplicate samples of bacterial isolates from ground *C.longa* sample; FX, FY and FZ are triplicate samples of bacterial isolates from raw *C.longa* sample; GX, GY and GZ are triplicate samples of bacterial isolates from ground *X. aethiopica* sample; EX and EY are duplicate samples of bacterial isolates from raw *X. aethiopica* sample

Table 3: Total Counts and Identity of Bacteria Isolates from *C.longa* and *X. aethiopica* Spice Samples on MacConkey Agar

Sample code	Total counts (cfu/g)	Colony code	Colonial characteristics	Microscopic characteristics	Suspected Microorganism
Raw *C.longa* Rhizomes (F)	1.1×10^8	FA	Dull and dry finger like projections Mucoid pinkish colonies	Gram negative, rod shaped in clusters Gram negative, rod shaped in clusters	*E. coli*
Raw *X. aethiopica* Pods (E)	6.4×10^8	EA	Mucoid pinkish colonies	Gram negative, rod shaped in clusters	*E. coli*
		EB	Mucoid pinkish colonies	Gram negative, rod shaped in clusters	*E. coli*
		EC	Mucoid pinkish colonies	Short gram negative rods predominantly in singles	*E. coli*
Ground *C.longa* (processed) (J)	NG	-	-	-	-
Ground *X. aethiopica* (processed) (G)	NG	-	-	-	-

NG = No Growth

Colony code: FA is bacterial isolates from raw *C.longa* sample; EA, EB and EC are triplicate samples of bacterial isolates from raw *X. aethiopica* sample

Table 4: Total Counts and Identity of Bacteria Isolates from *C.longa* and *X. aethiopica* Spice Samples on Brain Heart Infusion Agar (BHIA)

Sample code	Colony count (cfu/g)	Colony code	Colonial characteristics	Microscopic characteristics	Probable identity
Ground *C.longa* (processed) (J)	2.0×10^3	Ja	Dull and dry serrated flat colonies	Slender rods in chains	*Lactobacillus* spp.
		Jb	Small circular golden yellow colonies	Cocci in long chains	*Streptococcus* spp.
Raw *C.longa* Rhizomes (F)	3.0×10^6	Fa	Dull and dry serrated flat colonies	Slender rods in chains	*Lactobacillus* spp.
		Fb	Small circular golden yellow colonies	Cocci in long chains	*Streptococcus* spp.
Ground *X. aethiopica* (processed) (G)	1.0×10^3	Ga	Dull and dry serrated flat colon	Slender rods in chains	*Lactobacillus* spp.
		Gb	Mucoid and slimy cream colonies		*Bacillus* spp.
		Gc	Large gram positive rods with central spores	Cocci in long chains	*Streptococcus* spp.
Raw *X. aethiopica* Pods (E)	2.1×10^6	Ea	Dull and dry serrated flat colon	Slender rods in chains	*Lactobacillus* spp.
		Eb	Mucoid and slimy cream colonies	Large gram positive rods with central spores	*Bacillus* spp.

Colony code: Ja and Jb are duplicate samples of bacterial isolates from ground *C.longa* sample; Fa and Fb are duplicate samples of bacterial isolates from raw *C.longa* sample; Ga, Gb and Gc are triplicate samples of bacterial isolates from ground *X. aethiopica* sample; Ea and Eb are duplicate samples of bacterial isolates from raw *X. aethiopica* sample

Table 5: Biochemical and Carbohydrate Fermentation Reactions of the Bacterial Isolates

Catalase test	Oxidase test	Coagulase test	Indole test	Methyl red test	Voges-Proskaeur test	Citrate test	Urease test	NO_3	Glucose test	Sucrose test	Lactose test	Maltose test	Mannitol test	Xylose	Arabinose test	Fructose test	Identity of Bacterial isolates
+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	-	+	*Staphylococcus aureus*
-	-	-	-	+	-	+	-	+	+	+	+	-	+	-	-	-	*Enterococcus faecalis*
+	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	*Escherichia coli*
+	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	+	*Bacillus cereus*
+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	*Bacillus subtilis*
-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	*Lactobacillus brevis*
-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	+	+	*Streptococcus anginosus*
+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	*Micrococcus luteus*

APPENDIX V

RESULTS OF STATISTICAL ANALYSIS

Liquid extract

dependent factor	N	Subset for alpha = 0.05

ANOVA for alkaloid content of *Xylopia aethiopica* extracts

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	836.871	5	167.374	1825900.236	.000
	Within Groups	.001	6	.000		
	Total	836.871	11			
residue	Between Groups	2.697	5	.539	5394.400	.000
	Within Groups	.001	6	.000		
	Total	2.698	11			
total extractable	Between Groups	796.300	5	159.260	2827.518	.000
	Within Groups	.338	6	.056		
	Total	796.638	11			

Homogeneous Subsets

			f	e	d	c	b	a
Tukey HSD[a]	water Alkaloid from Xylopia sample	2	1.9000					
	acetone Alkaloid from Xylopia extract sample	2		2.6050				
	n-haxane Alkaloid from Xylopia sample	2			3.5900			
	methanol Alkaloids from Xylopia extract sample	2				7.5350		
	ethanol absolute Alkaloid from Xylopia sample	2					20.9250	
	ethanol 40 Alkaloid extract from Xylopia sample	2						21.5000
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	ethanol 40 Alkaloid extract from Xylopia sample	2	1.4850					
	water Alkaloid from Xylopia sample	2		1.5700				
	acetone Alkaloid from Xylopia extract sample	2			2.1550			
	ethanol absolute Alkaloid from Xylopia sample	2				2.2400		
	n-haxane Alkaloid from Xylopia sample	2					2.5350	
	methanol Alkaloids from Xylopia extract sample	2						2.7950
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	water Alkaloid from Xylopia sample	2	3.4750				
	acetone Alkaloid from Xylopia extract sample	2		4.7600			
	n-haxane Alkaloid from Xylopia sample	2			6.1250		
	methanol Alkaloids from Xylopia extract sample	2				10.3300	
	ethanol 40 Alkaloid extract from Xylopia sample	2					22.5600
	ethanol absolute Alkaloid from Xylopia sample	2					23.1650
	Sig.		1.000	1.000	1.000	1.000	.243

