EFFECT OF PROCESSING AND STORAGE ON THE RETENTION OF FLAVOUR COMPOUNDS IN *Piper nigrum* L. AND *Aframomum danielli* SPICE SAMPLES

A Ph.D. RESEARCH THESIS

BY

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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)

JULY 2018

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CERTIFICATION

I certify that this work "Evaluation of processing and storage on the retention of flavour compounds in *Piper nigrum* L and *Aframomum danielli* seeds spice samples was carried out by Adedokun, Isiaka Ishola, (Registration No: 20104738928), 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 research work is dedicated to God Almighty for His numerous favours, guide, love and protection towards me, my beloved wife Confidence Janet Adedokun, my children Azeez, Azizat and Zainab and my parents.

ACKNOWLEDGEMENTS

It is my pleasure to acknowledge the efforts, support of whose positive criticisms led to successes and gainful results achieved in this work. Most important are Prof (Mrs) A.Uzomah, Prof. C. N Ubbaonu and Prof (Mrs) J. N Nwosu for their relentless, painstaking supervision and contribution to the successful completion of this work. I extend my profound gratitude to Head of Department, Prof. (Mrs) N. C. Ihediohanma, Dean School of Engineering and Engineering Technology Engr. Prof. G.I Nwandikom and Associate Dean, Engr. Dr. M. S Nwakaudu. I also appreciate the support, constructive criticisms and suggestions of Prof. (Mrs) N. C Onuegbu, Prof. (Mrs) E. U Onyeka, Dr C. N Osuji, Engr. Dr. (Mrs) Omeire, Dr. (Mrs) N.O Kabuo, Dr. C.I Owuamanam, Dr. C. C Ogueke, Dr. J. G. Iwouno. I recognized the efforts of technologists Mr. C.E. Ndupu, Mrs Esther C. Utuagbaigwe, Udodiri Ufomadu, and Miss Kasarachi Ehiogu. Special thanks to my big brothers, great mentors and erudite academic scholars Prof. T. O Shittu and Prof. L. O Sanni of Department of Food Science and Technology, Federal University of Agriculture Abeokuta, Ogun State for their assistance. Also, I appreciated the effort of my colleagues Mr. A Ukozor and Ibe Chidiuto for good work. Furthermore, I thank Mr. Wason Ibrahim and Ekemenye Augusta for their assistance during the laboratory work.

On a special note I want to thank my lovely wife Chief (Mrs) Confidence Janet Oguguo Ajike Fatimah Adedokun who is not just a wife to me alone but also a good sister, mother for her patient, love, kindness and support during thin and thick of my work and toward successful completion of this work. Without God and you this dreamed work may not have been realized. To my beloved children Abdul Azeez Ikechukwu Promise, Hajia Azizat and Zainab I say thanks for their patient and courage. I extend my gratitude to my friend and sister Miss Ibeh Ukamaka Vivian and my God mother Mrs F.M Chukwudire, my brother Dr. Lukman Adegoke Agbabiaka and his family for their support. I say thank you to my mother Princess Adewuni S. Adedokun

and my father Mr. Adedokun A. Ojo, my uncles Mathew Ojo, Solomon Ojo and Adenrele Lateef Ijiola for their prayers and encouragement. To my siblings; big sister Modinat Toyosi, Ibrahim, Fatimah, Muideen, Ismail and Yakub I say thanks and God bless. A big thanks to my friend and brother Mr. Kabir Oyekan, I will not fail to recognize my in-laws Mrs Nina Mbata, Mr. Johnbosco Mbata, Gloria Mbata, Victor Mbata and also acknowledged the moral support of Mrs Chidinma Okegbade and her husband Olabode. Finally, all praise goes to Almighty Allah and His Prophet Mohammed (SAW) Amen.

TABLE OF CONTENTS **PAGE**

LIST OF TABLES **PAGE**

LIST OF FIGURES **PAGE**

LIST OF PLATES **PAGE**

ABSTRACT

Effects of processing and storage on the retention of flavour compounds in *Piper nigrum* L ('black pepper') and *Aframomum danielli* seeds spice samples was studied. Mature berries of *Piper nigrum* L. and pods of *Aframomum danielli* were procured from point of harvest and subjected to post-harvest treatment and dried respectively. Dried spice seeds were pulverized into powder prior to extraction of flavour compounds with six different solvents. The solvent extraction process was at 1:5w/v ratio of spice powder to solvent and the extract was vaporized to obtain 1:1w/v flavour extract from each solvent for preliminary investigation of flavour constituents. Three processing methods were applied for each spice: (i) pulverized sample, (ii) extract with aqueous 40% ethanol sample and (iii) ethanol extract-coated on suitable carriers (potato grits and rice grits). The pulverized and 40% ethanol extract were stored in amber coloured glass bottles while extract on suitable carriers were packaged in high density polyethylene bags. Each sample was stored at ambient temperature ($30\pm 3^{o}C$) for six months and analyzed at intervals. GC-MS was used to determine the chemical constituents and to monitor the flavour profile during storage. The results of moisture content, specific gravity, phyto-chemicals and flavour compounds in solvent extract samples were significantly ($p<0.05$) varied. Specific gravity of absolute ethanol flavour extract was 0.784 in *Piper nigrum* L. and 0.612 in *Aframomum danielli* and were significantly higher ($p<0.05$) than other solvent samples. In *Piper nigrum* L. n-hexane flavour extract showed 86 GC-MS peaks and 30 Major Flavour Principles (MFPs). This was followed by acetone extract with 78 GC-MS peaks and 26 MFPs. GC-MS peaks of 56 with 18 MFPs in absolute ethanol, 59 GC-MS peaks with 25MFPs in 40% ethanol extract, 42 GC-MS peaks with 20 MFPs in methanol and water extract had the least 5 peaks and 3MFPs. Piperine was the high major flavour principles (MFPs) in *Piper nigrum* L. flavour and the values ranged from 2.43% in hexane extract to 17.50% in ethanol extract. However 10 GC-MS peaks and 8 MFPs were found in absolute ethanol extract of *Aframomum danielli*, 9 GC-MS peaks and 7 MFPs in 40% ethanol extract sample, 22 GC-MS peaks and 6 MFPs in methanol sample, 28 GC-MS peaks and 7 MFPs in acetone flavour extract, 84 GC-MS peaks and 12 MFPs in hexane flavour extract and none in water extract of *Aframomum danielli*. The highest major flavour principle 'MFPs' of *Aframomum danielli* solvent extracts was eucalyptol with values ranging from 20.53% to 62.05%. Percentage retention (PR) of core volatile flavour principles in extract coated in suitable carriers in both spices reduced when the ratios of coating materials (rice grits and potato flour) increased in the encapsulated samples. Furthermore, the percentage retention of core volatile flavour principles in spice products samples studied varied at 6months storage. However 40% ethanol extract of *Piper nigrum* product sample (PP-EB) had the 91.31% piperine retention higher than other spice products samples in *Piper nigrum* while pulverized of *Aframomum danielli* product sample (AFD-PB) showed 83.46% highest eucalyptol retention among *Aframomum danielli* spice products. Sensory evaluation identified 5 and 8 sensory attributes of *Piper nigrum* and *Aframomum danielli* spice flavour product samples respectively. Pearson correlation value of 0.927 in *Piper nigrum* and 0.671 in *Aframomum danielli* for flavour pungency was the highest among other sensory attributes to overall acceptability. The total viable counts of fungi and bacteria were higher in raw spices than processed powder. Initial bacterial load was 1.21×10^{9}cfu/g in black pepper berries and 7.8×10^{8}cfu/g in *Aframomum danielli* raw seeds. These reduced to 0.9×10^{3}cfu/g and 1.21×10^{3}cfu/g pulverized spice powder.

Keywords: *Aframomum danielli,* eucalyptol, flavour, flavour retention, *Piper nigrum* L., phyto-chemical, piperine, pungency, potato grits and rice grits.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Spices can be defined as "any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms that contribute to flavour, whose primary function in food is seasoning rather than nutrition and that may contribute piquancy (spiciness) to the foods and beverages". In the most generally accepted sense, spices are plants or part of plants valued for their aromatic property and are often grown and harvested for these unique properties (Peter, 2004). 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. The term 'herb' is used as a subset of spice and refers to plants with aromatic leaves (Weiss, 2002).

The history of herbs and spices are as long as the history of mankind. People have used these plants since earliest times. No other commodity has played a more pivotal role in the development of modern civilization as spices (Brown, 1995). Spices have tremendous importance in the way we live, as ingredients in food, alcoholic beverages, medicine, perfumery, cosmetics, and coloring and also as garden plants. Spices are used in foods to impart flavour, pungency and color (Peter, 2004). They are also used to make food and confectionery more appetizing and palatable (Peter, 2004). Spices improve the palatability and the appeal of dull diets. They stimulate salivation and therefore promote digestion (Louise, 2002). Spices also have antioxidant, antimicrobial, pharmaceutical and nutritional properties. They are often referred to as food accessories or adjuncts because of their ability to stimulate appetite and increase the flow of gastric juice (Hirasa and Takemasa, 1998). Each spice has a unique flavour which is derived

from compounds known as phyto-chemicals (Srinivasa *et al*., 2004). Spices are rich in bioactive constituents especially in phenolics and they are utilized in several forms in human diets: as whole spices, as ground spices, or isolates from their extract (Srinivasa *et al*., 2004).

The most important spices traditionally traded throughout the world are products of tropical environments (Oboh and Akindahunsi, 2004). There are about 40 to 50 spices of global economic and culinary importance (Green and Schleichner, 1999). In Nigeria and other tropical regions of African, indigenous spices such as black pepper (*Piper nigrum*) one of the top three indigenous spices), *Aframomum danielli* and others, have not been utilized to their potentials as raw material in conventional food processing. Nowadays, there is increasing consumption of processed foods and ready to eat dishes, which often rely on food flavour, yet, indigenously grown spices in Nigeria are under-utilized.

Piper nigrum (Black pepper), the king of spices, is one of the oldest and the most popular spices in the world. It is a perennial, climbing vine and the hotly pungent spice made from its berries is one of the earliest spices known and was mentioned as far back as 1000 BC in ancient Sanskrit texts (Nair, 2004). It is widely cultivated in Nigeria. It is used as an important component of many recipes and to flavour foods. Many grades of these peppers are recognized in the spice trade. The major world producing countries include; Brazil, India, and Mexico and production ranged from about 3,053–29,034 kg/ha (2,720–25,860 lb/ac) (Weiss, 2002). In Africa Nigeria, Ghana, Cameroon countries are major producers of black pepper at high production tonnes that can meet domestic and international trade, yet, it is still under-utilized. In Nigeria, black pepper is found useful in the preparation of indigenous ethnic cuisines (in South East, South-South and South –West regions) or used in preparation of hot concoctions for women that just delivered a baby. Its use as spice product or raw material in food industry has not been adequately exploited.

Aframomum danielli belongs to the genus *Aframomum*, of the family *zingiberace.* It is a perennial succulent plant with yellow stamen. The fruit is initially green and later becomes reddish when ripen. It is commonly known as bastered meleguta in English language 'Atare Oburo'(Yoruba), Upoe' (Igbo) and 'Urima' (Edo) where they are mostly consumed (Adegoke and Skura, 1994). The fruits have has seeds that are used in a very small quantities in ground form as flavouring agent for traditional dishes and pepper- soups. It has been reported to have a broad spectrum of anti bacterial properties, mineral elements, and amino acids (Adegoke and Skura, 1994). *Aframomum danielli* has been reported to be a good antioxidant and have good inhibitory properties on some bacteria (Fasoyiro *et al* 2006; Martins *et al* 2001; Adegoke *et al.,* 1998) and on food spoilage yeasts (Adegoke *et al.* 2002; Adegoke *et al.,* 2000). In spite this importance of the spice *'Aframomum danielli'* in Nigeria, the crop is still under-utilized and has neither found any place in Nigerian markets or gained international trade recognition as spice product nor as raw material in food industry.

Nigerians demand food products with more natural flavour and increased safety and shelf-life. These demands have increased the importance and role of indigenous spices in many ethnic cuisines, the proof for this can be seen in most of 'Quick Service Food Industry' or 'Fast-food Restaurants in our society. The food industry and researchers have been very pro-active in promoting interest in exotic spice foods production in Nigeria as a promising sector; however, there is high importation of spice and spicy product flooding the markets in Nigeria. This is due to a number of factors which includes variation in standard and specification; inadequate packaging system and poor handling by indigenous retailers; decline in annual production by the farmers due to low income, lack of government attention and policy that favor spice production when compared with the government interest in cocoa, soybean, wheat, rice, cassava etc. This in-

turn has adverse effect on gross domestic product (GDP) aspect of the economy and possibility of junk products in the country. There is therefore the need to develop additional value-chain for indigenous spices such as black pepper and *Aframomum danielli* in order to salvage them from being endangered. This was done through adequate extraction method, improved packaging and shelf-life stability of spice bioactive flavour components for a new spice product that will meet indigenous demand and international trade needs.

1.2 STATEMENT OF PROBLEM

Spices and vegetable seasoning can be heavily contaminated with microorganisms because of the poor environment of post-harvest handling and processing conditions under which they are produced and distributed or for sales. The microbial load and high storage temperature can result in significantly loss of flavour, and the pungency of the spice. Inadequate post-harvest operation and quality specification are often the limiting factors in the establishment of a profitable enterprise based around spices, spice products and essential oils in developing countries Nigeria inclusive.

In spite of the abundant production of indigenous spices, processors in Nigeria have not been able to meet international trade importance and current global demand for spice products. Spice and spice products currently have high demand in food production and production of traditional cuisines. But Nigeria is still behind in developments and production of new products from spices either as a whole, single or blend product of international grade.

Nigeria focus on achievement of vision 20:2020 through Millennium Development Goals (MDGs) programmes and government policy has not been extended towards the promotion and utilization of indigenous spices as raw materials for the industries; rather great attention has been

focused mainly on root, tubers, cereals and legumes. This makes the farmers not to produce enough that will keep harvesters busy. The indigenous processors probably are lacking or unaware of technical knowledge on processing and storage methods. Thus cannot guarantee her product quality status as capable of meeting the demands of modern Nigerian home and much more, the foreign market. The exporter is sometimes embarrassed, because their product lacks the standard specifications that can give it the identity needed for an international market.

1.3 OBJECTIVES OF STUDY

The main objective of this work evaluated the effect of processing methods on the retention of flavour compounds in *Piper nigrum* L. (black pepper) and *Aframomum danielli* seeds extracts.

Specific objectives of the work were:

a. to investigate the effect of different extraction method(s) on the intensity of flavour components of the spices.
b. to investigate the effect of extraction methods on phytochemical components of the spices
c. to determine the retention of extracted spice flavour compounds on carriers (potato flour and rice grits)
d. to determine the effect of storage time on the flavour compounds retention on the spice samples.
e. to determine the sensory characteristics and consumer preference of different spice product samples.
f. to determine the microflora associated with the spices from harvest to the pulverized form.

1.4 JUSTIFICATION OF STUDY

This work will provide information on quality parameters and specifications to be monitored during the harvesting, processing and packaging of indigenous spices. The information developed from the indigenous spices (black peppers and *Aframomum danielli*) will create a wider range of interest on consumer preferences and reduce dependent on foreign flavour spice products. It is expected that the results from this research work will provide scientific information that can help many cottage producers to develop increasing interest on spices as raw materials in their operation. And this will consequently reduce importation of many spice based products in the country. The findings from the research will create opportunity and set standards for food industry of interest spice flavour production and indigenous producers who trade on spices. The research findings, hopefully, will also create value-chain-addition on Nigerian indigenous spices and attract the attention of government through concern agencies such as Ministries of Science and Technology as well as Commerce to promote spice trade across the national boundary through export promotion council of Nigeria.

1.5 SCOPE OF STUDY

This work focused mainly on examining the effect of post-harvest treatments on the microbial quality of the indigenous spices right from harvesting stage. Effect of extraction process, separation and concentration methods on the bioactive phytochemical properties of spices was determined. The impact of suitable edible carriers, improved packaging materials and storage on the threshold profile, stability and retention of flavour compounds was studied and evaluated. Product and consumer-acceptability studies were done to determine the consumer preference and food choice for the spice products through sensory evaluation.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORY OF BLACK PEPPER

Pepper family (*Piperaceae*) with family name of *Piperaceae* is derived from *piper*, the Latin word for pepper (Nair, 2004). The king of spices commonly called Black pepper (Plate 2.1) *Piper nigrum* with indigenous names of 'Iyere (Yoruba), Uziza (Igbo) is one of the most popular spices in the world. It is a perennial, climbing vine indigenous to the Malabar Coast of India. The hotly pungent spice made from its berries is one of the earliest spices known and was mentioned as far back as 1000 BC in ancient Sanskrit texts (Nair, 2004). Widely cultivated in the tropics of Southeast Asia, it became an important article of overland trade between India and Europe. It became a medium of exchange, and tributes were levied in black pepper in ancient Greece and Rome. In the middle Ages the Venetian and the Genoese became the main distributors, their virtual monopoly of the trade helping to instigate the search for an eastern sea route. The name pepper comes from the 'Sanskrit' word 'pippali' meaning berry. Today, apart from India, black pepper is widely cultivated throughout Indonesia, Malaysia, Thailand, tropical Africa, Brazil, Sri Lanka, Vietnam and China (Nair, 2004).

Pepper belongs to the *Piperaceae* family. Among the 700 different varieties there are bushy types, as well as tree-like, creeping, climbing and epiphytic sorts. *Piper nigrum* is a climbing plant, which, so long as it is not trimmed, can reach up to 10 m in height. The varieties differ in raceme length, leaf size, berry attributes (size, color, etc.), pest and disease resistance, quality parameters, and yield. The varieties also differ in suitability to various climates, such as wet conditions or well defined dry periods (Mathew *et al.,* 2006). The system of shoots is

distinguished between the main shoots, which grow upwards, and lateral, fruit- bearing shoots that grow horizontally. The main shoots form numerous nodes, on which grow adventitious roots for climbing, as well as lateral shoots, the stemmed, heart-shaped leaves and the blossom ears. The different varieties range from single-sexed to hermaphrodite, and are self pollinating. The syncarpy produces berry-like fruits (drupes) up to 15cm in length taking 6-8 months to develop from blossom to the ripe fruit (Nair, 2004).

2.2 GROWTH AND DEVELOPMENT OF BLACK PEPPER

Black pepper is a woody, climbing liana or vine. In cultivation, the plant is grown on a support such as a trellis. It may grow to a length of 10 m (33 ft) or more in length. During the third year after planting, a small crop can be harvested, with full production realized 7–8 years after planting. Plants are most productive at 8–20 years of age, but can continue bearing for 30 years. Ripe berries may be picked about 9 months after flowering. Berries ripen over a period of 2–6 months depending on climate or latitude. Berries are usually harvested every 7–14 days during the harvesting period. The harvesting calendar months vary throughout the world. For example, in India and some tropical African counties, black pepper is harvested from November through March, whereas in Madagascar the crop is harvested from June through October. There is potential for two crops per year in some regions. In Papaikou, Hawai, harvest occurs in February/March and in May/June (Nair, 2004).