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	42.101	5	8.420	9623.095	.000
	Within Groups	.005	6	.001		
	Total	42.106	11			
residue	Between Groups	1.362	5	.272	5447.067	.000
	Within Groups	.000	6	.000		
	Total	1.362	11			
total extractable	Between Groups	50.118	5	10.024	11455.446	.000
	Within Groups	.005	6	.001		
	Total	50.123	11			

Homogeneous Subsets

Liquid extract

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	water alkaloids from turmeric sample	2	1.9350					
	acetone alkaloids from turmeric extract sample	2		2.3950				
	ethanol absolute alkloid from Turmeric sample	2			3.0500			
	ethanol 40 alkaloids extract from turmeric sample	2				5.7650		
	methanol alkaloid from turmeric extract sample	2					6.0550	
	n-haxane alkaloids from turmeric sample	2						6.5450
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	**N**	**Subset for alpha = 0.05**					
			f	**e**	**d**	**c**	**b**	**a**
Tukey HSD[a]	methanol alkaloid from turmeric extract sample	2	1.0650					
	water alkaloids from turmeric sample	2		1.165				
	acetone alkaloids from turmeric extract sample	2			1.4650			
	ethanol absolute alkloid from Turmeric sample	2				1.5050		
	ethanol 40 alkaloids extract from turmeric sample	2					1.6150	
	n-haxane alkaloids from turmeric sample	2						2.1050
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	Subset for alpha = 0.05					
		f	**e**	**d**	**c**	**b**	**a**
Tukey HSD[a]	water alkaloids from turmeric sample	3.1000					
	acetone alkaloids from turmeric extract sample		3.8600				
	ethanol absolute alkloid from Turmeric sample			4.5550			
	methanol alkaloid from turmeric extract sample				7.1200		
	ethanol 40 alkaloids extract from turmeric sample					7.4100	
	n-haxane alkaloids from turmeric sample						8.6500
	Sig.	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA for flavonoid content

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	95.609	5	19.122	152974.627	.000
	Within Groups	.001	6	.000		
	Total	95.610	11			
residue	Between Groups	74.254	5	14.851	297016.667	.000
	Within Groups	.000	6	.000		
	Total	74.254	11			
total extractable	Between Groups	203.941	5	40.788	257609.526	.000
	Within Groups	.001	6	.000		
	Total	203.942	11			

Homogeneous Subsets for flavonoid content

Liquid extract

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	ethanol 40 Flavonoid extract from Xylopia sample	2	.3550					
	water Flavonoid from Xylopia sample	2		.5950				
	n-haxane Flavonoid from Xylopia sample	2			2.4050			
	ethanol absolute Flavonoid from Xylopia sample	2				3.6300		
	acetone Flavonoid from Xylopia extract sample	2					4.1100	
	methanol Flavonoids from Xylopia extract sample	2						8.8000
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	Subset for alpha = 0.05					
		f	e	d	c	b	a
Tukey HSD[a]	ethanol absolute Flavonoid from Xylopia sample	2.4950					
	n-haxane Flavonoid from Xylopia sample		3.0650				
	water Flavonoid from Xylopia sample			3.5050			
	methanol Flavonoids from Xylopia extract sample				5.4950		
	ethanol 40 Flavonoid extract from Xylopia sample					6.0450	
	acetone Flavonoid from Xylopia extract sample						9.8550
	Sig.	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	Subset for alpha = 0.05					
		f	e	d	c	b	a
Tukey HSD[a]	water Flavonoid from Xylopia sample	4.1000					
	n-haxane Flavonoid from Xylopia sample		5.4700				
	ethanol absolute Flavonoid from Xylopia sample			6.1250			
	ethanol 40 Flavonoid extract from Xylopia sample				6.4000		
	acetone Flavonoid from Xylopia extract sample					13.9650	
	methanol Flavonoids from Xylopia extract sample						14.2950
	Sig.	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA for turmeric flavonoids

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	37.374	5	7.475	8542.547	.000
	Within Groups	.005	6	.001		
	Total	37.379	11			
residue	Between Groups	62.299	5	12.460	166131.311	.000
	Within Groups	.000	6	.000		
	Total	62.300	11			
total extractable	Between Groups	59.235	5	11.847	6181.082	.000
	Within Groups	.011	6	.002		
	Total	59.247	11			

Homogeneous Subsets

Liquid extract

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	water flavonoids from turmeric sample	2	1.9350					
	acetone flavonoids from turmeric extract sample	2		2.4050				
	ethanol absolute alkloid from Turmeric sample	2			3.4050			
	ethanol 40 flavonoids extract from turmeric sample	2				5.0500		
	methanol alkaloid from turmeric extract sample	2					6.0550	
	n-haxane flavonoids from turmeric sample	2						6.5550
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	B	a
Tukey HSD[a]	acetone flavonoids from turmeric extract sample	2	.6650					
	n-haxane flavonoids from turmeric sample	2		1.0950				
	ethanol absolute alkloid from Turmeric sample	2			2.3600			
	methanol alkaloid from turmeric extract sample	2				2.6450		
	ethanol 40 flavonoids extract from turmeric sample	2					3.7150	
	water flavonoids from turmeric sample	2						7.5850
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	**N**	**Subset for alpha = 0.05**				
			e	**d**	**c**	**b**	**A**
Tukey HSD[a]	acetone flavonoids from turmeric extract sample	2	3.0700				
	ethanol absolute alkloid from Turmeric sample	2		5.7650			
	n-haxane flavonoids from turmeric sample	2			7.650		
	methanol alkaloid from turmeric extract sample	2				8.7000	
	ethanol 40 flavonoids extract from turmeric sample	2				8.7650	
	water flavonoids from turmeric sample	2					9.5700
	Sig.		1.000	1.000	1.000	.685	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA for phytic acid content

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	53.647	5	10.729	1417.995	.000
	Within Groups	.045	6	.008		
	Total	53.693	11			
residue	Between Groups	14.656	5	2.931	29311.733	.000
	Within Groups	.001	6	.000		
	Total	14.656	11			
total extractable	Between Groups	96.462	5	19.292	178083.031	.000
	Within Groups	.001	6	.000		
	Total	96.462	11			