2.3 USES AND PRODUCTS OF BLACK PEPPER

Aside from salt, pepper is the world's most important and valued spice. It is used as an important component of many recipes and to flavour foods. The berries of *Piper nigrum* are processed to

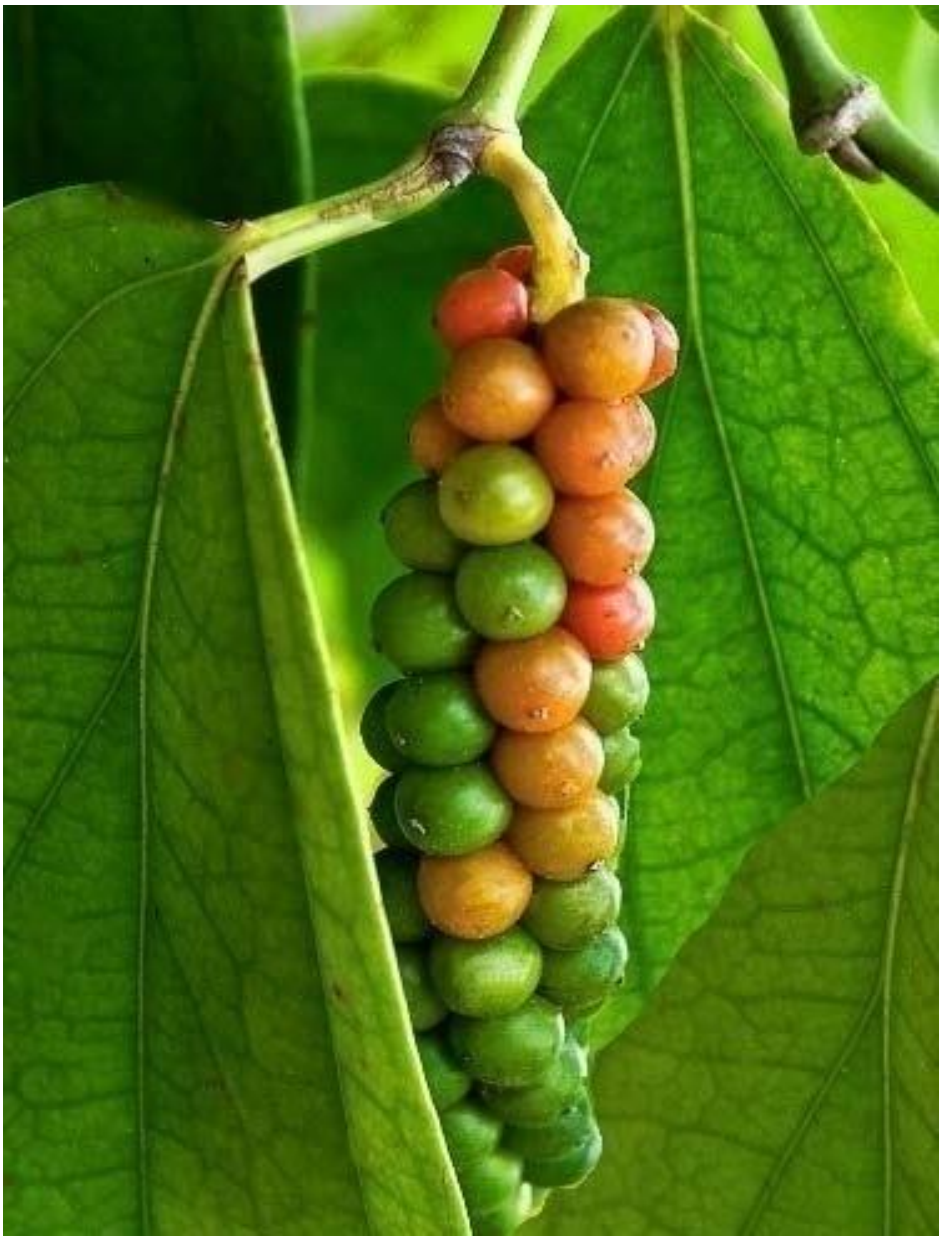

Plate 2.1: Mature Berries of *Piper nigrum* 'Black pepper'

several condiments: black pepper, white pepper, green pepper, and "Tellicherry" pepper (Nair, 2004). Many grades of these pepper products including its essential oil are recognized in the spice trade. According to Nair (2004) other important commercial products derived from the pepper plant are:

- Pepper oil (the vapor or steam distillation process widely used in fragrances or condiments; black pepper yields about 1–2.4% essential oil)
- Tea (pepper leaves combined with tea leaves)
- Perfumes (made from dried parts of the pepper plant)
- Sausage preservation

Commercial production of black pepper worldwide in 2000 was approximately 230,000 metric tons (MT) (254,000 T). Countries in the International Pepper Community, an inter-governmental organization of black pepper producing countries, produce 84% of the world's crop (Nair, 2004). Other countries such as Vietnam, China, and Madagascar produce the remaining 16%. Pacific island production probably comprises less than 1% of world production (Mathew et al., 2006; Nair, 2004).

The use of black pepper as a seasoning/condiment, on its own or in spice blends, is on the increase with the growing popularity of snacks, ethnic foods, ready-to-cook meals as well as healthy low-sugar-and-salt foods especially in the developed countries. Black pepper tastes strongest when freshly ground although pre-ground black pepper is often used in seasonings for convenience. White black pepper is less aromatic than black pepper but has special applications, as in white sauces where black pepper would give them an undesirable speckled appearance (Nair, 2004).

The value of black pepper as a natural preservative for meat and other perishable foods has been known for centuries. Studies have shown that this is due to the anti-oxidant and anti-microbial properties present in pepper (Mathew *et al.*, 2006). Black pepper is an important ingredient in Ayurvedic, Chinese and Unani and other traditional medicines. The major functional properties of black pepper are analgesic, anti-pyretic, anti-oxidant and anti-microbial. The three main therapeutic uses of black pepper are as a stomachic, digestive and tonic (Dobelis, 1986).

Black pepper has a number of medicinal uses, including the ability to control worm infestations. It is regarded as a purgative, an antidote for poisons, and an aphrodisiac. Black pepper can enhance digestion of food because after its ingestion, secretions of the pancreas and gastric system increase. The roots of black pepper also have medicinal qualities, as a stomach anesthetic (causes loss of feeling or awareness), analgesic (relieves pain without causing a complete loss of sensation), muscle relaxant, digestive stimulant, antiseptic, diuretic (increases urine flow), sudorific (diaphoretic, promotion of sweating), anxiolytic (reduces anxiety), and as a hypnotic (Nair, 2004). Black piperine, one of the alkaloids in pepper, is effective against houseflies, and gardeners use pepper sprays against several kinds of pests (Nair, 2004).

2.4 POSTHARVEST HANDLING AND PROCESSING OF BLACK PEPPER

Berries are harvested when their color is greenish yellow. In some places, the berries are dipped in boiling water for 10 minutes after harvest. This provides surface disinfestations and starts the fermentation process, which turns the berries black. Berries are dried in the sun after the hot water treatment. About 14 days are required for sun drying in order to reach a moisture content of approximately 12% and produce berries that have uniform color (dark brown to black), pungent aroma, and are free of mold. Shelf life for properly stored pepper is 12–18 months but at temperatures higher than 15–20°C and relative humidity of over 60%, harmful molds or

Aflatoxins (harmful substances produced by some molds) may form on peppercorns. About 100 kg (220 lb) of green pepper can produce approximately 35 kg (77 lb) of black pepper (Nair, 2004). Heat treatment lends a uniform, black luster to the peppercorns. Usually, the separated peppercorns are placed in a perforated basket or coarse fabric and dipped with the container into boiling water for one minute. Then the drained berries are spread onto a clean surface for sun drying.

2.5 OIL/RESIN EXTRACTION

The geographic origin of black pepper berries and the method of their preparation determine the chemical composition of pepper oil. The oil and/or oleoresins are produced through various methods such as fractionation, distillation, or extraction by solvents (such as ethanol, acetone, or dichloromethane). Oleoresins (used in pickles, canned meats, and dressings) may be produced by solvent extraction, and have similar pungency, odor, and flavour. The essential oils can also be obtained by cold pressing.

2.6 THE NUTRITIONAL COMPOSITION OF BLACK PEPPER

Black pepper is an excellent source of nutrient (Table 2.1.1) such as of 9.84% ash content, 12.52% crude fiber, 13.34% crude fat, 11.50% protein, 41.52% carbohydrate (Okonkwo and Ogu, 2014). Vitamins (Table 2.1.2) in black pepper are 8.12IU vitamin A, 0.04mg niacin, 0.88mg vitamin C, 0.44mg folate, 0.08IU vitamin E and 6.88mcg vitamin K (de Waard and Anunciado, 1999). Mineral elements in black pepper (Table 2.1.3) include 179.52mg/100g calcium, 35.54mg/100g magnesium, 98.52mg/100g potassium; 20.87mg/100g sodium, 217.70mg/100g phosphorous and 2.52mg/100g iron (Okonkwo and Ogu, 2014).

Table 2.1.1: Proximate composition of *Piper nigrum*

Composition	Value %
Moisture content	11.72
Dry matter	88.28
Ash	9.84
Crude fiber	12.52
Crude fat	13.34
Crude protein	11.50
Carbohydrate	41.52

Source: Okonkwo and Ogu **(2014)**

Table 2.1.2: Vitamins in *Piper nigrum*

Vitamin	Value (per 100g)
Vitamin A IU	8.12 IU
Vitamin A RE	0.80 RE
A—carotenoid	0.80 RE
A—beta carotene	4.88 mcg
Thiamin—B1	0.00 mg
Riboflavin—B2	0.00 mg
Niacin—B3	0.04 mg
Niacin equiv	0.04 mg
Vitamin C	0.88 mg
Vitamin E alpha equiv	0.04 mg
Vitamin E IU	0.08 IU
Vitamin E mg	0.04 mg
folate	0.44 mcg
Vitamin K	6.88 mcg

Source: de Waard and Anunciado 1999

Table 2.1.3: Mineral content of *Piper nigrum*

Composition	Value (mg/100g)
Calcium	179.52
Magnesium	35.54
Potassium	98.52
Sodium	20.87
Phosphorous	217.70
Iron	2.52

Source: Okonkwo and Ogu (2014)

2.7 NATURE, COMPOSITION AND IMPORTANCE OF *AFRAMOMUM DANIELLI*

Aframomum danielli (bastered meleguta) belongs to the botanical plant (Plate 2.2) family of *Zingiberace*. It is a perennial succulent plant with yellow stamen. The fruit is initially green and later becomes reddish when ripe. It is commonly known as "Atare-oburo" 'Upoe' and "Urima" among the Yoruba, Edo and Igbo ethnics' part of Nigeria (respectively) where they are mostly consumed. This spice has seeds that are used in a very small quantity in ground form as flavouring agent for traditional dishes and pepper-soups. The essential oil is used for perfumes and dye preparations. It has been reported to have a broad spectrum of anti bacterial properties. *Aframomum danielli* has been reported to be a good antioxidant and have good inhibitory properties on *Salmonella enteriditis, Pseudomonas fragi, Pseudomonas fluorescence, Proteus vulgaris, Stretococcus pyrogenes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis,* Gram-negative *Escherichia coli, Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, and Aspergillus niger* (Fasoyiro *et al*. 2001; Martins *et al.,* 2001; Adegoke *et al*., 1998) and on food spoilage yeasts and moulds (Adegoke *et al.,* 2000).

Nutritionally, the dried seeds of *Aframomum danielli* has been found to contain 5% moisture, 8.2% protein (dry matter basis), Calorific value of 469.7 Kcal/100g, varying amounts of minerals like calcium, magnesium, sodium, manganese, phosphorus, zinc and copper, as well as a number of Amino acids like L-Threonie, L-Serine, L-Valine, L-Proline, L-Glutamic acid, glycine, L-Leucine and L-Lysine. (Adegoke and Skrura, 1994). *Aframomum danielli* has been used in the preservation of a variety of food commodities, including maize and soybean against mould growth and insect infestation for 15 months without adversely affecting their viability (Adegoke *et al.,* 2002).

Plate 2.2: Bastered meleguta 'urima' (*Aframomum danielli*) plant and fruits

The seed of *A. danielli* are used as a traditional food spice among the Edo and Niger Delta people of Nigeria and also as an anti-inflammatory agent by rubbing of the alcohol and petrol extracts on the allergic and eczematous swelling. Previous studies on *A. danielli* have shown the presence of diterpenes as acids and aldehydes. The aldehyde 8β - 17 epoxy – 12 E-labolane 15, 16 – dial exhibited potent antifungal properties (Kimbu *et al.,* 1994; Kindu *et al*. 1987). Water extracts of the rhizome also had strong molluscidal activity against *Bulinus globosus* snails implicated in schistosomiasis (Iwu, 1993).

2.8 FLAVOUR RESEARCH

The acquisition of knowledge of the chemicals responsible for the characteristics odour and flavours of foods and other natural materials has long been the aim of researchers (Andew and Taylor, 2009; Howlett, 1992). Although modern instrumental techniques have considerably accelerated the pace of flavour research, there still remains much to be discovered in the field of biochemistry, odor and flavour appreciation and the many associated interfaces of these subjects, Howlett (1992) listed some of the specific benefits of flavour research as;

a. By understanding biosynthetic pathways, it is possible to improve on nature, obtaining better yields of flavour components, or removing objectionable flavour features in food stuffs.
b. Enabling the reconstitution of flavour using synthetic materials identical with those found in nature, giving to the flavourist and food technologist an ability to improve/enhance or regulate defects or eve to create novel food flavour,.
c. It is possible to specify the flavour attributes of foods and flavouring materials and thereby control in food products.

d. By understanding the mechanisms of olfaction and gustation, to create end-products having a more acceptable flavour profile.

The majority of the techniques used in flavour research are basically analytical methods capable of application to a wide range of problems: the flavourist becomes involved at the stage where the components have been quantitatively segregated and are available for the assessment of their odor and/or flavour characteristics and a judgment of their relative importance in the odor/flavour profile of the material from which they were derived.

The steps involved in flavour research have been described by Howlett (1992). The stage and techniques used in flavour research are; (i) selection of start material and sensory evaluation, (ii) isolation or Extraction which include aqueous distillation, adsorption, carbon dioxide reduced pressure distillation, expression, formation of derivatives, head space vapor collection, high vacuum, degassing, solvent partition, steam distribution and vacuum sublimation (iii) concentration methods use are adsorption / desorption, displacement, solvent recovery, freeze concentration, molecular distillation, vacuum distillation/fractionation and zone concentration (iv) methods for separation of components which include chemical fractionation, chromatography, column, gel permeation, gas –liquid chromatography, gas –solid, high pressure liquid, and thin layer chromatography (v) identification of compounds by retention index determination, sensory assessment of GLC effluents, spectroscopy, infrared, mass, nuclear magnetic resonance, ultraviolet (vi) organic synthesis methods are instrument analysis, sensory assessment.

2.8.1 Isolation methods

Aromatic components and their precursors are generally present in aqueous solution or as droplet in the cell sap, although some essential oils may exist discrete in oil sacs glandular hairs etc. it is obviously not practical to carry out an analysis on the original plant material per se and it is necessary to isolate the odor flavour complex as much as possible from the mass of inert cellular matter with the minimum amount of chemical change.

This may be achieved by several techniques depending on the nature of the start materials these include:

a. Expression – the physical extraction of aqueous juice from plant tissues of particular value in studies on fruit flavour
b. Solvent extraction- the solvent used may be either water from which the aromatic components may be recovered by high vacuum vaporization or low boiling point non polar solvent (e.g. ether cyclohexan methylene dichloride) or liquefied gaseous solvents (e.g. Freon's) or liquefied carbon dioxide. The solvent of choice depends on the physical nature of the start materials and its susceptibility to oxidative and other reaction leading to artifacts.
c. Steam distillation at atmospheric pressure or under vacuum
d. High vacuum degassing – applicable to the recovery of volatiles from fixed oils and foods having a high lipids contents
e. Head space vapor collection (Andew and Taylor, 2009; Howlett, 1992).

2.8.2 Extraction method

Methods involving solvent extraction are particularly difficult to control. Many plant materials have a coarse macroscopic structure requiring some degree of communication before treatment with a solvent. This grading is often accompanied by heat and may lead to volatile losses and chemical changes. Green plants are essentially wet and it is difficult to obtain a close mixture of aqueous cell contents and an immiscible solvent. Even when this is achieved by high speed homogenization process, the partition equilibrium may be slow and repeated extractions necessary to obtain a satisfactory yield. Extraction may be improved by using dried materials, but, again the chances of decomposition and the formation of artifacts are increased. Aromatic materials from natural sources differ widely in their physical and chemical properties, particularly in their volatility and solubility so that the choice of a solvent may be determined by the success of the extraction, very few solvents have a sufficiently, wide spectrum to recover all the active components and may call for multiple extraction using different solvents (Andew and Taylor, 2009; Howlett, 1992).

2.8.3 Distillation method

Steam distillation is a favoured method of isolation and recovery of aromatics from plant materials, although precautions must be taken to limit thermal degradation and other chemical changes in the components (Howlett, 1992). Initial separation by steam distillation followed by liquid –liquid partition extraction. These steps are mostly used for distillates obtained from material that have very low levels of aromatics.

2.8.4 Concentration Methods

The extracted isolates may be very weak in initial concentration of odor and flavouring constituents (Figure 2.1). In such cases it is necessary to carry out a concentration stage in order to increase the proportion of the desired aromatics by the removal of all or parts of the undesired diluents. Depending on the physical nature of the isolate this concentration may be achieved with minimal chemical change, by several methods including; adsorption/desorption, crystallization, dialysis, distillation, evaporation, freeze, concentration followed by centrifugation, freeze drying or partition solvent extraction of the above. The most widely-used method of concentration is by distillation (Andew and Taylor, 2009).

2.8.5 Molecular distillation

Molecular distillation is a process by which substances can be distilled under very high vacuum and low temperature of distillation. The distillation conditions ensure minimum damage to thermo labile components in plant materials. In conventional distillation, molecules leaving the surface of the boiling liquids may undergo many collisions with residual air molecule in the system and with other molecules. In molecular distillation, all air is removed from the system at a vacuum of 0.001mHg. The condensing surface is located close to the boiling liquid in such a way that no molecular collision takes place (i.e it is at the mean free path of the molecules present). The condensate is removed rapidly from the system. The method is particularly useful for the distillation of aromatic materials having a molecular weight between 400 and 1200 and distilling at 50^{o}C and 150^{o}C (Andew and Taylor, 2009; Barnitz, 1997).

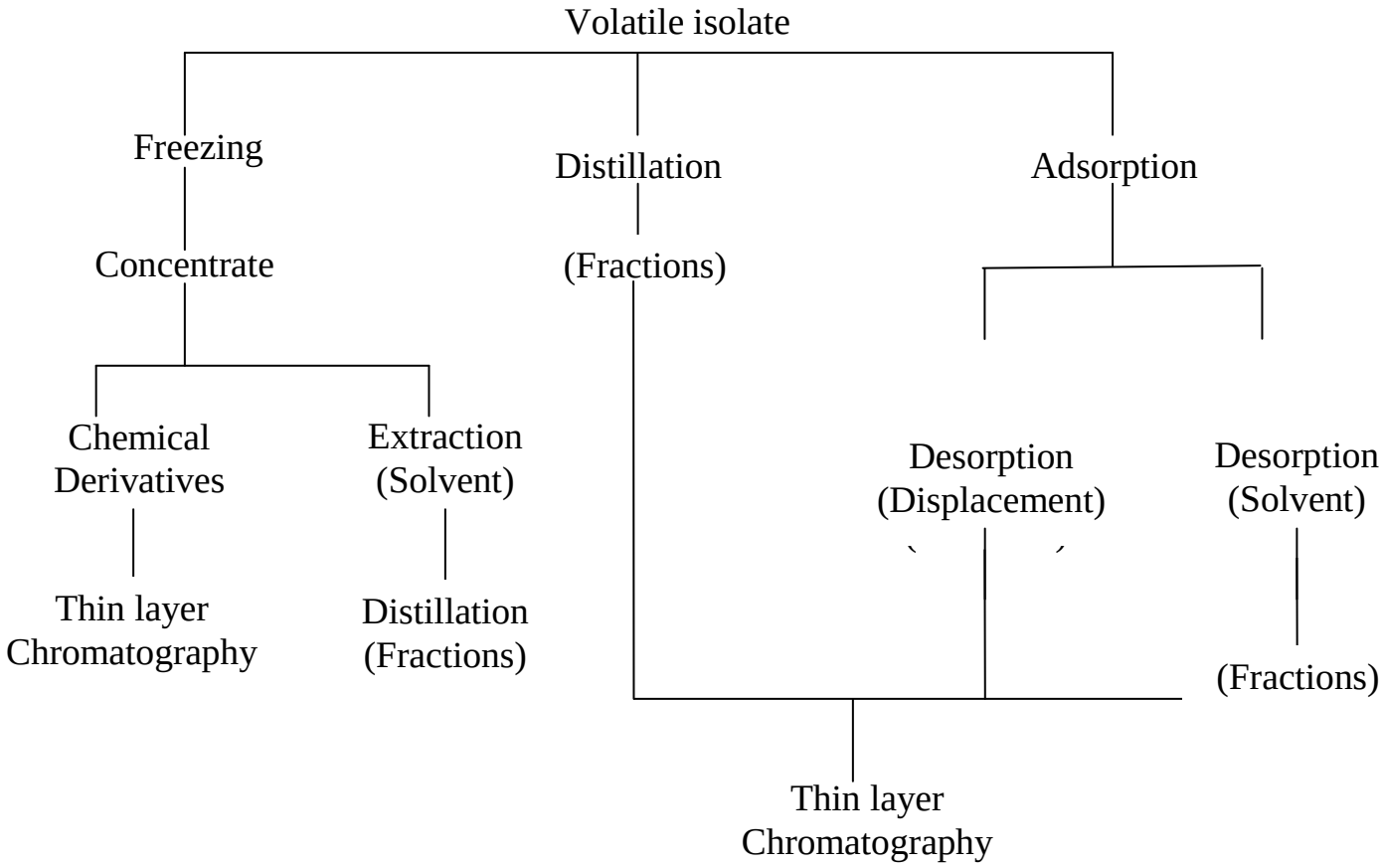

Figure 2.1: Scheme for the concentration of weak flavour isolates

2.8.6 Freeze concentration

Freeze concentration can be applied to aqueous and dilute alcoholic isolate (e.g fruit juice). The method consist of freezing the weak isolate and physical separating the water as ice crystal either on a cold finger or by centrifugation. Early researchers who used this method stressed the need for the mixture to be constantly agitated during the freezing to encourage the formation coarse crystal in order to reducing the entrapment of concentrate by occlusion. It is also necessary to stop the freezing while part of the isolate remains liquid. Concentration of between 10 and 20 times has been obtained by refreezing earlier concentrate (Andew and Taylor, 2009; Barnitz, 1997). Although the production of artifacts is eliminated, losses do occur due mainly to evaporation but also to occlusion adsorption adhesions to the ice surface or by diffusion into air channels in the ice. Some differential separation of components may take place during freeze concentration as some compounds tend to accumulate in the solid phase rather than remain in the liquid phase.

Whatever method is chosen, the resulting concentrated isolate is usually a highly complex mixture of organics which may require an initial separation into simpler fraction before being subjected to final analysis by any of the sensible instrumental techniques available for the separation of individual compounds. This coarse fractionation may be achieved on the basis of chemical identification (e.g. acid, basic neutral phenolic, etc) using techniques such as the formation of derivatives like 2, 4 dinitrophenly hydrazones, or on some physical character (e.g. boiling points) using fractional distillation or similar methods. Much work can be saved by the careful and critical sensory evaluation of the isolate before any fractionation is carried out. This ensures that the isolate tray represent the profile of the initial start material. Once fractionation

has been carried out, this essential control of the system is lost and hence the identification of a typical artifacts difficult to prove or disprove (Andew and Taylor, 2009; Barnitz, 1997).

2.8.7 Separation methods

Having obtained an isolate or fraction of an isolate, at an acceptable concentration with the minimal of chemical change or artifact production, the next stage is the separation of the complex mixture into its individual components. This leads to their characterization, classification and ultimate identification. Many chemical and physical techniques have been used to achieve this with varying degrees of success but without doubt the method having the widest application versatility and efficiency in this various forms is chromatography (Andew and Taylor, 2009).

2.8.8 Sensory evaluation

Of direct importance to the flavour chemist is the sensory assessment of Gas-Liquid-Chromatography effluents. The basic method was described by Ryder (1966) but depended on relatively low resolution columns to allow time for the sensory order response. Essentially, the method consists of sniffing the effluent gases as they emerge from the column and recording the sensory impression on the graph as the tracing is being recorded. However, researchers have warned that there is a danger of misleading information being obtained from this source due to an unexplained co-elution of trace components of widely different retention times. Terms used to describe effluent odour should be universally understandable, accurately definable and preferably could be of reference to some readily available chemical standard having the same attribute (Williams, 1975). The complexity of many natural odour and flavours may result in the overlapping of peaks, even with high resolution columns, and possible important trace

compounds may go unobserved by the presence of quantitatively major compound but may be sensorily less important constituents in this respect. The nose is usually more sensitive than most instrumental defectors and an odor may be observed in the effluent stream where there is no peak on the chromatogram to indicate a separate component (Williams, 1975).

2.9 MICROENCAPSULATION TECHNOLOGY AND APPLICATIONS

Microencapsulation (Mars and Scher, 1990) is a technique by which solid, liquid or gaseous active ingredients are packaged within a second material for the purpose of shielding the active ingredient from the surrounding environment. Thus the active ingredient is designated as the core material whereas the surrounding material forms the shell. This technique has been employed in a diverse range of fields from chemicals and pharmaceuticals to cosmetics and printing. For this reason, widespread interest has developed in microencapsulation technology.

Preparation of microcapsules dates back to 1950s when Green and Schleic (Green and Schleicher, 1957; Green, 1957) produced microencapsulated dyes by complex coacervation of gelatin and gum arabic, for the manufacture of carbonless copying paper. To this day, carbonless copy paper is one of the most significant products to utilize microencapsulation technology, and is still produced commercially. The technologies developed for carbonless copy paper have led to the development of various microcapsule products in later years. In the 1960s, microencapsulation of cholesteric liquid crystal by complex coacervation of gelatin and acacia was reported to produce a thermo-sensitive display material. Encapsulation technology has provided the enlargement of display areas and wider viewing angles. In defense applications this technology is used for fabrication of self-healing composites (Zhang, 2007) which forms an integral part of aerospace structures.

2.9.1 Microencapsulation techniques

Encapsulation of food ingredients into coating materials can be achieved by several methods. The selection of the microencapsulation process is governed by the properties (physical and chemical) of core and coating materials and the intended application of food ingredients. However, the microencapsulation processes that are used to encapsulate food ingredients are given in Table 2.2 which outlines various methods used for the preparation of microencapsulated food systems. Sophisticated shell materials and technologies have been developed and an extremely wide variety of functionalities can now be achieved through microencapsulation. Any kind of trigger can be used to prompt the release of the encapsulated ingredient, such as pH change (enteric and anti-enteric coating), mechanical stress, temperature, enzymatic activity, time, osmotic force, etc (Green and Schleicher, 1957; Green, 1957).

2.9.2 Microencapsulation by spray drying

The microencapsulation of flavour is the technology of converting liquid flavour materials into easy-to-handle solids. It also provides protection against degradative reactions and prevents the loss of flavour. In addition, it can be used to control the release of flavour during food processing and storage. Various encapsulation methods have been previously proposed (Gibbs *et al.,* 1999; Shahidi and Han 1993). Among them, spray drying is the most popular method of producing flavour powders, which presents the challenge of removing water by vaporization while retaining the flavours that are much more volatile than water. According to the selective diffusion concept the diffusion coefficient of flavour decreases at a higher rate than the diffusion coefficient of water during drying (Kashappa *et al.,* 2005). Numerous studies have been conducted to evaluate the retention of flavour during Spray drying. The properties of the volatile compounds in the capsule wall material (McNamee *et al.,* 2001; Buffo and Reineccius 2000), and the emulsion

(Liu *et al.,* 2001; Liu *et al.,* 2000; Mongenot *et al.,* 2000), along with the drying process conditions (Finney *et al.,* 2002), and the powder morphology during and after drying (Hecht and King 2000; Moreau and Rosenberg 1993; El-Sayed *et al.,* 1990) have been already reported. Recently, the main emphasis of the microencapsulation of flavour has concentrated on preventing flavour losses during spray drying and extending the shelf life of the products. This is intended to produce high quality flavour powders. Buffo and Reineccius (2000) reported the optimization of the gum acacia/modified starch/maltodextrin blending. The attempt to replace commonly used gum arabic with other carbohydrates has been investigated by McNamee, O'Riordan & O'Sullivan, (2001).

Furthermore, work has also been done on improving the properties of flavour (that is, combining flavour with additive materials). For example, Liu *et al.,* (2001) improved flavour retention by adding a weighting agent to the flavour. Therefore the processes of flavour development should be carried out to control the loss of flavour, Finney *et al.,* (2002) studied the effects of the type of atomization and processing temperature on the properties of the encapsulated flavour. The emulsion size in the feed liquid also influences flavour retention and the stability of the encapsulated flavour components. Using gum Arabic or Amiogum 23 as the carrier, Finney *et al.,* (2002) studied the effect of emulsion sizes of orange peel oil (0.9 mm ~4.0 mm) on flavour retention and shelf life. Their results suggested that a smaller emulsion size yields larger percentage retention of the orange oil with a smaller amount of surface oil, but did not produce a longer shelf life.

Sheu and Rosenberg (1995) suggested that the retention of volatiles during microencapsulation by spray drying could be enhanced by reducing the mean emulsion size of the dispersed core material during emulsification. Ethyl caprylate retention was improved by reducing the mean

emulsion size when a combination of whey protein and maltodextrin was used as the carriers. Furthermore Liu *et al.,* (2001) correlated improved eugenol retention by measuring the difference between the mean emulsion size and the mean particle size of the dry powder. Scanning Electron Microscopy (SEM) techniques have been developed for the study of the outer and inner structures of food microencapsulation, including procedures for embedding and sectioning or polishing the embedded specimen by Liu *et al.,* (2001). SEM techniques have since been further developed and become an important technique in studying microencapsulation as a tool for the selection of wall materials, for the study of core material distribution in microcapsules, and for elucidating the mechanisms of capsule formation.

Table 2.2: Various microencapsulation techniques and the processes involved in each technique

No	Microencapsulation techniques	Major steps in encapsulation
	Spray-drying	a. Preparation of the dispersion b. Homogenization of the dispersion c. Atomization of the in-feed dispersion d. Dehydration of the atomized particles
	Spray-cooling	a. Preparation of the dispersion b. Homogenization of the dispersion c. Atomization of the in-feed dispersion
	Fluidized-bed coating	a. Preparation of coating solution b. Fluidization of core particles. c. Coating of core particles
	Extrusion	a. Preparation of molten coating solution b. Dispersion of core into molten polymer c. Cooling or passing of core-coat mixture through dehydrating liquid
	Centrifugal extrusion	a. Preparation of core solution b. Preparation of coating material solution c. Co-extrusion of core and coat solution through nozzles
	Lyophilization	a. Mixing of core in a coating solution b. Freeze-drying of the mixture
	Coacervation	a. Formation of a three-immiscible chemical phases b. Deposition of the coating c. Solidification of the coating
	Centrifugal suspension separation	a. Mixing of core in a coating material b. Pour the mixture over a rotating disc to obtain encapsulated tiny particles c. Drying
	Cocrystallization	a. Preparation of supersaturated sucrose solution b. Adding of core into supersaturated solution c. Emission of substantial heat after solution reaches the sucrose crystallization temperature
	Liposome entrapment	a. Micro-fluidization b. Ultrasonication c. Reverse-phase evaporation

Source: Kashappa *et al.*, (2005)

2.9.3 Applications of microencapsulation

1. Food Industry

Currently there is a trend towards a healthier way of living, which includes a growing awareness by consumers for what they eat and what benefits certain ingredients have in maintaining good health. Preventing illness by diet is a unique offering of innovative so called "functional foods", many of which are augmented with ingredients to promote health. However simply adding ingredients to food products to improve nutritional value can compromise their taste, color, texture and aroma. Sometimes they slowly degrade and lose their activity, or become hazardous by oxidation reactions. Ingredients can also react with components present in the food system, which limit bioavailability. Microencapsulation techniques may be used to overcome all these challenges by providing viable texture blending, appealing aroma release, and taste, odor and color masking (Kirby, 1991). The technology enables food companies to incorporate minerals, vitamins, flavour and essential oils. In addition, microencapsulation can simplify the food manufacturing process by converting liquids to solid powder, decreasing production costs by allowing batch processing using low cost, powder handling equipment. Microcapsules also help fragile and sensitive components to be protected, handled and stored during processing, packaging and distribution.

2. Agriculture application

One of the most important applications of microencapsulated products is in the area of crop protection (Scher *et al.,* 1998). Nowadays insect pheromones are becoming viable as a bio-rational alternative to conventional hard pesticides. Specifically, sex attractant pheromones can reduce insect populations by disrupting their mating process. Hence small amounts of species-

specific pheromone are dispersed during the mating season, raising the background level of pheromone to the point where it hides the pheromone plume released by its female mate (Ilichev *et al*., 2006). Polymer microcapsules, polyurea, gelatin and gum arabic serve as efficient delivery vehicles to deliver the pheromone by spraying the capsule dispersion. Further, encapsulation protects the pheromone from oxidation and light during storage and release (Scher, 1998).

2.9.4 Recent developments in microencapsulation of food ingredients

Microencapsulation is defined as a technology of packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Cho *et al*., 2000). The microencapsulation technology has been used by the food industry for more than 60 years. In a broad sense, encapsulation technology in food processing includes the coating of minute particles of ingredients (e.g., acidulants, fats, and flavours) as well as whole ingredients (e.g., raisins, nuts, and confectionary products), which may be accomplished by microencapsulation and macro-coating techniques, respectively (Kirby, 1991). More specifically, the microcapsule has the ability to preserve a substance in the finely divided state and to release it as occasion demands (Cho *et al*., 2000; Kirby, 1991). These microcapsules may range from sub micrometer to several millimeters in size and have a multitude of different shapes, depending on the materials and methods used to prepare them. The food industry applies microencapsulation process for a variety of reasons:

- encapsulation/entrapment can protect the core material from degradation by reducing its reactivity to its outside environment (e.g., heat, moisture, air, and light),
- evaporation or transfer rate of the core material to the outside environment is decreased/retarded,

- the physical characteristics of the original material can be modified and made easier to handle,
- the product can be tailored to either release slowly over time or at a certain point (i.e., to control the release of the core material to achieve the property delays until the right stimulus),
- the flavour of the core material can be masked,
- the core material can be diluted when only very small amounts are required, yet still achieve a uniform dispersion in the host material, and
- it can be employed to separate components within a mixture that would otherwise react with one another (Kirby, 1991)

Various properties of microcapsules that may be changed to suit specific ingredient applications include composition, mechanism of release, particle size, final physical form, and cost. The architecture of microcapsules is generally divided into several arbitrary and overlapping classifications. One such classification is known as matrix encapsulation. This is the simplest structure, in which a sphere is surrounded by a wall or membrane of uniform thickness resembling that of a hen's egg. In this design, the core material is buried to varying depths inside the shell. This microcapsule has been termed a single-particle structure. It is also possible to design microcapsules that have several distinct cores within the same microcapsule or, more commonly, number numerous core particles embedded in a continuous matrix of wall material. This type of design is termed the aggregate structure (Chio *et al.*, 2002).

In order to improve the properties of food ingredients, immobilization of food ingredients onto a suitable polymer or addition of antimicrobial agents are common practices in the food industries, (Chio *et al.*, 2002) For example, an important bacteria used in the food industry, lactic acid

bacteria, was first immobilized in 1975 on Berl saddles and *Lactobacillus lactis* was encapsulated in alginate gel beads years later. Chio *et al.*, (2002) suggested that immobilized lactic acid bacteria could be used to continuously produce yogurt. However, the alginate gel beads leaked large quantities of cells. The use of microencapsulated food ingredients allows food ingredients to be carefully tailored to the specific release site through the choice and microencapsulation variables, specifically, the method and food ingredients-.polymer ratio, (Kirby, 1991) The total amount of ingestion and the kinetics of release are variables that can be manipulated to achieve the desired result, (Kirby, 1991). Using innovative microencapsulation technologies and varying the copolymer ratio, molecular weight of the polymer, etc., microcapsules can be developed into an optimal food ingredient device (Kirby, 1991).