Homogeneous Subsets

Liquid extract

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	B	a
Tukey HSD[a]	ethanol absolute Phytic acid from Xylopia sample	2	2.9050				
	methanol Phytic acids from Xylopia extract sample	2		3.7100			
	acetone Phytic acid from Xylopia extract sample	2			4.1150		
	ethanol 40 Phytic acid extract from Xylopia sample	2			4.2600		
	n-haxane Phytic acid from Xylopia sample	2				6.2050	
	water Phytic acid from Xylopia sample	2					9.2450
	Sig.		1.000	1.000	.592	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	B	a
Tukey HSD[a]	acetone Phytic acid from Xylopia extract sample	2	.9150				
	ethanol absolute Phytic acid from Xylopia sample	2		1.4700			
	n-haxane Phytic acid from Xylopia sample	2			2.8750		
	methanol Phytic acids from Xylopia extract sample	2				3.4200	
	water Phytic acid from Xylopia sample	2					3.7150
	ethanol 40 Phytic acid extract from Xylopia sample	2					3.7250
	Sig.		1.000	1.000	1.000	1.000	.902

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	B	a
Tukey HSD[a]	ethanol absolute Phytic acid from Xylopia sample	2	4.3750					
	acetone Phytic acid from Xylopia extract sample	2		5.0300				
	methanol Phytic acids from Xylopia extract sample	2			7.1300			
	ethanol 40 Phytic acid extract from Xylopia sample	2				8.140		
	n-haxane Phytic acid from Xylopia sample	2					9.0800	
	water Phytic acid from Xylopia sample	2						12.9600
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA phytic acid of turmeric samples

		Sum of Squares	df	Mean Square	F	Sig.
liquidextract	Between Groups	96.710	5	19.342	257892.378	.000
	Within Groups	.000	6	.000		
	Total	96.710	11			
residue	Between Groups	14.041	5	2.808	56162.667	.000
	Within Groups	.000	6	.000		
	Total	14.041	11			
total extractable	Between Groups	124.241	5	24.848	119270.952	.000
	Within Groups	.001	6	.000		
	Total	124.242	11			

Homogeneous Subsets

Liquid extract

	dependent factor	**Subset for alpha = 0.05**					
		f	**e**	**d**	**c**	**b**	**a**
Tukey HSD[a]	ethanol absolute phytic acid from Turmeric sample	6.9250					
	n-haxane phytic acidfrom turmeric sample		7.495				
	acetone phytic acidfrom turmeric extract sample			9.5250			
	water phytic acidfrom turmeric sample				9.69		
	ethanol 40 phytic acidextract from turmeric sample					12.0	
	methanol phytic acid from turmeric extract sample						14.95
	Sig.	1.000	1.000	1.000	1.000	1.00	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	acetone phytic acidfrom turmeric extract sample	2	1.1				
	methanol phytic acid from turmeric extract sample	2		1.3450			
	n-haxane phytic acidfrom turmeric sample	2		1.3650			
	ethanol absolute phytic acid from Turmeric sample	2			1.7850		
	ethanol 40 phytic acidextract from turmeric sample	2				3.6	
	water phytic acidfrom turmeric sample	2					3.6750
	Sig.		1.000	.178	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	Subset for alpha = 0.05				
		f	e	D	c	b
Tukey HSD[a]	ethanol absolute phytic acid from Turmeric sample	8.7100				
	n-haxane phytic acidfrom turmeric sample		8.8600			

	acetone phytic acidfrom turmeric extract sample			10.6300		
	water phytic acidfrom turmeric sample				13.3700	
	methanol phytic acid from turmeric extract sample					16.29
	ethanol 40 phytic acidextract from turmeric sample					
	Sig.	1.000	1.000	1.000	1.000	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA FOR SAPONIN

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	13.752	5	2.750	55008.667	.000
	Within Groups	.000	6	.000		
	Total	13.752	11			
residue	Between Groups	.614	5	.123	2455.467	.000
	Within Groups	.000	6	.000		
	Total	.614	11			
total extractable	Between Groups	12.429	5	2.486	24857.933	.000
	Within Groups	.001	6	.000		
	Total	12.430	11			

Homogeneous Subsets

Liquid extract

	dependent factor	N	Subset for alpha = 0.05					
			f	e	D	c	b	a
Tukey HSD[a]	ethanol absolute Saponins from Xylopia sample	2	.2150					
	ethanol 40 Saponins extract from Xylopia sample	2		.5050				
	water Saponins from Xylopia sample	2			.8350			
	acetone Saponins from Xylopia extract sample	2				1.3550		
	n-haxane Saponins from Xylopia sample	2					1.4750	
	methanol Saponins from Xylopia extract sample	2						3.4950
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05				
			e	d	C	b	A
	acetone Saponins from Xylopia extract sample	2	.1550				
	methanol Saponins from Xylopia extract sample	2	.1750	.1750			
	ethanol absolute Saponins from Xylopia sample	2		.1950	.1950		
Tukey HSD[a]	n-haxane Saponins from Xylopia sample	2			.2050		
	ethanol 40 Saponins extract from Xylopia sample	2				.3850	
	water Saponins from Xylopia sample	2					.7950
	Sig.		.178	.178	.721	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	**N**	**Subset for alpha = 0.05**					
			f	**e**	**D**	**c**	**b**	**a**
	ethanol absolute Saponins from Xylopia sample	2	.4100					
	ethanol 40 Saponins extract from Xylopia sample	2		.8900				
	acetone Saponins from Xylopia extract sample	2			1.5100			
Tukey HSD[a]	water Saponins from Xylopia sample	2				1.6300		
	n-haxane Saponins from Xylopia sample	2					1.6800	
	methanol Saponins from Xylopia extract sample	2						3.6700
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	2.863	5	.573	11450.800	.000
Liquid extract	Within Groups	.000	6	.000		
	Total	2.863	11			
	Between Groups	.003	5	.001	12.400	.004
residue	Within Groups	.000	6	.000		
	Total	.003	11			
	Between Groups	2.830	5	.566	5660.800	.000
total extractable	Within Groups	.001	6	.000		
	Total	2.831	11			