Microcapsule-based systems increase the life span of food ingredients and control the release of food ingredients. Various properties of microcapsules that may be changed to suit specific ingredient applications include composition, mechanism of release, particle size, final physical form, and cost. Before considering the properties desired in encapsulated products, the purpose of encapsulation must be clear. In designing the encapsulation process, the following questions are taken into consideration:

1. What functionality should the encapsulated ingredients provide the final product?
2. What kind of coating material should be selected?
3. What processing and storage conditions must the encapsulated ingredient survive before releasing its content?
4. What is the optimal concentration of the active ingredient in the microcapsule?
5. By what mechanism will the ingredient is released from the microcapsules?
6. What are the particle size, density, and stability requirements for the encapsulated ingredient?

7. What are the cost constraints of the encapsulated ingredient? (Kirby, 1991)

2.9.5 Coating Materials Used For Microencapsulation in the Food Industry

As important as the choice of microencapsulation technique is, the selection of wall material also plays a crucial role in the development of particle properties of microencapsulated food ingredients. Generally, shell materials are required to have some of the following characteristics: film-forming, pliable, tasteless, non-hygroscopic, soluble in an aqueous media or solvent, and/or able to exhibit a phase transition, like melting or gelling (Kirby, 1991). Specifically, for food use the coating material should also:

_ be easily digested by the body,

_ have no interaction with the core material,

_ be non-sticky,

_ be impervious to water,

_ be inexpensive,

_ should not impart sensory changes,

_ comply with food regulations and indigenous customs (Kirby, 1991).

Numerous coating materials have been used in food ingredient microencapsulation. Most of them are natural or are derivatives of plant or animal food products, which have been approved by FDA as GRAS (generally recognized as safe) materials. Table 2.3 lists some commonly used

Table 2.3: Coating materials for microencapsulation of functional food additives

Category	Coating materials	Widely used methods
Carbohydrate	Starch, maltodextrins, chitosan, corn syrup solids, dextran, modified starch, cyclodextrins	Spray- and freeze-drying, extrusion, coacervation, inclusion complexation
Cellulose	Carboxymethyl cellulose, methyl cellulose, ethylcellulose, cellulose acetate-phthalate, cellulose acetate butylate-phthalate	Coacervation, spray-drying, and edible films
Gum	Gum acacia, agar, sodium alginate, carrageenan	Spray-drying, syringe method (gel beads)
Lipids	Wax, paraffin, beeswax, diacylglyerols, oils, fats	Emulsion, liposomes, film formation
Protein	Gluten, casein, gelatin, albumin, peptides	Emulsion, spray-drying

Source: Kashappa *et al.,* (2005)

coating materials for microencapsulation of food ingredients and their applicable techniques.

In developing a microencapsulation system, the techniques and coating materials need to be considered together, as they usually influence each other. In the current study, decision was made to investigate glassy carbohydrates, including cellulose derivatives, as coating materials for encapsulating iron extrudates in a fluidized-bed process. Also, due to their unique film-forming properties and phase transition, they are expected (Kashappa *et al.,* 2005) to protect the stability of the core ingredients (i.e., micronutrients) in dried forms while achieving desirable bioavailability through instant release when the iron particles are released in the digestive system. More importantly, these materials are relatively inexpensive and widely available even in developing countries. On the other hand, certain gums, e.g., sodium alginate, will be used in the internal gelation for making extruded rice analogues. This polymer has been used extensively in numerous applications due to its well-known gelling effect. The broad availability and relative low cost of the materials will encourage the implementation of the successful formulations developed in this study. Encapsulating water-soluble core materials such as minerals, water-soluble vitamins, enzymes, acidulants, and some flavours can be possible through use of coating materials (Kashappa *et al.,* 2005).

2.10 NATURE AND DETERIORATIVE CHARACTERISTICS OF SPICES AND SPICE PRODUCTS

Spices are the generic name for products derived from a variety of plant parts: bark, bud, flower, fruit, root, seed or secretion. FDA describes spices as .any aromatic vegetable substance in the whole, broken or ground form that is used primarily to season food rather than to contribute nutrients. . The main quality contributing factors like aroma, flavour and color are sensitive to vagaries of climate and during storage. They are affected by factors like high temperature and

humidity, oxygen, respiration, heat, insects, pests, microorganisms, rodents and birds which work in combination to cause the following deteriorations:

1. Loss of aroma and flavour: Loss of aroma and flavour is 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.
2. Bleaching of color occurs in spices like green and bell pepper, green cardamom, turmeric, red chillies, paprika and saffron which contain natural pigments. This deterioration is caused by oxygen and accelerated by light, humidity and temperature and favored by oxygen.

3. 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 odour does occur at higher relative humidity (RH).

4. Microbial spoilage at and above 70% RH; microbial spoilage occurs in spices due to moisture sorption. The microorganisms usually seen on raw spices may not be harmful but give rise to problems when used in food preparation. So it is very important to check them.

5. Insect infestation: The problem of insect infestation in spices is quite serious. 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. If warehouses are not ensured of adequate protection against rodents, they also cause a great deal of spoilage.

2.11 PACKAGING OF SPICES AND SPICE PRODUCTS

2.11.1 Packaging

Processed plant materials should be packaged as quickly as possible to prevent deterioration of the product and as a protection against exposure to pest attacks and other sources of contamination. Continuous in-process quality control measures should be implemented to eliminate substandard materials, contaminants and foreign matter prior to and during the final stages of packaging. Processed plant materials should be packaged in clean, dry boxes, sacks, bags or other containers in accordance with standard operating procedures and national and/or regional regulations of the producer and the end-user countries. Materials used for packaging should be non-polluting, clean, dry and in undamaged condition and should conform to the quality requirements for the plant materials concerned. Fragile plant materials should be packaged in rigid containers. Whenever possible, the packaging used should be agreed upon between supplier and buyer. Reusable packaging material such as jute sacks and mesh bags should be disinfected and thoroughly dried prior to reuse, so as to avoid contamination by previous contents. Packaging materials should be stored in a clean and dry place that is free from pests and inaccessible to livestock, domestic animals and other sources of contamination (UNIDO/FAO, 2005; Indiramma, 1995; Balasubramanyam and Indirama, 1989; Anadaraman and Reincium, 1980).

A label affixed to the packaging should clearly detail the product name of the spice, the plant name, the place of production, the harvest date and the names of the grower and the processor, and quantitative information. The label should also contain information indicating quality approval and comply with other national and/or regional labeling requirements. The label should bear a number that clearly identifies the production batch. Additional information about the production and quality of the plant materials may be added in a separate certificate, which is clearly linked to the package carrying the same batch number. Records should be kept of batch packaging, and should include the product name, place of origin, batch number, weight, assignment number and date. They should be retained for a period of three years or as required by national and regional authorities.

UNIDO/FAO (2005) reported that the International Trade Centre (UNCTAD/WTO) has produced a packaging manual (1999) for dried herbs and spices (ISBN 92-9137-114-9) and this reviews products and relevant packaging standards; explains various types of packaging methods and packaging materials used for handling and storage of such products; outlines current trends and highlights health, safety and environmental issues affecting spice packaging. There is continuing development with packaging materials and an example is CTMP (Chemi-Thermo-Mechanical Pulp) board which is aluminum-free but has aroma –barrier properties. There is also an increasing usage of irradiation by importing countries to sterilize herbs and spices and this requires special packaging by the exporting processors. The American Society for Testing and Materials has a standard guide (F1640-03) for packaging materials for foods to be irradiation of dried spices, herbs, and vegetable seasonings to control pathogens and other organisms (UNIDO/FAO, 2005). The International Atomic Energy Agency also lists packaging approved in

the USA and in the UK, for the packaging and irradiation of food. Some specific packaging requirements for major spice crops are detailed below (UNIDO/FAO. 2005).

2.11.2 Packaging for seeds and fruits

Vanilla, for most of the world trade, has grades of whole and split beans which are subdivided according to length into bundles, each containing 70-100 beans and packed into wax-lined cardboard boxes that hold 20-40 bundles. Beans remaining from the grading and sorting, such as short or broken beans, are bulked together and loosely packed into boxes.

Cardamom, when dried, needs to be kept in polypropylene bags to ensure the flavour components are retained and to prevent re-adsorption of moisture, mould growth and reduce insect pests. A sealing machine with a timer will aid efficient bagging.

Cloves when bagged should be stored in cool, clean dry buildings. Sound, properly dried cloves are relatively free from storage pests. Clove oils should be stored in full, airtight containers, and remain sealed until required. Bulk oil is usually transported in 200 litre metal drums.

Nutmeg and mace is graded according to size, and the bigger the mace, the better the premium. After drying, the nutmeg seeds are put into bags and transported to the processing factory for kernel separation and grading. After grading, nutmeg is bagged, labeled and fumigated prior to export. Nutmegs are usually packed in double-layered linen, jute, sisal or woven bags. If other packaging is used, care should be taken to avoid materials that might lead to sweating and mould development. Mace follows a similar process but there is a need for three months curing before bagging and fumigation and top quality mace is packaged in plastic bags.

2.11.3 Packaging for leaves and stems

It is essential that all material is dry to below 10% moisture content. The leaves and stems of dried herbs should be stored in cool, dark and dry areas of low humidity and polyethylene bags or packs used.

2.11.4 Packaging for flowers and buds

Saffron stigma, when dried and graded, should be stored in airtight containers, in a cool dry place out of the light.

2.11.5 Packaging for roots and rhizomes

Ginger when fresh should be stored in polyethylene bags with 2% ventilation to prevent dehydration and mould development. Ginger rhizome for bulk shipping can be packed in jute sacks, wooden boxes or lined corrugated cardboard boxes. Processed, dry ginger should be packaged in laminated bags that have low oxygen permeability, and stored in a cool dry environment.

Turmeric is mostly traded as whole rhizome and then processed into powder or oleoresin. The colour constituents of turmeric deteriorate with light, and to a lesser extent under heat and oxidative conditions. Ground turmeric should be stored in UV protective packaging and a cool dry environment.

2.11.6 Packaging for bark, wood and resins

Cinnamon and Cassia quills are graded on colour and pressed into cylindrical bales in jute cloth or corrugated cardboard cartons. Cinnamon and cassia, especially if ground, require polypropylene packaging and polyethylene is not recommended as the flavour components diffuse through it.

2.12 MAJOR SPICE CROPS IN WORLD TRADE

In terms of world trade value, the most important spice crops from the tropical regions are pepper, capsicums, nutmeg/mace, cardamom, allspice/pimento, vanilla, cloves, ginger, cinnamon and cassia, and turmeric. Coriander, cumin, mustard, and sesame seeds and the herbs sage, oregano, thyme, bay and the mints are the most important spice crops from non-tropical environments. The characteristics and environmental needs of the crops dominating the global spice trade are described below (UNIDO/FAO, 2005)

2.12.1 Black pepper

Black pepper (*Piper nigrum*) is a perennial vine, which produces a small berry fruit, which is dried to become pepper. Black pepper is a plant of the humid tropics requiring adequate rainfall and warmth for its growth. It is grown successfully between latitudes of 20° North and 20° South and from sea level up to an altitude of 2400m. The crop can tolerate a temperature range between 10° and 40°C but the optimum is between 25°C-40°C. A well-distributed rainfall in the range of 1250mm-2000mm is considered necessary for pepper production (UNIDO/FAO, 2005).

2.12.2 Capsicums, Chilli peppers and Paprika

Capsicums (*Capsicum annuum var. annuum; C. chinense; C. frutescens*) are the dried and processed fruit of these annual peppers. A rainfall of 600-1250mm is desirable. Rainfall is needed over the growing season but is not needed as the fruits ripen. Heavy rain during flowering adversely affects pollination and wetness at ripening encourages fungal spoilage. Capsicums flourish in warm sunny conditions, require 3-5 months with a temperature range of 18°C-30°C; below 5°C growth is retarded, and frost kills plants at any growth stage. A seedbed temperature of 20-28°C is the optimum for germination (UNIDO/FAO, 2005)

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2.12.3 Nutmeg, Mace and By-products

The perennial nutmeg tree (*Myristica fragrans*) grows to a height of 20m. Nutmeg is the kernel of the seed, while mace is the net like crimson coloured leathery outer growth (aril) covering the shell of the seed. The tree requires an optimal growing temperature between 20-30°C and the annual rainfall should be between 1500-2500mm (UNIDO/FAO, 2005)

2.12.4 Cardamom

Cardamom (*Elettaria cardamomum; E. major; E. speciosa*) is a tall growing (<5m) perennial herb and the fruit, borne on panicles at the base of the plants, is a trilocular capsule that contains 15-20 seeds. The natural altitudinal growing range is between 750-1500m while the most productive cultivated zone is between 1000 and 1200m. The annual rainfall is usually 2500-4000mm in the monsoon belt. A temperature range of 10-35°C occurs over the production areas with a lower limit of about 17°C and an optimum temperature within 22-24°C favoured.

Cardamom grows naturally in shade but will produce good yields in only partial shade if well watered (UNIDO/FAO, 2005).

2.12.5 Allspice – Pimento

Allspice (*Pimenta dioica*) is a forest tree cultivated to produce the dried fruit berries which are the product allspice. It grows in semi-tropical lowland forests with a mean temperature of 18-24°C and an annual rainfall of 1500-1750mm. Evenly spread rainfall is desirable but trees will grow well with rainfall between 1200-2500mm(UNIDO/FAO, 2005)

2.12.6 Vanilla

Vanilla **(***Vanilla planifolia, Mexican vanilla*); *V. pompona* (West Indian vanilla); *V. tahitensis* (Tahitian vanilla) are perennial vines which produce vanilla beans. Vanilla grows well in humid tropical climates with a well-distributed annual rainfall of 1900 – 2300mm but with no prolonged dry period. A warm humid climate with temperatures ranging between 24°C-30°C is preferred with a mean close to 27°C (UNIDO/FAO, 2005).

2.12.7 Cloves

The clove tree (*Syzygium aromaticum*) is an evergreen which grows up to 15m in height. The clove tree produces buds which are used whole or ground as a spice. Bud and stem oils and oleoresins, and leaf oil, are used principally as a source of eugenol. An optimal rainfall is 1750-2500mm, with a dry season and a temperature range of 15-30°C in a maritime environment (UNIDO/FAO, 2005).

2.12.8 Ginger

Ginger (*Zingiber officinale*) rhizome is the source of the most important ginger products which are dried rhizome, whole or ground as spice with ginger oil and oleoresins used as flavourings. A well-distributed yearly rainfall of 2500-3000mm is the optimum with a minimum rainfall of 1500mm. The crop flourishes under warm sunny conditions but day temperatures above 30°C can cause leaf scorching while temperatures above 37°C without humidity and water can cause plant death. Ground temperatures of 25-30°C are optimum for initial rhizome growth. Frost will kill the foliage and rhizomes near the surface but altitude as such is not a constraint to ginger production (UNIDO/FAO, 2005)

2.12.9 Cinnamon and Cassia

Cinnamon and cassia spices (*Cinnamomum verum; Cinnamomum cassia* (China); *C. burmannii* (Indonesia), and *C. loureirii* (Vietnam), are the prepared dried bark of the trees belonging to the genus *Cinnamomum*. Cinnamon is the hardiest among the tree spices, tolerating a wide range of soil and climatic conditions. The optimum climate has an average temperature between 27-30°C and 2000-2500mm of rainfall. Cinnamon is an evergreen tree that is kept to a height of 2-3m. The soil conditions are very important, as waterlogged soil will produce a bitter cinnamon bark (UNIDO/FAO, 2005)

2.12.10 Turmeric

Turmeric (*Curcuma longa*) is a plant of open forests with partial or intermittent shade desirable, although recent research has shown crop yields can be higher in open cultivation. An annual rainfall between 1000-2000mm is necessary with 1500mm being optimum. Adequate soil

moisture is the most significant factor affecting rhizome yield, the target product. Temperature is important, as the optimum varies with crop growth. High heat (30-35°C) is needed to encourage sprouting, 25-30°C during tillering, 20-25°C as rhizomes appear and 18-20°C during enlargement. The major spice crops outside the tropical environments are the flower stigma of saffron, the fruits ("seeds") of coriander and cumin, seed of mustard and sesame, and the leaves of bay, sage, origanum and thyme (UNIDO/FAO, 2005).

2.12.11 Saffron

Saffron (*Crocus sativus*) is a sterile hybrid and re-propagates annually by producing replacement daughter corms. The dried tri-lobed stigma of the saffron crocus flower is the commercial product. The autumn flowering saffron grows best in Mediterranean environments with cool moist winters and hot dry summers, and under this type of environment strategic irrigation will aid flower production (UNIDO/FAO, 2005)

2.12.12 Coriander

Coriander (*Coriandrum sativum*) is a strong smelling annual herb extensively grown in many climates throughout the world. In commerce, coriander is broadly divided into two types according to the size of the fruit, which in turn determines the volatile oil content and end use. The small fruited type var. *microcarpum* (diameter 1.5-3mm) is grown widely in cooler temperate regions while the larger fruited type var. vulgare (diameter 3-5mm) is grown in tropical and subtropical environments. The small-fruited types contain higher oil (0.5-2%), which is extracted for its essential oil while the larger fruit with a lower oil (<1%), is used for grinding and blending. The fresh leaf (cilantro) is used in Asian cuisine (UNIDO/FAO, 2005).

2.12.13 Cumin

Cumin (*Cuminum cyminum*), a small annual herb native to the Mediterranean region, which is grown for its aromatic dried fruit that is widely used in cooking. Cumin prefers areas with low atmospheric humidity during the period of flowering, seed formation and ripening (UNIDO/FAO, 2005).

2.12.14 Mustard seed

White Mustard (*Sinapis alba*) and Indian mustard (pictured; *Brassica juncea*) are annual or biennial herbs. They are cultivated world-wide for dried ripe seeds, and used extensively in prepared mustards or in food products. The extracted mustard oil is also widely used as a food flavour ingredient (UNIDO/FAO, 2005)

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2.12.15 Sesame seed

Sesame (*Sesamum indicum*), an annual herb, is now widely grown between 25°N and 25°S and can be grown to 40°N and 35°S. Sesame is grown for its seed in a wide range of environments from tropical to temperate and needs mean temperatures above 10°C and to 40°C maximum. The plant is killed by frost and, depending on cultivar, matures within 40 to 180 days (UNIDO/FAO, 2005)

2.13 ECONOMIC IMPACT AND TRADE

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). The major spices of international

trade have well known stable long-term markets but most species tend to be commodities and as such there is a competition and price fluctuation. The value of global spice imports is estimated at US$2 to 2.4 billion and in 2002 pepper topped the list with 20% of the total value followed by capsicum (18%), vanilla (13%), nutmeg/mace/cardamom (9%), spice seeds (8%) and ginger (6%). The major spice production is in the tropics from developing and least developed countries. There is also a very significant domestic consumption of spices in many spice-producing countries. The supply side of the industry has always been dynamic and has been punctuated by periodic relocations of the major production areas. To remain competitive, countries such as India are moving into the value-added sector, producing spice, essential oils, oleoresins, powders, specialty extracts and blends (UNIDO/FAO, 2005).

In addition, India has established spice Agri-Export Zones and they are actively developing capabilities in quality management, improved packaging and technology innovation in production and processing. The largest spice importer is the European Union (with Germany being the leading country in the EU). The USA and Japan are the two largest single country importers of spices. In the EU countries, 55-60% of the total spice and herb use is for industrial consumption, 35-40% by the retail sector and 10-15% by the catering sector (UNIDO/FAO, 2005). The high industrial sector use reflects the growing popularity of ready-to-use spice mixtures. Another reason is the increasing consumption of processed foods and ready to eat dishes, which often rely on spices and herbs to retain and enhance food flavour.

The ever more stringent regulations governing the international trade in spices and their derivatives for culinary use in food processing will mean that spice producers who ignore such specifications will eventually lose their markets to quality producers. For example, the EU

(European Union) has aflatoxin regulations to detect contaminated consignments and restrict their import while adulteration of spices with colourants has led to product bans.

Some two hundred essential oils are produced and traded internationally in volumes that range from 20-30,000 tonnes for orange oil to less than 100 kg for some specialty flower extracts. Prices vary widely, but for the majority of oils traded in volume, they fall within the range of US$4-$60/kg and for specialist minor oils, the price can be many hundreds of US$/kg (UNIDO/FAO, 2005). The citrus oils (orange, lemon, tangerine, lime, manadarin, grapefruit etc.) plus the mint oils have by far the largest number of important applications in tonnages as well as variety of flavours. The seasoning oils of spices and herbs (clove bud, coriander, cinnamon, garlic, nutmeg, onion etc) are traded in lesser quantities and have more selected applications. The majority of other oils is used in even lower quantities and has more targeted applications (UNIDO/FAO, 2005).

Estimating world production and trade of essential oils is fraught with difficulties (Verlet, 1993). In many countries domestic production statistics are not recorded and export statistics are recorded for some of the high volume oils and the rest are often included in codes that encompass a range of products. Therefore publications of global production must be treated with a high degree of suspicion as they are based on limited statistical data from a few countries and generally ignore factors such as domestic consumption. Mature markets, where demand for essential oils is highly developed, have low potential growth because of low population growth. The European Union is the world's biggest importer of essential oils (with France, Germany and UK being the major importing countries). The USA is the world's largest importing country of essential oils followed by Japan. Most of the oils go into mainstream industries and the number of mainstream users continues to decline through mergers and acquisitions. Aromatherapy is

seen as a potential high value growth area; however, it represents less than 1-2% of the total essential oil trade. It is predicted that the growth in essential oils will be led by the flavour oils (Green, 2002).

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 (Figure 2.2). The structure of the supply tree shows there are number 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 of herbs and spices is dried and cleaned for use in a crude form without further processing. The top three exporting countries for specific spices or groups of spices show the leading producing countries are in tropical environments, while countries in summer dry Mediterranean or continental environments are the major producers of spice fruits and seeds, saffron, thyme and bay leaves. The major spice trading countries are China, Madagascar, Indonesia and India while Guatemala, Brazil, Vietnam and Sri Lanka are significant traders. The value of that trade varies annually, and fluctuates about $US2.5 (Green, 2002).

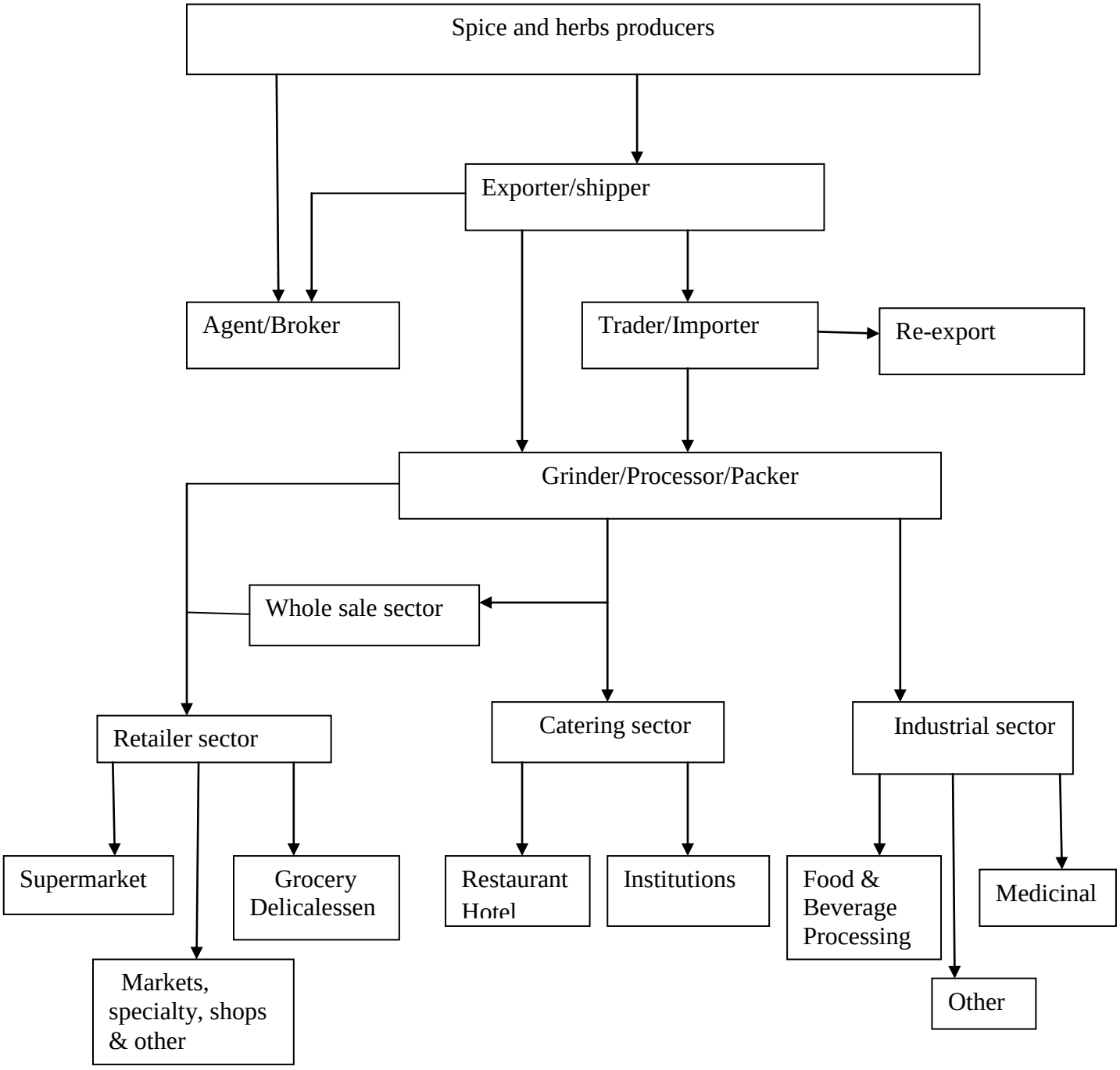


Figure 2.2: Trade distribution structure for spices and spice products

(Source: UNIDO/FAO, 2005**)**

2.14 SPICE PRODUCTS

Primary products

The primary products harvested for spice or essential oil production can be broadly divided into six categories: seeds and fruits, leaves and stems, flowers and buds, roots and rhizomes, and bark, wood and resins (UNIDO/FAO, 2005).

Secondary and Derived Products

The secondary and derived products are many and varied but the most common are spice mixtures (e.g. curry powders) and compounds extracted from the plant material such as essential oils or oleoresins. In cases where the primary spice does not meet the quality specification as a primary product it will often be purchased as a low value product extracted to produce the essential oil, oleoresin or aroma compounds. There is also considerable advantage to the industrial food processor purchasing standardized extracts of known quality, which have no microbial or other contaminants (UNIDO/FAO, 2005)

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Materials collection

The mature ripe *Piper nigrum* berries (Plate 3.1) was harvested from Aba-Adimula Farm in Fiditi town area in Afijio Indigenous Government Area of Oyo State. Mature *Aframomum danielli* (Plate 3.2) was harvested from Akpojeota farms in Effurun town near Warri in Uwie L.G.A, Delta State. Rice (*Oryza sativa*) NERICA variety was obtained from Nigeria Cereal Research Institute Apata, Ibadan, Oyo State. Orange flesh sweet potato (*Ipomea batatas)* variety was purchased at Ama-Hausa market along Owerri-Onitsha express way, Owerri, Imo State. Packaging materials used including Amber Plastic bottle was purchased from A&J Pharmaceutical Ltd, Egbu town, Owerri and High Density Polyethylene (HDPE) bag was purchased from a supplier located at Industrial layout, Irete, Owerri, Imo State.

The microbial media used were potato dextrose agar, Nutrient agar, Brain Heart Infusion Agar (BHIA) and MacConkey agar were purchased from Finlab Laboratory Chemical Supplier, Owerri, Imo State and Labstock Nig. Ltd, Yaba, Lagos State. Solvents used for extraction of flavour compounds include De-ionized water, Ethanol (98% assay), 40% Ethanol, Methanol, Acetone, n-Hexane and purchased from Finlab Laboratory Chemical Supplier, Owerri, Imo State. High performance liquid chromatography and gas chromatograph-mass spectrum grade reagents used are Acetonitrile, acetic acid, ethanol and methanol. These analytical grade reagents were purchased from Labstock Nig. Ltd., Lagos State.

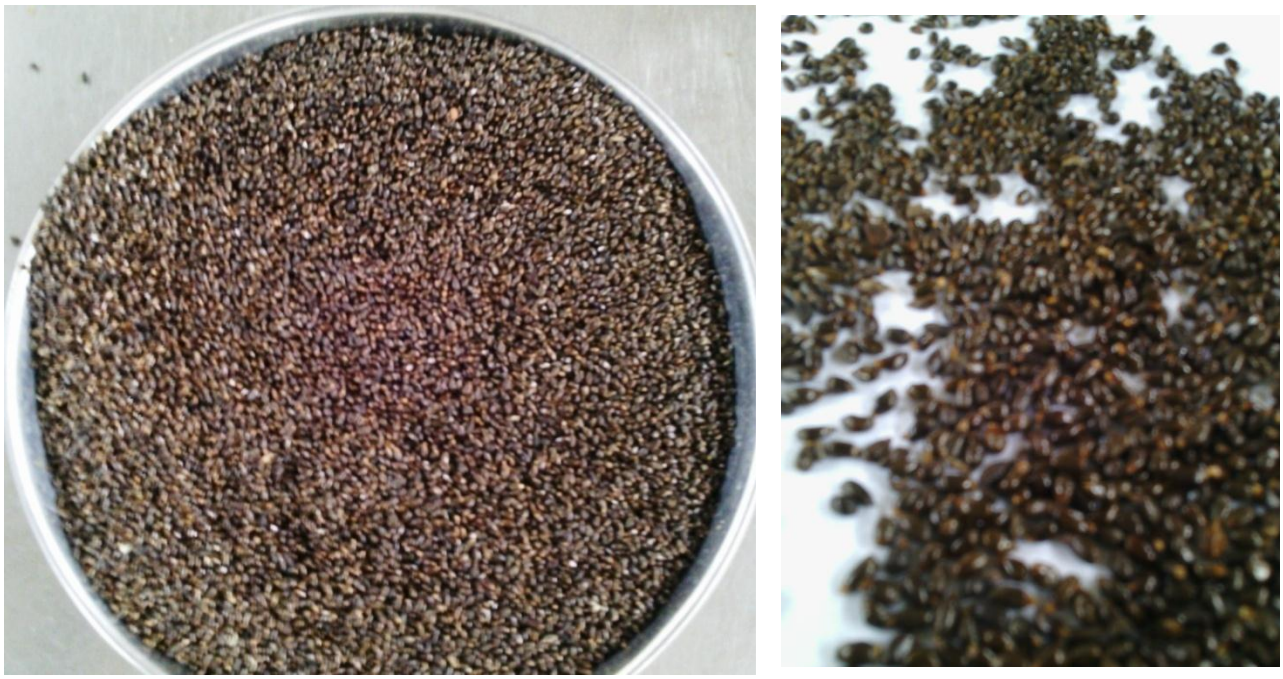
Plate 3.1: Harvested Black pepper berries

3.2 METHODS

3.2.1 Post harvest preparation of black pepper and *Aframomum danielli* spices

The method of International Phytosanitary requirements for pepper described by Nair (2004) was modified for the post-harvest preparation of these spices. Harvested black pepper berries (Plate 3.1) were sorted manually to remove all the extraneous matter. The fresh berries were dipped in hot water of 65^{o}C for 10minutes to reduce vegetative microbial load and aid the drying. Then the berries were subjected to sun-drying for 5-7days by spreading on high density black Polythene in order to reduced the moisture content. The dried product was dried to ≤10% final moisture content and stored in air tight plastic containers for further work.

The mature *Aframomum danielli* pods were sorted to separate ripe from unripe pod. Then, the pods were dipped in hot water (65^{o}C) for 15 minutes and drained. Drained pods were subjected to sun-drying process for two (2) weeks or till a moisture level ≤10^{o}C was obtained. *Aframomum danielli* seeds (Plate 3.3) were removed by breaking the dried pods with the aid of kitchen knife, and the seeds weight was determined, as well as final moisture content. The sample was packaged in an air tight plastic container for further use.



Aframomum danielli ‘bastered meleguta’ seeds

Plate 3.2: *Aframomum danielli* seeds

3.2.2 Rice grits (carrier) preparation

The method described by Singh (1997) was modified for the processing of high quality rice grits. The rice paddy was parboiled at 70^{o}C for 15 min and then dried in a hot oven at 65^{o}C for 8h. It was then passed through abrasive dehulling machine several times to remove the husk and bran. The grains collected were blanched at 65^{o}C for 20min, then washed and drained to remove excess water. Blanched rice grains were dried at 75^{o}C in a cabinet dryer and milled into grits with a Warring blender (model: HGB2WTG4). The milled grits was sieved through 120microns mesh sieve size and obtained 120micons rice grits particle size (Jillavenkatesa *et al*., 2001). The rice grits was stored and packaged in an air tight polyethylene bag for further use. The Figure 3.1 showed the steps used for the production of rice grits.

3.2.3 Potato grits (carrier) preparation

The method described by Tewe *et al*., (2003) was used for potato grits preparation. The potato was subjected to some preliminary operations such as peeling under water that contained 0.2% Potassium meta-bisulphate in order to prevent browning, washing, slicing (5mm thick), blanched at 65^{o}C for 20min and drying at 75^{o}C in a cabinet dryer for 8h. The dried potato slices was cooled at ambient temperature (30$\pm$ 2^{o}C) and milled into grits using Warring blender (model: HGB2WTG4. The grits was sieved through a 120microns mesh sieve and obtained 120micons potato grits particle size (Jillavenkatesa *et al*., 2001). The potato grits was stored in an air tight polyethylene bag for further use. The Figure 3.2 showed the steps used for the production of potato grits.

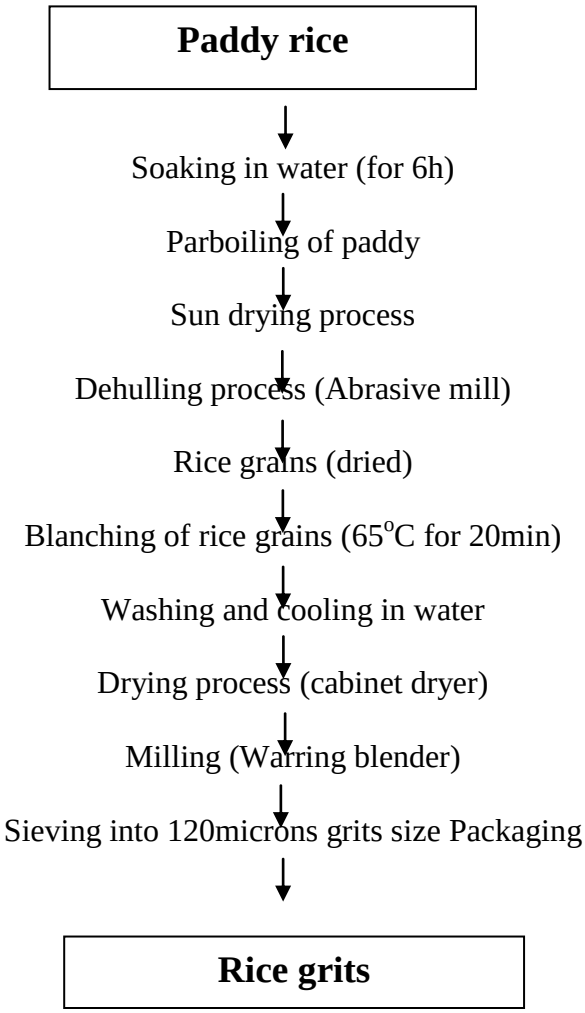


Figure 3.1: Flow chart for white rice grits carrier processing

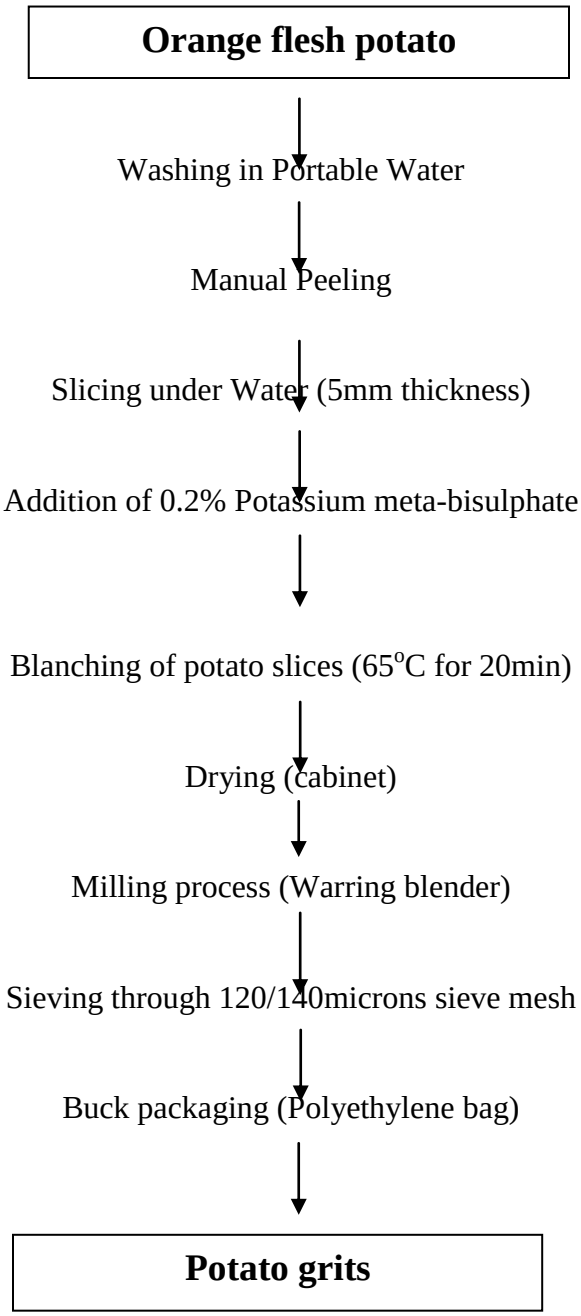


Figure 3.2: Flow diagram for processing of potato grits carrier

3.3 PREPARATION OF DIFFERENT FORMS OF SPICES

The following forms of spices were prepared: pulverized powder; liquid-extract and extract-coated form (Plate 3.3.1 and Plate 3.3.2) were prepared from each spicy seeds.