Homogeneous Subsets

Liquid extract

	dependent factor	**N**	**Subset for alpha = 0.05**					
			f	**e**	**d**	**c**	**b**	**a**
	acetone Tannin from Xylopia extract sample	2	.5650					
	n-haxane Tannin from Xylopia sample	2		.8650				
	ethanol 40 Tannin extract from Xylopia sample	2			.9750			
Tukey HSD[a]	ethanol absolute Tannin from Xylopia sample	2				1.1950		
	water Tannin from Xylopia sample	2					1.2250	
	methanol Tannin from Xylopia extract sample	2						2.1350
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05	
			b	A
	ethanol absolute Tannin from Xylopia sample	2	.1150	
	methanol Tannin from Xylopia extract sample	2	.1150	
	acetone Tannin from Xylopia extract sample	2	.1150	
Tukey HSD[a]	n-haxane Tannin from Xylopia sample	2	.1350	.1350
	ethanol 40 Tannin extract from Xylopia sample	2		.1450
	water Tannin from Xylopia sample	2		.1550
	Sig.		.178	.178

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	acetone Tannin from Xylopia extract sample	2	.6800					
	n-haxane Tannin from Xylopia sample	2		1.00				
	ethanol 40 Tannin extract from Xylopia sample	2			1.120			
	ethanol absolute Tannin from Xylopia sample	2				1.3100		
	water Tannin from Xylopia sample	2					1.3800	
	methanol Tannin from Xylopia extract sample	2						2.2500
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA for tannin of turmeric

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	2.631	5	.526	10522.667	.000
	Within Groups	.000	6	.000		
	Total	2.631	11			
residue	Between Groups	.007	5	.001	27.200	.000
	Within Groups	.000	6	.000		
	Total	.007	11			
total extractable	Between Groups	2.583	5	.517	5166.933	.000
	Within Groups	.001	6	.000		
	Total	2.584	11			

Homogeneous Subsets

Liquid extract

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	n-haxane TANNINS from turmeric sample	2	.1950					
	water TANNINS from turmeric sample	2		.2250				
	ethanol absolute TANNINS from Turmeric sample	2			.6350			
	acetone TANNINS from turmeric extract sample	2				.7650		
	methanol alkaloid from turmeric extract sample	2					1.2050	
	ethanol 40 TANNINS extract from turmeric sample	2						1.4650
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05	
			B	a
Tukey HSD[a]	ethanol 40 TANNINS extract from turmeric sample	2	.1050	
	n-haxane TANNINS from turmeric sample	2	.1050	
	methanol alkaloid from turmeric extract sample	2	.1150	
	acetone TANNINS from turmeric extract sample	2	.1250	
	water TANNINS from turmeric sample	2	.1250	
	ethanol absolute TANNINS from Turmeric sample	2		.1750
	Sig.		.178	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	n-haxane TANNINS from turmeric sample	2	.3000					
	water TANNINS from turmeric sample	2		.3500				
	ethanol absolute TANNINS from Turmeric sample	2			.8100			
	acetone TANNINS from turmeric extract sample	2				.8900		
	methanol alkaloid from turmeric extract sample	2					1.3200	
	ethanol 40 TANNINS extract from turmeric sample	2						1.5700
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA for oxalate content

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	1.136	5	.227	4543.067	.000
	Within Groups	.000	6	.000		
	Total	1.136	11			
residue	Between Groups	.908	5	.182	3630.000	.000
	Within Groups	.000	6	.000		
	Total	.908	11			
total extractable	Between Groups	1.755	5	.351	2106.560	.000
	Within Groups	.001	6	.000		
	Total	1.756	11			

ANOVA for oxalate of turmeric samples

		Sum of Squares	df	Mean Square	F	Sig.
Liquid extract	Between Groups	1.806	5	.361	7224.667	.000
	Within Groups	.000	6	.000		
	Total	1.806	11			
residue	Between Groups	.141	5	.028	423.500	.000
	Within Groups	.000	6	.000		
	Total	.142	11			
total extractable	Between Groups	1.956	5	.391	3610.415	.000
	Within Groups	.001	6	.000		
	Total	1.956	11			

Homogeneous Subsets

Liquid extract

	dependent factor	N	Subset for alpha = 0.05					
			f	E	d	c	b	a
Tukey HSD[a]	acetone oxalate from turmeric extract sample	2	.4550					
	ethanol absolute alkloid from Turmeric sample	2		.5450				
	water oxalate from turmeric sample	2			.7250			
	methanol oxalate from turmeric extract sample	2				.8150		
	ethanol 40 oxalate extract from turmeric sample	2					1.085	
	n-haxane oxalate from turmeric sample	2						1.6150
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Residue

	dependent factor	N	Subset for alpha = 0.05				
			f	E	d	c	b
Tukey HSD[a]	ethanol 40 oxalate extract from turmeric sample	2	.2650				
	ethanol absolute alkloid from Turmeric sample	2		.3650			
	water oxalate from turmeric sample	2			.4100		
	acetone oxalate from turmeric extract sample	2				.4550	
	n-haxane oxalate from turmeric sample	2				.4750	
	methanol oxalate from turmeric extract sample	2					.6200

	Sig.		1.000	1.000	1.000	.271	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

total extractable

	dependent factor	N	Subset for alpha = 0.05				
			e	D	c	b	a
	ethanol absolute alkloid from Turmeric sample	2	.9100				
	acetone oxalate from turmeric extract sample	2	.9100				
	water oxalate from turmeric sample	2		1.1300			
Tukey HSD[a]	ethanol 40 oxalate extract from turmeric sample	2			1.3500		
	methanol oxalate from turmeric extract sample	2				1.4350	
	n-haxane oxalate from turmeric sample	2					2.0900
	Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

Homogeneous Subsets on product test on sensory properties of turmeric product

Colour

	dependent factor	N	Subset for alpha = 0.05	
			b	a
Tukey HSD[a]	3:1wheat grits encapsulated flavour product from turmeric	7	5.0000	
	2:1wheat grits encapsulated flavour product from turmeric	7	5.1429	5.1429
	2:1 yam grits encapsulated flavour product from turmeric	7	5.2857	5.2857
	3:1 yam grits encapsulated flavour product from turmeric	7	5.4286	5.4286
	powder turmeric flavour product	7	5.7143	5.7143
	solvent flavour extract from turmericr flavour product	7		6.2857
	Sig.		.506	.079