3.3.1 Spice pulverized powder

The powded product from each spice was prepared using the method described by Fasoyiro *et al.,* (2006). Six hundred grams (600g) each of dried spice materials (*Piper nigrum* and *Aframomum danielli*) was ground using Warring blender (model: HGB2WTG4). The milled powder was sieved by passing through sieve (200μm sieve aperture), allowed to cool and then packaged in an air-tight polyethylene bag.

3.3.2 Solvent extraction of flavour compound(s)

The methods described by Fasoyiro *et al.,* (2006) and Chang *et al*., (1988) were combined for the extraction of flavour components (3.4) from spice materials. Each of the pulverized spice powder samples was mixed at a ratio of 1:5 (w/v) into the solvent (which include; 98% ethanol, 40% ethanol, acetone, n-Hexane, methanol and de-ionized water as control). The mixture was centrifuged at 3000rpm for 10min and transferred into a beaker covered with lid and kept at ambient condition for a period of 48h. The mixture was filtered through Whatman No. 2 filter paper. The filtrate was further concentrated by evaporation in a rotary vacuum evaporator at 40^{o}C and obtained 1:1 ratio of spice bioactive flavour extract to solvent. The spice extracts from the different solvents was evaluated for intensity of flavour principles perceived, desirability of the aroma, cost and food compatibility of solvent were used to select just one solvent for continuation of the study.

LIQUID EXTRACT, POWDERIZED AND ENCAPSULATED BASTERED MELEGUTA *AFRAMOMUM DANIELLI*' FLAVOUR SAMPLES

LIQUID EXTRACT, POWDERIZED AND ENCAPSULATED
PIPER NIGRUM 'UZIZA' FLAVOUR SAMPLES

Plate 3.3.1: Packaged flavour samples from *Aframomum danielli and Piper nigrum*

FLAVOUR PRODUCTS FROM BASTERED MLEGUTA
'*Aframomum danielli seeds'*

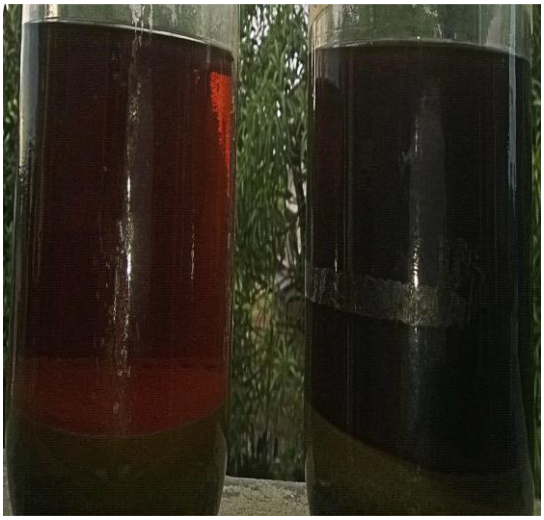
FLAVOUR PRODUCTS FROM BLACK PEPPER
'*Piper nigrum*

Plate 3.3.2: Flavour products from *Piper nigrum* and *Aframomum danielli*'

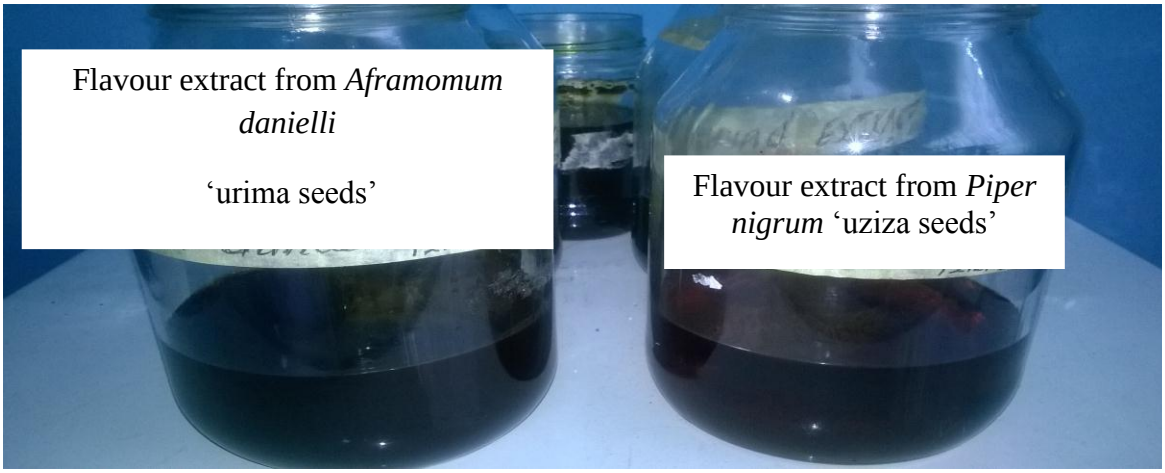
Piper nigrum and *Aframomum danielli*

BEFORE FILTRATION PROCESS

ZERO HOUR EXTRACTION PROCESS TIME OF *Piper nigrum* and *Aframomum danielli*' FLAVOUR



48 HOUR EXTRACTION PROCESS TIME OF *Piper nigrum* and *Aframomum danielli*'FLAVOUR



CONCENTRATED FLAVOUR EXTRACTED FROM 1:1 (W/V) *Piper nigrum* AND *Aframomum danielli*

Plate 3.4: Stages in solvent extraction of flavour compounds from *Piper nigrum* and *Aframomum danielli*'

3.3.3 Encapsulated spice flavour compounds samples

Microencapsulation or entrapment of the extracts flavour compounds from the chosen solvent (40% ethanol) for each spice was carried out using a modified method of Jung and Sung (2000). The development of the encapsulated flavour compounds extracted was carried out as described in Table 3.1. Two types of edible carrier's base or food vehicles (grits from potato and rice respectively) were used for the encapsulation of the spice flavour extracts. The spice extracts from chosen food compatible solvent with highest flavour intensity was dispersed into each carrier (food vehicle). The formulation was done using varied ratios (2:1, 3:1 w/v) of edible carrier and liquid flavour extract. This mixture was homogenized in a shear homogenizer for 5 min at 800-1000rpm until the carrier and extracts are mixed completely. The resultant slurry was lyophilized in a Telstar freeze-dryer (model: Bomb as de Vacio-2G6). The lyophilized sample was packaged in high density polyethylene bags and stored for further examination and study.

3.4 PACKAGING AND STORAGE METHODS FOR SPICE PRODUCTS

Each sample of the spice products: (powder, liquid extract and encapsulated spice product) was packaged in two types of packaging materials which include plastic bottle (amber type) and HDPE container. The samples were stored in desiccators under tropical ambient temperature ($28 \pm 3^{o}C$) for six months. Retention of flavour principles of the spice products was determined at two weeks interval for the first two months of storage and 4weeks interval for the remaining four months.

Table 3.1: Formulation of encapsulated spice extract product

Sample code	Spice	Extraction	Carrier type	Encapsulation ratio
PP_{ERC1}	*Piper nigrum*	40% Ethanol	Rice grits	1:2
PP_{ERC2}	*Piper nigrum*	40% Ethanol	Rice grits	1:3
PP_{EPC1}	*Piper nigrum*	40% Ethanol	Potato grits	1:2
PP_{EPC2}	*Piper nigrum*	40% Ethanol	Potato grits	1:3
AFD_{ERC1}	*Aframomum danielli*	40% Ethanol	Rice grits	1:2
AFD_{ERC2}	*Aframomum danielli*	40% Ethanol	Rice grits	1:3
AFD_{EPC1}	*Aframomum danielli*	40% Ethanol	Potato grits	1:2
AFD_{EPC2}	*Aframomum danielli*	40% Ethanol	Potato grits	1:3

Key:

$PP_{ERC=}$ 40% ethanol extract of *Piper nigrum* coated in Rice-grits Carrier
$PP_{EPC=}$ *Piper nigrum* Extract with Potato-grits Carrier
$AFD_{ERC=}$ 40% ethanol extract of *Aframomum danielli* Extract with Rice-grits Carrier
$AFD_{EPC=}$ *Aframomum danielli* Extract with Potato-grits Carrier

3.5 CHEMICAL ANALYSIS

3. 5.1 Moisture Content

The standard method of the A.O.A.C (2010) was used. Two gram-portions of each grated samples was weighed into previously weighed dry crucibles with lid. The crucibles with samples were then dried in an oven (Astel Hearson, England), at 105^0C for 24 h. The crucibles with contents was cooled in a desiccators and weighed, then put back into the oven and the operation was repeated until a constant weight was obtained. The loss in weight obtained represented the moisture content and calculated with the following formula:

$$\% \text{ Moisture content} = \frac{W_1 - W_2}{W_1} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{Equation 3.7}$$

where:

W_1 = is the weight of sample before drying.

W_2 = is the weight of sample after drying.

3.5.2 Determination of Specific gravity of Liquid Extract

The specific gravity of the liquid extract samples was determined using the AOAC (2010) recommended method. A known volume of distil water was filled in a specific gravity bottle and take the weight of both bottle and water was taken. The distilled water was removed and dried the bottle in an oven at 40^oC for 10min. This was followed by filling the dried bottle with liquid spice flavour extract and determined the weight.

$$\text{Calculation: } \frac{\text{Weight of bottle with distil water}}{\text{Weight of the bottle + liquid flavour extract}} \quad \ldots\ldots\ldots\ldots\ldots\text{Equation 3.8}$$

3.5.3 Determination of Alkaloids

This was done by the alkaline precipitation gravimetric method described by AOAC (2010). Two grams (2g) of sample (from each spice flavour extracts, spice residue) was weighed and dispersed in 10% acetic acid solution in ethanol in a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28^{o}C. It was later filtered via Whatman No. 42 grade of 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 alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80^{o}C for 4h. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Calculation:

$$\% \text{ Alkaloid} = \frac{\text{Weight of precipitate}}{\text{Initial weight of sample}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 3.1}$$

3.5.4 Determination of Flavonoid

This was determined according to the method of AOAC (2010). Five grams of the sample (from each spice flavour extracts, spice residue) 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 of the extract was treated with equal volume of ethyl acetate starting with drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Calculation:

$$\text{Flavonoid (\%)} = \frac{\text{Weight of precipitate}}{\text{Initial weight of sample}} \times \frac{100}{1} \quad \text{..... Equation 3.2}$$

3.5.5 Tannins

The method of AOAC (2010) was used for the determination of tannin contents of the differently prepared samples (from each spice flavour extracts, spice residue). Zero point two grams (0.2 g) of finely ground sample was measured into a 50 ml beaker. A 20 ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80^{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. A 1 ml of sample extract was pipetted into 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2C0_3 was added into the mixture. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20min when bluish-green coloration developed. A standard Tannic Acid solution of range 0-10ppm was treated similarly as 1ml of sample above. The absorbance of the Tannic acid standard solutions as well as samples was read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm.

Percentage tannin was calculated using the formula:

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample}} \times 100 \quad \text{.....Eqn 3.3}$$

3.5.6 Saponin

The Spectrophotometry method of AOAC (2010) was used for Saponin analysis. One gram (1g) of sample (from each spice flavour extracts, spice residue) 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. 1mL of the colourless solution was pipette into 50 mL volumetric flask and 2mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. A 0-10ppm standard Saponin solution was prepared from Saponin stock solution. The standard solution was treated similarly with 2mL of 5% FeCl solution as done for 1mL sample 3 above. The absorbance of the sample as well as standard Saponin solutions was read after colour development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm.

Percentage Saponin was calculated using the formula:

$$\text{Saponin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x}} \times 100 \quad \text{……..Eqn 3.4}$$

3.5.7 Phytate

This was determined according to the method of AOAC (2010). One gram/mL of sample material from each spice flavour extracts, spice residues) was added with 0.2N HCl such that we have 30µg ml phytate solution and 0.5ml of extract was pipetted into a test tube fitted with a ground glass stopper, then, added 1mL of solution (2), cover the tube with the stopper with the aid of clip. The tube was heated in a boiling water bath for 30 minutes. Care was taken to ensure

that the first 5min the tube remains well stoppered, after cooling in ice water for 15 minutes, they were allow to adjust to room temperature. Once the tubes have reached room temperature, the mix content of the tube was centrifuged for 30min at 3000rpm. Then 1ml of the supernant was transferred into another test tube and added 1.5ml of solution (3). Measure the absorbance at 519nm against distilled water. The method was calibrated with the reference solutions as a substitute for the sample solution with each set of analyses. Preparation of the calibration curve was carried out by plotting the concentrations of the reference solutions against their corresponding absorbance. Then the absorbance of the test sample is used to obtain the concentration from the calibration curve.

Phytate =

$$\frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample}} \times 100 \quad \ldots\ldots \text{Eqn } 3.5$$

3.5.8 Oxalates Determination by Titration Method

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

Digestion:

Two grams (2g) of sample was suspended in 190mL of distil water in a 250ml volumetric flask. This was followed by the addition of 10mL of 6M HC1 and the suspension was digested at 100°C for 1hour. It was cooled to 30^{o}C and then made up to 250ml mark before filtration.

Oxalate Precipitation:

Duplicate portions of 125ml of the filtrate are 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 changes from salmon pink colour to a faint yellow colour (pH 4 - 4.5). Each portion was then heated to 90°C, cooled and filtered to remove precipitate 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 minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

Permanganate Titration:

At this point, the total filtrate resulting from 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.

$$\frac{T \times (Vme)(Df) \times 10^5}{(ME) \times Mf} \quad (mg/100g) \qquad \text{Equation 3.6}$$

where

T = the litre of $KMnO_4$ (ml),

Vme = 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 flour used.

3.6 DETERMINATION OF FLAVOUR PRINCIPLES (COMPOUNDS)

3.6.1 Analysis of Flavour principles

The flavour principles was analyzed using GC-MS (Shimadzu prominence model: GCMS-QP2010 ultra fast mass spectrometry and auto sampler, by Shimadzu Scientific Instrument, Kyoto, Japan) and MS Detector.

The procedure described by Vipul *et al.* (2013) was modified for the identification and quantification of flavour compounds in *Piper nigrum* 'black pepper spice samples. The operation conditions for identification and quantification of chemical components in spice flavour samples include GC initial temperature of 50^{o}C, injection temperature of the system was 250^{o}C and the injection mode was split 1:2 and gas type used was helium as mobile phase. The column name is Rtx.5MS 30m x 0.25um x 0.25mm and Column Oven Temperature Program: 50^{o}C (2min) to 9^{o}C/min to 300^{o}C (2min). Mass spectrometry (MS Detector) used was ion source temperature 200^{o}C, interface temperature of 250^{o}C and acquisition mode was scan mode. Library search for each chromatogram spectrums was carried out using Wiley GC-MS library.

The method described by Adegoke *et al.*, (2013) was used for the determination of flavour principles in *Aframomum danielli* samples. The operation conditions for determination of chemical components in *Aframomum danielli* flavour samples include GC initial temperature 32^{o}C, injection temperature was 250^{o}C, injection mode of sample was splitless and the gas type for mobile phase was Helium. The column oven temperature program was 32^{o}C (2min) to 10^{o}C/min to 300^{o}C and column name was Rtx.5MS 30m x 0.25um x 0.25mm. Mass spectrometry (MS Detector) was ion source temperature 200^{o}C, interface temperature of 250^{o}C and acquisition Mode was scan mode. Library search was carried out using Wiley GC-MS library.

The operation condition for Scanning of flavour compounds include: GC initial temperature of 32^{o}C, injection temperature was 250^{o}C, injection mode was split 1:1 and gas type was Helium. The column oven temperature program was 32^{o}C (2min) to 10^{o}C/min to 300^{o}C (2min) Column name was Rtx.5MS 30m x 0.25um x 0.25mm. Mass spectrometry (MS Detector) was ion Source temperature 200^{o}C, interface temperature of 250^{o}C and acquisition Mode was scan mode. Library search was carried out using Wiley GC-MS library.

3.7 SENSORY EVALUATION

The sensory evaluation was carried out using the scoring test method described by Iwe (2002). The sensory characteristics of the products such as taste, aroma (flavour) and general acceptability were examined by a team of twenty (20) semi-trained panelists for product-oriented test and fifty (50) panelists for consumer preference test. These tests were done in order to determine perceptible difference on the sensory attributes of the samples during and after development.

Consumer-oriented test was conducted to determine product acceptability and preference on selected food dishes using the 7-points hedonic scale structured questionnaire (appendix Ia). Also the panelists were asked to recommend foods for each of the spice products has provided in the questionnaire (appendix Ib).

Three forms of each spice samples were used to spice most recommended dishes ‘pepper soup’. Spice flavoured pepper soup was prepared by making pepper soup broth. A 1L of pepper soup broth was made by boiling 1.5L of portable water with added 500g of beef meat cuts, pinch of grounded fresh pepper, one grated onion and pinch of salt to taste. The mixture was boiled for 30min and divided into 6 portions. A 200mL of the pepper soup stock was added with 5g of each

spice sample (pulverized, liquid flavour extract and 2:1v/w rice grits or potato grits extract-coated flavour form). The mixture was stirred thoroughly and allowed to cool to 40^{o}C prior to sensory evaluation. These 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.

3.8 MICROBIOLOGICAL EXAMINATIONS

The total plate count, heterotrophic fungi plate count and biochemical characterization of the microflora were determined on raw spice samples and pulverized *Piper nigrum* and *Aframomum danielli*.

3.8.1 Determination of Microbial Population

Microbial count was done according to ICMSF (2002). Ten grams (10g) of each powder was dispersed in 90mL of peptone water and serially diluted into 10-folds dilution using sterile peptone water, and then homogenized by shaking vigorously. An aliquot portion (0.1ml) of 3rd up to 9th dilution was inoculated in duplicate onto the potato dextrose agar (PDA), nutrient agar (NA) and MacConkey agar (MA) for the isolation of heterotrophic fungi and bacteria 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 at ambient temperature (28$^{o}\pm$02^{o}C) for fungal growth. The nutrient agar plates were spread evenly with a sterile spreader and incubated for 24-48h at a temperature of 37^{o}C for total viable count (Pelezar *et al.* 1993). The MacConkey plate agars were incubated at 37^{o}C for 24h for coliform bacteria (Pelezar *et al*. 1993; Cheesbrough, 2000). The Brain Heart Infusion Agar plates were incubated at 48-50^{o}C 24-48h for spore formers (ICMSF, 2002).

3.8.2 Spore Staining Test

The spore staining was used to confirm the presence of spores when indicated in the Gram stain. Isolates were heat-fixed on a slide and flooded with 5% malachite green. It was steamed for 3 minutes (without allowing it to boil), dried, cooled and then rinsed off and stained with Safranin for 30 seconds. This was also rinsed, dried with filter paper and viewed under the microscope using oil immersion lens. It is expected that positive spores will be green while the non-spore former will be stained pink (ICMSF, 2002).

3.8.3 Identification of Isolates

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 was matched against those in Buchannan and Gibbon (1994), while those of the yeast isolates were matched with features presented in Harrigan and McLance (1990).Cultures of moulds were identified based on macro and micro morphology, reverse and surface coloration of colonies grown on PDA (Harrigan and McLance, 1990).Bacterial colony count was done using the Gallenkamp electronic colony counter. A magnifying lens was used in counting fungal colonies.

3.8.4 Biochemical tests

3.8.4.1 Catalase test

This was carried out to determine the ability of isolates to produce catalase, which liberates oxygen from hydrogen peroxide. Each isolate was picked using sterile inoculating loop and emulsified in a drop of hydrogen peroxide on a clean glass slide. The appearance of gas bubbles after a few seconds indicated a positive catalase test (ICMSF, 2002).

3.4.2 Coagulase test

This test differentiates Staphylococcus aureus which produces the enzyme, coagulase from *S. epidermidis* and *S. saprophyticus* which do not produce coagulase. Physiological saline solution of 0.85% NaCl was prepared and a drop was placed on a clean slide. A colony of the test organism was emulsified in 1ml of dilute plasma to give a milky suspension, then thoroughly mixed within 10 seconds, clumping (coagulation) showed a positive result, while non-clumping showed a negative result (Benson, 2005).

3.8.4.3 Oxidase test

Oxidase test is an important differential procedure that should be performed on all gram negative bacteria for their rapid identification. 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 which all Staphlococci are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram negative bacteria) the N, N-dimethhyl-p-phenylenediamine oxalate and ∞-naphthol react to indophenol blue. *Pseudomonas aeruginosa* is an oxidase positive organism. Hence, the oxidase reagent, 1% tetramethyl-p-phenylene-diamine dihydro-chloride was used. It was prepared by measuring little quantity (5ml) of oil then dissolved in 100ml of distilled water. Strips of whatman No 1 filter paper was soaked in a freshly prepared 1% solution of the reagent. The various isolates were streaked on a wetted stips of filter paper. Deep purple color appearance within 10 seconds indicates oxidase positive. The absence of purple coloration indicates a negative oxidase reaction (Holt, 1984).

3.8.4.4 Sugar fermentation/oxidation

This test is used to differentiate between bacteria groups that oxidize carbohydrate such as members of *Enterobacteriaceae*. One milliliter (1ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes containing 9ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. 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, one control sample 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 indicates acid production in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction (ICMSF, 2002).

3.8.4.5 Hydrogen sulphide production (H_2S) test

The test isolates from pure culture was aseptically inoculated into a tube containing Kligler iron agar (KIA) by streaking the surface of the slant agar. 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 indicates H_2S production indicates positive H_2S and no blackening means no H_2S production (Clarke, 1953).

3.8.4.6 Urease test

Urease Agar slant was prepared in a McCartney bottle, and then inoculated the agar slant by streak-inoculate the entire agar surface with heavy with inoculum. The bottle containing the

inoculate was anaerobically incubated att 35^{o}C for 24hours. A pink colour in the medium indicated a positive result (Collin *et al*., 1995).

3.8.4.7 IMViC test

This test consists of four different tests; they are Indole production, Methyl-Red test, Voges Proskaeur test and Citrate utilization test. This test is 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

Production of indole is detected using Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

Procedure:

Bacteria from the pure culture were inoculated in peptone water, which contains amino acid tryptophan and incubated overnight at 37^{o}C. Following incubation few drops of Kovac's reagent was added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol and con. HCl. Ehrlich's reagent is more sensitive in detecting indole production in anerobes and non-fermenters. Formation of a red or pink coloured ring at the top was taken as positive (Cheesbrough, 2000)

Methyl red (MR) test

This was done to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose

fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less.

Procedure:

The bacteria from isolate were inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37^{o}C for 48 hours. Over the 48 hours the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium was tested by the addition of 5 drops of MR reagent. Development of red color was taken as positive. MR negative organisms produce yellow color (Cheesbrough, 2000).

Voges Proskauer (VP) test

While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol producers.

Procedure:

The bacteria from pure culture isolate was inoculated into glucose phosphate broth and incubated for at least 48 hours. A 0.6 ml of alpha-naphthol was added to the test broth and shaken. A 0.2 ml of 40% KOH was added to the broth and shaken. The tube was allowed to stand for 15 minutes. Appearance of red color was taken as a positive test. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents (Cheesbrough, 2000).

Citrate utilization test

This test was done to detect the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH

indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen.

Procedure:

Bacterial colonies from pure culture was picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37^{o}C. If the organism has the ability to utilize citrate, the medium changes its color from green to blue. The blue colour observed indicate positive result (Cheesbrough, 2000).

3.9 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A 2x6 factorial design comprising of 2 types of indigenous spices (*Piper nigrum* and *Aframomum danielli*) and six solvents giving a total of twelve (12) samples were used at the preliminary phase (up to 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 when adopted during the product development phase of this research work. The total number of samples was 36 in all. Performance data were presented as tables', graphs and charts where necessary. All data was subjected to descriptive test, and analysis of variance (ANOVA). Multiple comparison tests (turkey and Duncan's tests) were used to separate means where significant differences exist. Statistical package for social science (SPSS) 20.0 Software Inc. USA was used.

The data obtained from different analyses were subjected to various statistical analyses which include simple descriptive mean and standard deviation, analyses of variance (ANOVA), while turkey's test was used to separate the means using Statistical Package for Social Science (SPSS) 20.0 Software Inc. USA.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

Table 4.1: Moisture content of pulverized and encapsulated flavour principles

Spice sample	Sample code	Moisture % (*Piper nigrum*)	Moisture content % (*Aframomum danielli*)
Powder	Powder	$8.22^{a}\pm0.02$	$7.01^{a}\pm0.01$
Rice grits coated spice extract at ratio 1:2	RG-C_1	$6.88^{c}\pm0.03$	$6.45^{b}\pm0.02$
Rice grits coated spice extract at ratio 1:3	RG-C_2	$6.08^{e}\pm0.01$	$6.12^{c}\pm0.02$
Potato grits coated spice extract at ratio 1:2	PG-C_1	$7.11^{b}\pm0.02$	$6.96^{a}\pm0.01$
Potato grits coated spice extract at ratio 1:3	PG-C_2	$6.32^{d}\pm0.01$	$6.01^{d}\pm0.02$
LSD ($p<0.05$)	-	0.14250	0.07890

($\pm$) Standard deviation of triplicate sample, mean scores with different superscript in the column are significantly different ($p<0.05$). LSD=Least significant difference

Table 4.2 Specific gravity values of solvents and spice extracts

Flavour extract	**Specific gravity of solvents**	***Piper nigrum* flavour extract**	***Aframomum danielli* extract**	**Increase Specific gravity of *Piper nigrum* flavour extract**	**Increase Specific gravity of *Aframomum danielli* extract**
Absolute ethanol extract	0.795	$1.579^{a}\pm0.02$	$1.407^{c}\pm0.02$	$0.784^{a}\pm0.02$	$0.612^{b}\pm0.01$
40% ethanol extract	0.935	$1.561^{b}\pm0.01$	$1.505^{a}\pm0.02$	$0.626^{c}\pm0.02$	$0.570^{c}\pm0.01$
Methanol extract	0.791	$1.541^{c}\pm0.01$	$1.423^{b}\pm0.03$	$0.750^{b}\pm0.03$	$0.632^{a}\pm0.01$
Acetone extract	0.790	$1.366^{e}\pm0.01$	$1.282^{e}\pm0.01$	$0.576^{d}\pm0.01$	$0.492^{d}\pm0.01$
n-Hexane extract	0.659	$0.97^{f}\pm0.02$	$0.884^{f}\pm0.01$	$0.320^{f}\pm0.0$	$0.228^{f}\pm0.01$
Water extract	1.0	$1.435^{d}\pm0.02$	$1.320^{d}\pm0.01$	$0.435^{e}\pm0.01$	$0.320^{e}\pm0.0$
LSD ($p<0.05$)	-	0.0511	0.0301	0.0523	0.0355

($\pm$) Standard deviation of triplicate sample, mean values with different superscript in the column are significantly different ($p<0.05$). LSD=Least significant difference

4.3 PHYTO-CHEMICAL IN THE SOLVENT EXTRACTS OF *PIPER NIGRUM* AND *AFRAMOMUM DANIELLI*

Table 4.7: Values of Phyto-chemicals in the solvent extracts of *Piper nigrum* and *Aframomum danielli*

Type of solvent extract	*Piper nigrum* ('Uziza' spice)			*Aframomum danielli* ('Urima' spice)		
	*Solvent extract	*Spice Extraction residue	*Total	*Solvent extract	*Spice Extraction residue	*Total
			Alkaloid (%)			
Ethanol (absolute)	$6.54^{a}\pm0.01$	$0.97^{f}\pm0.01$	$7.51^{a}\pm0.03$	$7.08^{a}\pm0.01$	$1.82^{b}\pm0.02$	$8.89^{a}\pm0.02$
Ethanol 40%	$3.91^{b}\pm0.01$	$1.89^{b}\pm0.01$	$5.79^{b}\pm0.02$	$4.12^{b}\pm0.01$	$2.64^{a}\pm0.01$	$6.76^{b}\pm0.01$
Methanol	$2.08^{c}\pm0.01$	$1.49^{c}\pm0.02$	$3.57^{d}\pm0.01$	$3.72^{c}\pm0.01$	$0.38^{e}\pm0.02$	$4.09^{e}\pm0.02$
Acetone	$1.24^{d}\pm0.01$	$1.24^{e}\pm0.02$	$2.47^{e}\pm0.01$	$2.83^{f}\pm0.01$	$0.67^{d}\pm0.01$	$3.50^{f}\pm0.01$
n-hexane	$1.04^{e}\pm0.01$	$1.42^{d}\pm0.01$	$2.46^{e}\pm0.0$	$2.98^{e}\pm0.01$	$1.25^{c}\pm0.0$	$4.23^{d}\pm0.02$
Distilled water	$2.11^{c}\pm0.01$	$2.26^{a}\pm0.01$	$4.37^{c}\pm0.03$	$3.10^{d}\pm0.01$	$1.79^{b}\pm0.01$	$4.89^{c}\pm0.02$
LSD ($p<0.05$)	0.01118	0.01472	0.02102	0.01323	0.00764	0.01915
			Flavonoid (%)			
Ethanol (absolute)	$5.66^{a}\pm0.01$	$2.42^{a}\pm0.01$	$8.08^{a}\pm0.03$	$6.45^{a}\pm0.01$	$1.71^{d}\pm0.02$	$8.16^{a}\pm0.0$
Ethanol 40%	$5.56^{b}\pm0.01$	$2.29^{b}\pm0.02$	$7.86^{b}\pm0.04$	$5.10^{a}\pm0.01$	$0.09^{f}\pm0.01$	$5.19^{e}\pm0.0$
Methanol	$4.90^{d}\pm0.02$	$0.41^{d}\pm0.01$	$5.32^{c}\pm0.01$	$3.27^{a}\pm0.02$	$2.58^{b}\pm0.02$	$5.84^{d}\pm0.01$
Acetone	$5.18^{c}\pm0.01$	$0.07^{e}\pm0.01$	$5.25^{c}\pm0.0$	$3.22^{a}\pm0.02$	$1.53^{e}\pm0.01$	$4.74^{f}\pm0.01$
n-hexane	$2.05^{f}\pm0.01$	$1.34^{c}\pm0.02$	$3.37^{e}\pm0.02$	$3.91^{a}\pm0.03$	$2.74^{a}\pm0.03$	$6.65^{b}\pm0.01$
Distilled water	$4.02^{e}\pm0.02$	$0.47^{d}\pm0.03$	$4.49^{d}\pm0.01$	$2.41^{a}\pm0.02$	$2.09^{c}\pm0.03$	$6.51^{c}\pm0.02$
LSD ($p<0.05$)	0.01323	0.01472	0.01915	01.16052	0.020	0.01756
			Phytic acid (%)			
Ethanol (absolute)	$7.72^{a}\pm0.02$	$1.56^{c}\pm0.01$	$9.28^{a}\pm0.0$	$4.19^{a}\pm0.0$	$1.03^{f}\pm0.02$	$5.22^{b}\pm0.03$
Ethanol 40%	$5.91^{b}\pm0.02$	$3.02^{a}\pm0.0$	$8.92^{b}\pm0.03$	$3.11^{b}\pm0.01$	$1.42^{e}\pm0.03$	$4.53^{e}\pm0.04$
Methanol	$5.82^{c}\pm0.01$	$1.21^{e}\pm0.02$	$7.03^{d}\pm0.0$	$2.83^{c}\pm0.0$	$2.0^{d}\pm0.02$	$4.82^{d}\pm0.01$
Acetone	$4.20^{f}\pm0.01$	$1.36^{d}\pm0.01$	$5.56^{f}\pm0.0$	$2.51^{d}\pm0.0$	$2.85^{b}\pm0.0$	$5.35^{a}\pm0.03$
n-hexane	$4.85^{e}\pm0.02$	$1.13^{f}\pm0.0$	$5.98^{e}\pm0.02$	$2.25^{e}\pm0.02$	$2.98^{a}\pm0.01$	$5.22^{b}\pm0.03$
Distilled water	$5.59^{d}\pm0.01$	$2.45^{b}\pm0.01$	$8.04^{c}\pm0.03$	$3.10^{b}\pm0.01$	$1.84^{c}\pm0.01$	$4.94^{c}\pm0.03$
LSD ($p<0.05$)	0.01683	0.01384	0.01384	0.01291	0.01893	0.02661

*Mean of triplicate sample, ± standard deviation, mean scores with different letter within the same column are significantly different ($p< 0.05$).

Table 4.3 (continued): Values of Phyto-chemicals in the solvent extracts of *Piper nigrum* and *Aframomum danielli*

Type of solvent extract	*Piper nigrum* ('Uziza' spice)			*Aframomum danielli* ('Urima' spice)		
	*Solvent extract	*Spice Extraction residue	*Total	*Solvent extract	*Spice Extraction residue	*Total
			Saponin (%)			
Ethanol (absolute)	$0.71^{a}\pm0.01$	$0.25^{b}\pm0.01$	$0.96^{a}\pm0.03$	$0.82^{a}\pm0.01$	$1.49^{d}\pm0.02$	$2.29^{b}\pm0.02$
Ethanol 40%	$0.64^{b}\pm0.0$	$0.20^{c}\pm0.0$	$0.84^{b}\pm0.0$	$0.82^{a}\pm0.0$	$2.14^{a}\pm0.01$	$2.94^{a}\pm0.03$
Methanol	$0.40^{d}\pm0.01$	$0.18^{cd}\pm0.0$	$0.58^{c}\pm0.02$	$0.30^{b}\pm0.02$	$1.91^{b}\pm0.01$	$2.21^{b}\pm0.04$
Acetone	$0.06^{e}\pm0.01$	$0.51^{a}\pm0.01$	$0.57^{c}\pm0.02$	$0.14^{c}\pm0.0$	$1.27^{e}\pm0.02$	$1.40^{d}\pm0.01$
n-hexane	$0.10^{e}\pm0.01$	$0.14^{d}\pm0.02$	$0.24^{d}\pm0.02$	$0.07^{d}\pm0.0$	$1.34^{e}\pm0.02$	$1.41^{d}\pm0.02$
Distilled water	$0.52^{c}\pm0.01$	$0.28^{b}\pm0.02$	$0.79^{b}\pm0.02$	$0.13^{c}\pm0.0$	$1.76^{c}\pm0.03$	$1.84^{c}\pm0.02$
LSD ($p<0.05$)	0.01225	0.01118	0.01936	0.00866	0.02566	0.03122
			Tannin (%)			
Ethanol (absolute)	$1.33^{a}\pm0.01$	$0.12^{c}\pm0.03$	$1.45^{a}\pm.0.01$	$0.46^{a}\pm0.04$	$0.14^{c}\pm0.01$	$0.61^{b}\pm0.02$
Ethanol 40%	$0.82^{b}\pm0.0$	$0.13^{bc}\pm0.03$	$0.94^{b}\pm0.0$	$0.44^{b}\pm0.01$	$0.29^{b}\pm0.03$	$0.74^{a}\pm0.0$
Methanol	$0.76^{c}\pm0.0$	$0.14^{b}\pm0.01$	$0.89^{d}\pm0.01$	$0.22^{d}\pm0.01$	$0.11^{dc}\pm0.03$	$0.33^{e}\pm0.02$
Acetone	$0.69^{d}\pm0.0$	$0.14^{b}\pm0.0$	$0.83^{e}\pm0.01$	$0.14^{e}\pm0.03$	$0.32^{a}\pm0.04$	$0.45^{d}\pm0.03$
n-hexane	$0.49^{e}\pm0.01$	$0.11^{d}\pm0.0$	$0.59^{f}\pm0.0$	$0.23^{c}\pm0.02$	$0.09^{e}\pm0.01$	$0.30^{f}\pm0.0$
Distilled water	$0.76^{c}\pm0.01$	$0.15^{a}\pm0.01$	$0.91^{c}\pm0.0$	$0.22^{d}\pm0.01$	$0.07^{f}\pm0.01$	$0.55^{c}\pm0.01$
LSD ($p<0.05$)	0.01118	0.00866	0.01528	0.00957	0.01683	0.14841
			Oxalate (mg/100g)			
Ethanol (absolute)	$2.51^{a}\pm0.01$	$1.16^{a}\pm0.01$	$3.67^{a}\pm0.03$	$0.98^{a}\pm0.01$	$0.26^{d}\pm0.03$	$1.24^{a}\pm$o.03
Ethanol 40%	$2.43^{b}\pm0.01$	$0.55^{d}\pm0.04$	$2.63^{b}\pm0.02$	$0.36^{b}\pm0.02$	$0.44^{a}\pm0.01$	$0.79^{b}\pm0.02$
Methanol	$0.98^{e}\pm0.01$	$0.35^{f}\pm0.01$	$1.33^{c}\pm0.03$	$0.26^{c}\pm0.01$	$0.32^{b}\pm0.02$	$0.58^{d}\pm0.04$
Acetone	$1.17^{d}\pm0.01$	$0.62^{c}\pm0.1$	$1.79^{d}\pm0.02$	$0.17^{d}\pm0.0$	$0.44^{a}\pm0.03$	$0.61^{c}\pm0.0$
n-hexane	$0.71^{f}\pm0.01$	$0.96^{b}\pm0.02$	$1.67^{e}\pm0.02$	$0.18^{d}\pm0.01$	$0.09^{e}\pm0.01$	$0.27^{e}\pm0.01$
Distilled water	2.15c±0.01	$0.43^{e}\pm0.02$	$2.5^{c}\pm0.0$	$0.27^{c}\pm0.0$	$0.31^{c}\pm0.02$	$0.58^{d}\pm0.01$
LSD ($p<0.05$)	0.01323	0.20236	0.02217	0.01118	0.01732	0.02291

*Mean of triplicate sample, ± standard deviation, mean values with different letter within the same column are significantly different ($p< 0.05$).