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Pungency

	dependent factor	N	Subset for alpha = 0.05
			a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from turmeric	7	5.0000
	2:1 yam grits encapsulated flavour product from turmeric	7	5.0000
	2:1wheat grits encapsulated flavour product from turmeric	7	5.2857
	3:1wheat grits encapsulated flavour product from turmeric	7	5.4286
	powder turmeric flavour product	7	6.0000
	solvent flavour extract from turmeric flavour product	7	6.0000
	Sig.		.390

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Sweetness

	dependent factor	N	Subset for alpha = 0.05	
			b	a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from turmeric	7	1.2857	
	2:1 yam grits encapsulated flavour product from turmeric	7	1.2857	
	2:1wheat grits encapsulated flavour product from turmeric	7	2.1429	
	3:1wheat grits encapsulated flavour product from turmeric	7		3.2857
	powder turmeric flavour product	7		3.5714
	solvent flavour extract from turmeric flavour product	7		4.0000
	Sig.		.061	.172

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Minty

	dependent factor	N	Subset for alpha = 0.05	
			b	a
	2:1wheat grits encapsulated flavour product from turmeric	7	.8571	
	3:1 yam grits encapsulated flavour product from turmeric	7	1.1429	
	2:1 yam grits encapsulated flavour product from turmeric	7	1.1429	
Tukey HSD[a]	solvent flavour extract from turmeric flavour product	7	1.5714	1.5714
	3:1wheat grits encapsulated flavour product from turmeric	7	1.5714	1.5714
	powder turmeric flavour product	7		2.1429
	Sig.		.247	.485

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Sweetness

	dependent factor	N	Subset for alpha = 0.05
			a
	3:1wheat grits encapsulated flavour product from turmeric	7	.2857
	3:1 yam grits encapsulated flavour product from turmeric	7	.2857
	2:1 yam grits encapsulated flavour product from turmeric	7	.4286
Tukey HSD[a]	powder turmeric flavour product	7	.8571
	solvent flavour extract from turmeric flavour product	7	.8571
	2:1wheat grits encapsulated flavour product from turmeric	7	1.1429
	Sig.		.211

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Bitterness

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	2:1 yam grits encapsulated flavour product from turmeric	7	1.4286			
	2:1wheat grits encapsulated flavour product from turmeric	7	1.5714			
	3:1 yam grits encapsulated flavour product from turmeric	7	1.8571	1.8571		
Tukey HSD[a]	3:1wheat grits encapsulated flavour product from turmeric	7		2.5714	2.5714	
	solvent flavour extract from turmeric flavour product	7			3.1429	3.1429
	powder turmeric flavour product	7				3.7143
	Sig.		.666	.151	.362	.362

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Harshness

	dependent factor	N	Subset for alpha = 0.05	
			b	a
Tukey HSD[a]	3:1wheat grits encapsulated flavour product from turmeric	7	1.5714	
	3:1 yam grits encapsulated flavour product from turmeric	7	1.5714	
	2:1 yam grits encapsulated flavour product from turmeric	7	1.5714	
	2:1wheat grits encapsulated flavour product from turmeric	7	1.7143	
	solvent flavour extract from turmeric flavour product	7	1.8571	
	powder turmeric flavour product	7		3.1429
	Sig.		.979	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

hotness

	dependent factor	N	Subset for alpha = 0.05	
			b	a
Tukey HSD[a]	2:1wheat grits encapsulated flavour product from turmeric	7	1.1429	
	3:1 yam grits encapsulated flavour product from turmeric	7	1.5714	
	solvent flavour extract from turmeric flavour product	7	1.7143	1.7143
	3:1wheat grits encapsulated flavour product from turmeric	7	1.7143	1.7143
	2:1 yam grits encapsulated flavour product from turmeric	7	1.7143	1.7143
	powder turmeric flavour product	7		2.5714
	Sig.		.377	.056

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

after-taste

	dependent factor	N	Subset for alpha = 0.05
			a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from turmeric	7	5.0000
	3:1wheat grits encapsulated flavour product from turmeric	7	5.4286
	2:1wheat grits encapsulated flavour product from turmeric	7	5.7143
	powder turmeric flavour product	7	5.8571
	solvent flavour extract from turmeric flavour product	7	5.8571
	2:1 yam grits encapsulated flavour product from turmeric	7	5.8571
	Sig.		.174

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

	overall acceptability		
	dependent factor	**N**	**Subset for alpha = 0.05**
			a
Tukey HSD[a]	2:1wheat grits encapsulated flavour product from turmeric	7	5.1429
	3:1wheat grits encapsulated flavour product from turmeric	7	5.2857
	2:1 yam grits encapsulated flavour product from turmeric	7	5.2857
	3:1 yam grits encapsulated flavour product from turmeric	7	6.0000
	powder turmeric flavour product	7	6.2857
	solvent flavour extract from turmeric flavour product	7	6.4286
	Sig.		.103

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Statistical analysis on product test on sensory properties of turmeric flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	7.619	5	1.524	2.630	.040
	Within Groups	20.857	36	.579		
	Total	28.476	41			
pungency	Between Groups	7.262	5	1.452	1.578	.191
	Within Groups	33.143	36	.921		
	Total	40.405	41			
sweetness	Between Groups	49.262	5	9.852	32.668	.000
	Within Groups	10.857	36	.302		
	Total	60.119	41			
minty	Between Groups	7.262	5	1.452	4.067	.005
	Within Groups	12.857	36	.357		
	Total	20.119	41			
sweet-taste	Between Groups	4.500	5	.900	1.890	.120
	Within Groups	17.143	36	.476		
	Total	21.643	41			
bitterness	Between Groups	29.619	5	5.924	20.733	.000
	Within Groups	10.286	36	.286		
	Total	39.905	41			
harshness	Between Groups	13.333	5	2.667	4.732	.002
	Within Groups	20.286	36	.563		
	Total	33.619	41			
hotness	Between Groups	7.548	5	1.510	5.141	.001
	Within Groups	10.571	36	.294		
	Total	18.119	41			
after-taste	Between Groups	4.190	5	.838	1.920	.115
	Within Groups	15.714	36	.437		
	Total	19.905	41			
overall acceptability	Between Groups	11.262	5	2.252	2.810	.030
	Within Groups	28.857	36	.802		
	Total	40.119	41			

Homogeneous Subsets on product test on sensory properties of *Xylopia aethiopica* flavour products

colour

	dependent factor	N	Subset for alpha = 0.05
			a
Tukey HSD[a]	powder *Xylopia aethiopica* flavour product	7	5.2857
	solvent flavour extract from *Xylopia aethiopica* flavour product	7	5.4286
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	5.5714
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	5.8571
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	6.0000
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	6.2857
	Sig.		.453

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

pungency of flavour

	dependent factor	N	Subset for alpha = 0.05		
			c	b	a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	4.4286		
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	4.7143		
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7		5.7143	
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7		5.7143	
	powder *Xylopia aethiopica* flavour product	7		6.2857	6.2857
	solvent flavour extract from *Xylopia aethiopica* flavour product	7			6.7143
	Sig.		.923	.392	.691

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

sweetness

	dependent factor	N	Subset for alpha = 0.05
			a
Tukey HSD[a]	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	1.0000
	powder *Xylopia aethiopica* flavour product	7	1.7143
	solvent flavour extract from *Xylopia aethiopica* flavour product	7	1.8571
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	1.8571
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	1.8571
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	2.0000
	Sig.		.148

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

minty

	dependent factor	N	Subset for alpha = 0.05
			a
Tukey HSD[a]	solvent flavour extract from *Xylopia aethiopica* flavour product	7	.8571
	powder *Xylopia aethiopica* flavour product	7	1.0000
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	1.0000
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	1.0000
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	1.1429
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	1.1429
	Sig.		.989

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

sweetness

	dependent factor	**N**	**Subset for alpha = 0.05**
			a
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	.7143
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	.8571
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	1.0000
Tukey HSD[a]	solvent flavour extract from *Xylopia aethiopica* flavour product	7	1.1429
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	1.2857
	powder *Xylopia aethiopica* flavour product	7	1.4286
	Sig.		.542

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

bitterness

	dependent factor	**N**	**Subset for alpha = 0.05**	
			b	**a**
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	3.5714	
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	4.0000	
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	4.1429	
Tukey HSD[a]	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	4.4286	
	powder *Xylopia aethiopica* flavour product	7		5.8571
	solvent flavour extract from *Xylopia aethiopica* flavour product	7		6.0000
	Sig.		.314	.999

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

harshness

	dependent factor	N	Subset for alpha = 0.05
			a
Tukey HSD[a]	powder *Xylopia aethiopica* flavour product	7	1.0000
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	1.0000
	solvent flavour extract from *Xylopia aethiopica* flavour product	7	1.1429
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	1.1429
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	1.2857
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	2.0000
	Sig.		.284

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

hotness

	dependent factor	N	Subset for alpha = 0.05		
			c	b	a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	3.1429		
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	3.7143	3.7143	
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	3.7143	3.7143	
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7		4.2857	
	powder *Xylopia aethiopica* flavour product	7			5.7143
	solvent flavour extract from *Xylopia aethiopica* flavour product	7			5.8571
	Sig.		.644	.644	.999

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

after-taste

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	3.5714			
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	4.2857	4.2857		
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	4.7143	4.7143	4.7143	
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7		5.1429	5.1429	5.1429
	solvent flavour extract from *Xylopia aethiopica* flavour product	7			5.8571	5.8571
	powder *Xylopia aethiopica* flavour product	7				6.0000
	Sig.		.096	.342	.096	.342

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

	overall acceptability			
	dependent factor	**N**	**Subset for alpha = 0.05**	
			b	**a**
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	4.4286	
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	7	5.1429	5.1429
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7	5.2857	5.2857
Tukey HSD[a]	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	7		5.7143
	powder *Xylopia aethiopica* flavour product	7		5.8571
	solvent flavour extract from *Xylopia aethiopica* flavour product	7		6.1429
	Sig.		.314	.169

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Statistical analysis product test on sensory properties of *Xylopia aethiopica* flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	4.976	5	.995	.965	.452
	Within Groups	37.143	36	1.032		
	Total	42.119	41			
pungency of flavour	Between Groups	27.262	5	5.452	18.079	.000
	Within Groups	10.857	36	.302		
	Total	38.119	41			
sweetness	Between Groups	4.571	5	.914	1.646	.173
	Within Groups	20.000	36	.556		
	Total	24.571	41			
minty	Between Groups	.405	5	.081	.110	.989
	Within Groups	26.571	36	.738		
	Total	26.976	41			
sweet-taste	Between Groups	2.500	5	.500	.808	.552
	Within Groups	22.286	36	.619		
	Total	24.786	41			
bitterness	Between Groups	36.190	5	7.238	12.324	.000
	Within Groups	21.143	36	.587		
	Total	57.333	41			
harshness	Between Groups	4.976	5	.995	1.320	.278
	Within Groups	27.143	36	.754		
	Total	32.119	41			
hotness	Between Groups	44.690	5	8.938	18.462	.000
	Within Groups	17.429	36	.484		
	Total	62.119	41			
after-taste	Between Groups	30.500	5	6.100	9.854	.000
	Within Groups	22.286	36	.619		
	Total	52.786	41			
overall acceptability	Between Groups	13.143	5	2.629	4.476	.003
	Within Groups	21.143	36	.587		
	Total	34.286	41			

Statistical analysis on consumers' preference on sensory properties of *Xylopia aethiopica* flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	17.742	5	3.548	6.106	.000
	Within Groups	66.250	114	.581		
	Total	83.992	119			
pungency of flavour	Between Groups	40.367	5	8.073	22.838	.000
	Within Groups	40.300	114	.354		
	Total	80.667	119			
aroma	Between Groups	38.900	5	7.780	20.203	.000
	Within Groups	43.900	114	.385		
	Total	82.800	119			
overall acceptability	Between Groups	58.967	5	11.793	32.791	.000
	Within Groups	41.000	114	.360		
	Total	99.967	119			

Homogeneous Subsets on sensory properties *Xylopia aethiopica* flavour products

colour

	dependent factor	N	Subset for alpha = 0.05	
			b	a
Tukey HSD[a]	powder *Xylopia aethiopica* flavour product	20	5.1000	
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20		5.9000
	solvent flavour extract from *Xylopia aethiopica* flavour product	20		5.9500
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20		6.0500
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20		6.1500
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20		6.3000
	Sig.		1.000	.561

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

pungency of flavour

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20	4.6500			
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20	4.7500			
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20	5.0000	5.0000		
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20		5.5000	5.5000	
	powder *Xylopia aethiopica* flavour product	20			5.9000	5.9000
	solvent flavour extract from *Xylopia aethiopica* flavour product	20				6.2000
	Sig.		.431	.092	.281	.603

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Aroma

	dependent factor	N	Subset for alpha = 0.05		
			c	b	a
Tukey HSD[a]	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20	4.5500		
	solvent flavour extract from *Xylopia aethiopica* flavour product	20	4.8000		
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20		5.4500	
	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20		5.5000	
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20		6.0000	6.0000
	powder *Xylopia aethiopica* flavour product	20			6.1000
	Sig.		.799	.064	.996

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

overall acceptability

	dependent factor	N	Subset for alpha = 0.05		
			c	b	a
Tukey HSD[a]	3:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20	4.5500		
	3:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20	4.6000		
	2:1wheat grits encapsulated flavour product from *Xylopia aethiopica*	20		5.6500	
	2:1 yam grits encapsulated flavour product from *Xylopia aethiopica*	20		5.8000	5.8000
	powder *Xylopia aethiopica* flavour product	20			6.2500
	solvent flavour extract from *Xylopia aethiopica* flavour product	20			6.2500
	Sig.		1.000	.969	.175

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Statistic on concentration of Curcumin during storage of turmeric spice flavour samples

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
TMRPB	Between Groups	395.408	8	49.426	206899.785	.000
	Within Groups	.002	9	.000		
	Total	395.411	17			
TMREB	Between Groups	57.793	8	7.224	21316.943	.000
	Within Groups	.003	9	.000		
	Total	57.796	17			
TMRWC1	Between Groups	9.668	8	1.208	5438.137	.000
	Within Groups	.002	9	.000		
	Total	9.670	17			
TMRWC2	Between Groups	11.735	8	1.467	6140.169	.000
	Within Groups	.002	9	.000		
	Total	11.737	17			
TMRYC1	Between Groups	42.305	8	5.288	19830.656	.000
	Within Groups	.002	9	.000		
	Total	42.308	17			
TMRYC2	Between Groups	17.095	8	2.137	12019.719	.000
	Within Groups	.002	9	.000		
	Total	17.096	17			

Homogeneous Subsets

TMREB (Turmeric 40% ethanol extract)

	Dependent factor	N	Subset for alpha = 0.05						
			g	F	E	d	c	b	a
Tukey HSD[a]	6-Months storage	2	5.6500						
	5-Months storage	2		6.9300					
	4-Months storage	2			8.0350				
	3-Months storage	2				8.4650			
	2-Months storage	2					9.8950		
	6WKS storage	2						10.6300	
	4WKS storage	2						10.6850	10.6850
	2WKS storage	2							10.7100
	Zero hr storage	2							10.7350
	Sig.		1.000	1.000	1.000	1.000	1.000	.184	.261

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

TMRPB (Turmeric powder)

	Dependent factor	N	Subset for alpha = 0.05							
			h	g	f	e	d	c	b	a
Tukey HSD[a]	6-Months storage	2	39.6700							
	5-Months storage	2		42.0200						
	4-Months storage	2			46.5800					
	3-Months storage	2				47.1750				
	2-Months storage	2					50.0600			
	6WKS storage	2						50.4900		
	4WKS storage	2							53.0850	
	2WKS storage	2								53.2150
	Zero hr storage	2								53.2700
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	.086

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

TMRWC1 (Turmeric wheat grit encapsulation 1:2 spice extract to carrier)

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	D	c	b	a
Tukey HSD[a]	6-Months storage	2	3.0750								
	5-Months storage	2		3.180							
	4-Months storage	2			3.6650						
	3-Months storage	2				3.9450					
	2-Months storage	2					4.3000				
	6WKS storage	2						4.3950			
	4WKS storage	2							4.6500		
	2WKS storage	2								5.0500	
	Zero hr storage	2									5.3000
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

TMRWC2 (Turmeric wheat grit encapsulation 1:3 spice extract to carrier)

	Dependent factor	N	Subset for alpha = 0.05						
			g	f	e	d	c	b	a
Tukey HSD[a]	6-Months storage	2	2.0250						
	5-Months storage	2		2.7650					
	4-Months storage	2			3.0900				
	3-Months storage	2				3.6700			
	2-Months storage	2					4.1000		
	4WKS storage	2						4.3100	
	6WKS storage	2						4.3100	
	2WKS storage	2						4.3600	4.3600
	Zero hr storage	2							4.3950
	Sig.		1.000	1.000	1.000	1.000	1.000	.133	.442

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

TMRYC1 (Turmeric yam grit encapsulation 1:2 spice extract to carrier)

	Dependent factor	N	Subset for alpha = 0.05						
			g	f	e	d	c	b	a
Tukey HSD[a]	6-Months storage	2	5.4650						
	5-Months storage	2		6.0450					
	4-Months storage	2			6.5900				
	3-Months storage	2				7.6850			
	2-Months storage	2					9.1000		
	6WKS storage	2						9.1900	
	4WKS storage	2							9.4100
	2WKS storage	2							9.4400
	Zero hr storage	2							9.4650
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	.111

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

TMRYC2 (Turmeric wheat grit encapsulation 1:3 spice extract to carrier)

	Dependent factor	N	Subset for alpha = 0.05						
			g	f	E	d	c	b	a
Tukey HSD[a]	6-Months storage	2	2.5700						
	5-Months storage	2		3.4600					
	4-Months storage	2			4.0150				
	3-Months storage	2				4.1200			
	6WKS storage	2					5.0050		
	2-Months storage	2					5.0050		
	4WKS storage	2						5.2700	
	2WKS storage	2							5.5400
	Zero hr storage	2							5.5850
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	.110

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Statistic on common predominant compounds in *Xylopia aethiopica* flavour extract

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Beta-pienene	Between Groups	793.153	5	158.631	705024.837	.000
	Within Groups	.001	6	.000		
	Total	793.154	11			
Beta-phellandrene	Between Groups	664.090	5	132.818	388735.868	.000
	Within Groups	.002	6	.000		
	Total	664.092	11			
androgaphile	Between Groups	71.075	5	14.215	65607.292	.000
	Within Groups	.001	6	.000		
	Total	71.076	11			

Homogeneous Subsets

Beta-pienene

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
Tukey HSD[a]	methanol *Xylopia aethiopica* flavour extract	2	.0000			
	hexane *Xylopia aethiopica* flavour extract	2	.0000			
	aqueous *Xylopia aethiopica* flavour extract	2	.0000			
	acetone *Xylopia aethiopica* flavour extract	2		8.1250		
	ethanol absolute *Xylopia aethiopica* flavour extract	2			15.4950	
	ethanol 40% *Xylopia aethiopica* flavour extract	2				20.2950
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Beta-phellandrene

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	aqueous *Xylopia aethiopica* flavour extract	2	4.2850					
	acetone *Xylopia aethiopica* flavour extract	2		7.3950				
	hexane *Xylopia aethiopica* flavour extract	2			12.1000			
	ethanol 40% *Xylopia aethiopica* flavour extract	2				20.2950		
	methanol *Xylopia aethiopica* flavour extract	2					21.5550	
	ethanol absolute *Xylopia aethiopica* flavour extract	2						23.9450
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

andrographile

	Dependent factor	N	Subset for alpha = 0.05				
			e	**d**	**c**	**b**	**a**
Tukey HSD[a]	aqueous *Xylopia aethiopica* flavour extract	2	.0000				
	methanol *Xylopia aethiopica* flavour extract	2		4.2050			
	hexane *Xylopia aethiopica* flavour extract	2		4.2050			
	ethanol absolute *Xylopia aethiopica* flavour extract	2			5.4300		
	ethanol 40% *Xylopia aethiopica* flavour extract	2				7.0800	
	acetone *Xylopia aethiopica* flavour extract	2					7.3000
	Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Statistical on concentration beta-phellandrene of xylopia flavour extract samples

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
XPPB	Between Groups	70.702	8	8.838	24473.908	.000
	Within Groups	.003	9	.000		
	Total	70.706	17			
XPEB	Between Groups	49.772	8	6.222	116.206	.000
	Within Groups	.482	9	.054		
	Total	50.254	17			
XPEWC1	Between Groups	70.406	8	8.801	24371.369	.000
	Within Groups	.003	9	.000		
	Total	70.409	17			
XPEWC2	Between Groups	93.613	8	11.702	39741.245	.000
	Within Groups	.003	9	.000		
	Total	93.615	17			
XPEYC1	Between Groups	177.762	8	22.220	95229.482	.000
	Within Groups	.002	9	.000		
	Total	177.764	17			
XPEYC2	Between Groups	199.956	8	24.995	61630.329	.000
	Within Groups	.004	9	.000		
	Total	199.960	17			

Homogeneous Subsets

XPPB (*Xylopia aethiopica* powder)

	dependent factor	N	Subset for alpha = 0.05							
			h	g	F	E	d	c	b	a
Tukey HSD[a]	6months storage	2	14.2350							
	5months storage	2		14.7750						
	4months storage	2			15.0200					
	3months storage	2				15.9100				
	2months storage	2					17.4750			
	6wks storage	2						18.0100		
	4wks storage	2							19.1700	
	2wks storage	2								19.4050
	Zero Hrs	2								19.4350
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	.796

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

XPEB (40% ethanol *Xylopia aethiopica* extract)

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
Tukey HSD[a]	6months storage	2	19.5650			
	5months storage	2	19.9700	19.9700		
	4months storage	2	20.1150	20.1150		
	3months storage	2		20.5300		
	2months storage	2			21.4950	
	6wks storage	2				23.0250
	4wks storage	2				23.1900
	2wks storage	2				23.8050
	Zero Hrs	2				23.9400
	Sig.		.390	.372	1.000	.050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

XPEWC1 (*Xylopia aethiopica* wheat grit encapsulation 1:2 spice extract to carrier)

	dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	6months storage	2	13.5600								
	5months storage	2		14.9450							
	4months storage	2			15.8400						
	3months storage	2				17.0050					
	2months storage	2					17.9750				
	6wks storage	2						18.3500			
	4wks storage	2							19.0000		
	2wks storage	2								19.1350	
	Zero Hrs	2									19.6750
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

XPEWC2 (*Xylopia aethiopica* wheat grit encapsulation 1:3 spice extract to carrier)

	dependent factor	N	Subset for alpha = 0.05								
			i	h	g	F	e	d	c	b	a
Tukey HSD[a]	6months storage	2	8.9700								
	5months storage	2		9.8100							
	4months storage	2			10.6650						
	3months storage	2				12.8400					
	2months storage	2					13.1450				
	6wks storage	2						13.8750			
	4wks storage	2							14.0750		
	Zero Hrs	2								15.1950	
	2wks storage	2									15.9700
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

XPEYC1 (*Xylopia aethiopica* yam grit encapsulation 1:2 spice extract to carrier)

	dependent factor	N	Subset for alpha = 0.05							
			h	G	f	E	d	c	b	a
Tukey HSD[a]	6months storage	2	9.6650							
	5months storage	2		9.7750						
	4months storage	2			10.4200					
	3months storage	2				13.7500				
	2months storage	2					14.8450			
	4wks storage	2						16.6550		
	6wks storage	2						16.6550		
	2wks storage	2							17.0200	
	Zero Hrs	2								17.9350
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

XPEYC2 (*Xylopia aethiopica* yam grit encapsulation 1:3 spice extract to carrier)

	dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
	6months storage	2	7.0450								
	5months storage	2		8.1300							
	4months storage	2			9.3700						
	3months storage	2				11.295					
Tukey	2months storage	2					12.6700				
HSD[a]	6wks storage	2						14.5950			
	4wks storage	2							15.6250		
	2wks storage	2								16.040	
	Zero Hrs	2									16.1650
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.