Table 4.7 shows the phyto-chemicals solvent extracts of *Piper nigrum* and *Aframomum danielli* seeds.

Table 4.4.1: Values of the Major Flavour Principles in ethanol extract of *Piper nigrum*

Chemical compound identified	Molecular formula	Concentration %
Beta-pinene	$C_{10}H_{16}$	2.57
Copaene	$C_{15}H_{24}$	2.07
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- $$ Cyclohexane, 2,4-diisopropenyl-1-methyl-1-vinyl-,	$C_{15}H_{24}$	4.01
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	$C_{15}H_{24}$	5.22
Cis-beta-famescence	$C_{15}H_{24}$	6.39
Humulene	$C_{15}H_{24}$	2.02
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	$C_{15}H_{24}$	1.34
Beta-bisabolene	$C_{15}H_{24}$	6.81
n-Hexadecanoic	$C_{16}H_{32}O_2$	2.70
9,12-octadecadienoic acid	$C_{18}H_{32}O_4$	1.15
3-Adamantan-1-yl-3-oxo-propionitrile	$C_{13}H_{17}NO$	2.15
Kauran-18-al, 17-(acetyloxy-4-beta)	$C_{22}H_{34}O_3$	1.30
ethylpiperroncyanoacetrate	$C_{13}H_{13}NO_4$	14.47
G-Eicosyne	$C_{20}H_{38}$	2.66
Methanone, (2-amino-6,7-dihydro-5H-pyrrolo[1,2-a]imidazol-3-yl)(2,4-dimethoxyphenyl)-	$C_{15}H_{17}N_3O_3$	8.38
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	$C_{16}H_{17}NO_3$	11.05
Piperine	$C_{17}H_{19}NO_3$	17.53
9,12-Octadecadienoyl chloride (z,z)	$C_{18}H_{31}ClO$	1.45

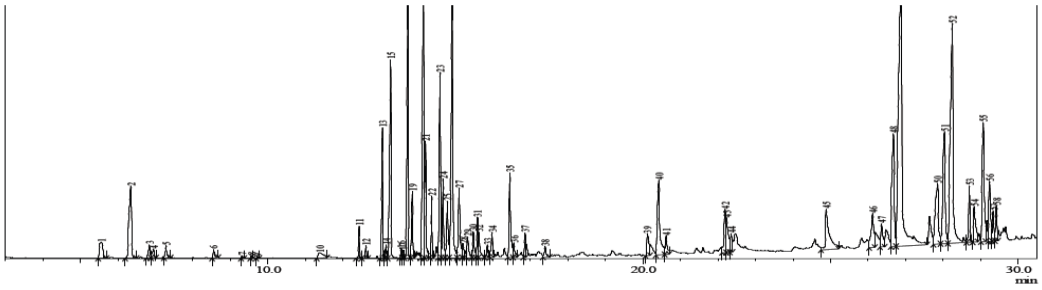


Figure 4.1: Chromatogram peaks of chemical components in absolute ethanol extract of *Piper nigrum*

Table 4.4.2: Values of the Major Flavour Principles in Aqueous 40% Ethanol Extract of *Piper nigrum*

Chemical compound identified	Molecular formula	Concentration %
Copaene	$C_{15}H_{24}$	1.84
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	$C_{15}H_{24}$	4.26
[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	5.09
Gamma-elemene	$C_{15}H_{24}$	1.29
Beta-famescene	$C_{15}H_{24}$	6.55
Humulene	$C_{15}H_{24}$	2.65
Beta-tlangene	$C_{15}H_{24}$	3.63
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	$C_{15}H_{24}$	1.52
Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	$C_{15}H_{24}$	1.51
Beta-bisabolene	$C_{15}H_{24}$	7.22
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	1.92
Apiol	$C_{12}H_{14}O_4$	2.33
n-Hexadeconic acid	$C_{16}H_{32}O_2$	2.96
Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	1.63
9,12-Octadecadienoic acid ethy ester	$C_{20}H_{36}O_2$	1.14
Ethyl Oleate	$C_{20}H_{38}O_2$	1.22
Benzamide, N-(2-hydroxyphenyl)-2,6-dimethoxy	$C_{11}H_{15}IO$	2.66
9-Eicosyne	$C_{20}H_{38}$	3.91
Benzaminde,(-2-hydroxypheny)-2,6-dimethrox	$C_{15}H_{15}NO_4$	5.33
Methanone, (2-amino-6,7-dihydro-5H-pyrrolo[1,2-a]imidazol-3-yl)(2,4-dimethoxyphenyl)-	$C_{15}H_{17}N_3O_3$	11.72
Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-	$C_{20}H_{34}O$	1.35
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	$C_{16}H_{17}NO_3$	1.67
Piperine	$C_{17}H_{19}NO_3$	15.42
9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	2.19
n-Octadeca-6,9,12,15-tetraenoylpyrrolidide	$C_{22}H_{35}NO$	1.25

Table 4.4.2 shows the identified major flavour chemical compounds in aqueous 40% ethanol extract of *Piper nigrum.*

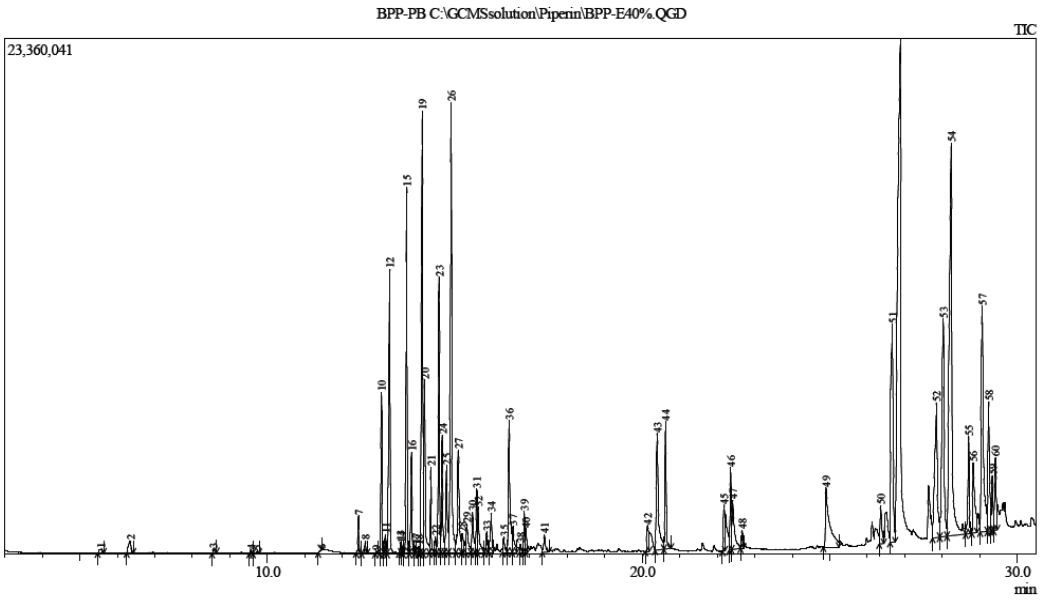


Figure 4.2: Chromatogram peaks of chemical components in 40% ethanol extract of *Piper nigrum* spice

Table 4.4.3: Values of the Major Flavour Principles in Methanol Extract of *Piper nigrum*

Chemical compound identified	Molecular formula	Concentration %
Bicycle[3,1,1]leptain,6,6-dimethyl-2-methyene- (IS)	$C_{10}H_{16}$	1.65
Alpha-guaiene	$C_{15}H_{24}$	1.92
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	$C_{15}H_{24}$	3.21
(z-z) Alpha farnesene	$C_{15}H_24$	1.68
Beta-bisadolene	$C_{15}H_{24}$	2.14
Cyclohexane,3-(1,5-dimethyl-4-hexenyl)6-methylene, [S-(R*,S*)]	$C_{15}H_{24}$	5.24
Diethyl phthalate	$C_{12}H_{18}O_4$	10.92
2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane	$C_{20}H_{18}O_6$	6.13
1-Dodecanone, 2-(imidazol-1-yl)-1-(4-methoxyphenyl)-	$C_{22}H_{32}N_2O_2$	1.40
2-Hexanone,3-cyclohexylidene-4-ethyl	$C_{14}H_{24}O$	1.50
Tricyclo[3.3.1.1(3,7)]decane-1-carboxylic acid, 2,2,3,3,4,4,5,5,6,6,7,7,-dodecafluoroheptyl ester	$C_{18}H_{18}F_{12}O_2$	1.99
2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one, 9-[[(1,3-benzodioxol-5-ylmethyl)amino]methyl]octahydro-2,5a-dimethyl-	$C_{23}H_{29}NO_5$	7.96
Piperine	$C_{17}H_{19}O_3$	6.38
Cholest-22-ene-21-ol,3,5-dehydro-6-methyoxyl, pivalate	$C_{33}H_{54}O_3$	8.21
5aH-3a,12-Methano-1H-cyclopropa[5',6']cyclodeca[1',2':1,5]cyclopenta[1,2-d][1,3]dioxol-13-one, 1a,2,3,9,12,12a-hexahydro-9-hydroxy-10-(hydroxymethyl)-	$C_{23}H_{32}O_5$	9.02
2-Benzofuranmethanol, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-, cis-	$C_{12}H_{20}O_2$	6.15
Silane, [[(3.beta.)-gorgost-5-en-3-yl]oxy]trimethyl-	$C_{33}H_{58}OSi$	4.65
22,26-Oxido-4,17-cholestadien-3.beta.,16.alpha.-diol $$ (17Z)-20-(5-Methyltetrahydro-2H-pyran-2-yl)pregna-4,17-diene-3,16-diol	$C_{27}H_{42}O_3$	4.52
Piperine	$C_{17}H_{19}NO_3$	5.52
Ethyliso-allocholate	$C_{26}H_{44}O_5$	1.42

The contents of the major flavour principles in methanol extract of *Piper nigrum* is presented in Table 4.4.3. The result includes formula and concentration.

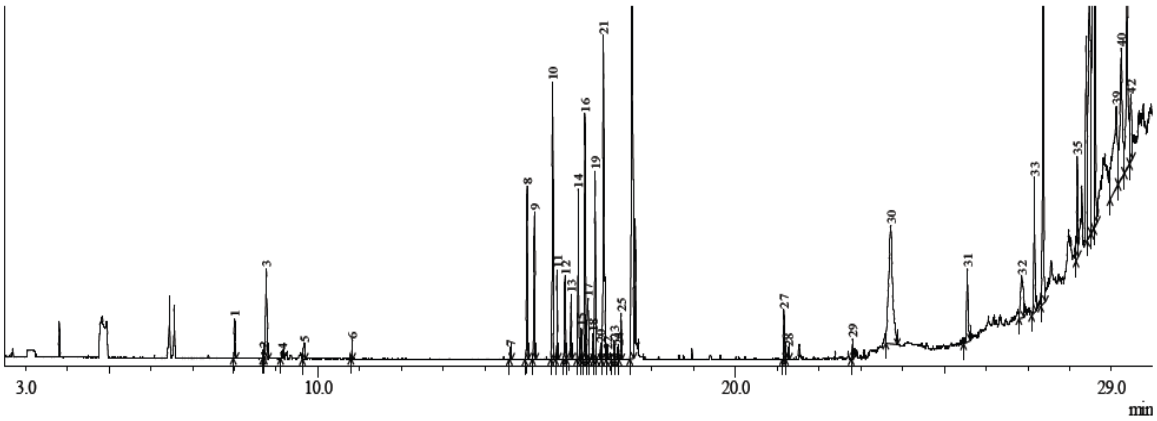


Figure 4.3: Chromatogram peaks of chemical components in methanol extract of *Piper nigrum* spice

Table 4.4.4: Values of the Major Flavour Principles in Acetone Extract of *Piper nigrum*

Chemical compound identified	Molecular formula	Concentration %
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	$C^{10}H^{16}$	1.44
Alfa-copane	$C^{14}H^{24}$	2.38
1-Pentadecene	$C^{15}H^{30}$	1.59
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	$C^{14}H^{24}$	2.93
Caryophyllene	$C^{14}H^{24}$	4.11
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	$C^{14}H^{24}$	1.44
(E)-Beta-famescene	$C^{14}H^{24}$	1.43
Humulene	$C^{14}H^{24}$	1.18
Alpha-Guaiene	$C^{14}H^{24}$	2.28
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	$C^{14}H^{24}$	3.79
Cyclohexene,1,3-disopropenyl-6-methyl	$C^{11}H^{12}O^{3}$	2.26
Beta-Bisabolene	$C^{14}H^{24}$	2.50
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C^{14}H^{24}$	5.80
Bromoacetic acid, pentadecyl ester	$C_{17}H_{33}BrO_2$	4.05
1-Nonadeaene	$C_{19}H_{38}$	1.55
3-Adamantan-1-yl-3-oxo-propionitrite	$C_{13}H_{17}NO$	1.19
1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose	$C_{21}H_{23}NO_8S$	3.59
Beta-sitosterol	$C_{29}H_{50}O$	1.63
3-Amino-4-piperronyl-5-pyrazolene	$C_{11}H_{11}N_3O_3$	8.41
5aH-3a,12-Methano-1H-cyclopropa[5',6']cyclodeca[1',2':1,5]cyclopenta[1,2-d][1,3]dioxol-13-one, 1a,2,3,9,12,12a-hexahydro-9-hydroxy-10-(hydroxymethyl)-	$C_{23}H_{32}O_5$	2.18
Piperine	$C_{17}H_{19}NO_3$	0.96
(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol	$C_{11}H_{20}O$	1.83
3,4-Dimethoxbenzoicanhydride	$C_{18}H_{18}O_7$	3.88
Isoxaben	$C_{18}H_{24}N_2O_4$	5.29
Piperine	$C_{17}H_{19}NO_3$	5.05
alpha.-Santonin	$C_{15}H_{18}O_3$	1.36

Table 4.4.4 present the profile and content of major flavour principles in acetone extract o *Piper nigrum*. The result includes the formula and concentration.

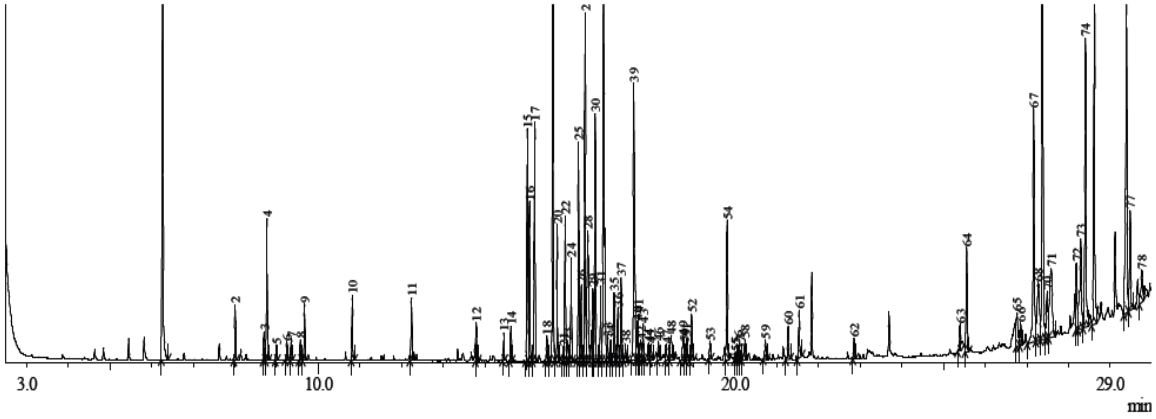


Figure 4.4: Chromatogram peaks of chemical components in acetone extract of *Piper nigrum* spice

Table 4.4.5: Values of the Major Flavour Principles in n-hexane Extract of *Piper nigrum*

Chemical compound identified	Molecular formula	Concentration %
Alpha-copaene	$C_{15}H_{24}$	2.90
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	$C_{15}H_{24}$	4.10
Caryophyllene	$C_{15}H_{24}$	5.10
Cis-beta-farnescene	$C_{15}H_{24}$	2.99
Humulene	$C_{15}H_{24}$	1.98
Alpha-Guaiene	$C_{15}H_{24}$	3.05
Gamma-Murolene	$C_{15}H_{24}$	1.24
-beta-copaene	$C_{15}H_{24}$	5.17
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	$C_{15}H_{24}$	1.58
1,3-Benzodioxide,4-methyoxy-6-2-propenyl	$C_{11}H_{12}O_3$	2.76
beta-Humulene	$C_{15}H_{24}$	1.52
-beta-Bisabolene	$C_{15}H_{24}$	3.54
1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	$C_{11}H_{12}O3$	2.15
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	6.08
Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	1.29
Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]- $$ o-Menth-8-ene-4-methanol, .alpha.,.	$C_{15}H_{26}O$	1.21
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	1.93
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	$C_{15}H_{26}O$	2.47
Caryophyllene oxide	$C_{15}H_{24}O$	1.64
9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane	$C_{15}H_{24}O$	1.27
Alpha-Bisabolol	$C_{15}H_{26}O$	1.22
Beta-Santalol	$C_{15}H_{24}O$	1.28
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.69
9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	1.64
1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose	C21H23NO8S	1.79
3-Ethyl-5-hydroxy-5-trifluoromethyl-2524,5-dihydropyrazol-1-yl)-(3-hydroxy-2-m332ethylphenyl)methanone	$C_{14}H_{15}F_3N_2O_3$	6.78
(R)-(-)-28514-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	1.39
Isoxaben	$C_{18}H_{24}N_2O_4$	3.87
Piperine	$C_{17}H_{19}NO_3$	2.43
(R)(-)-14-Methy-8-hexadecyn-1-ol	$C_{17}H_{32}O$	1.06

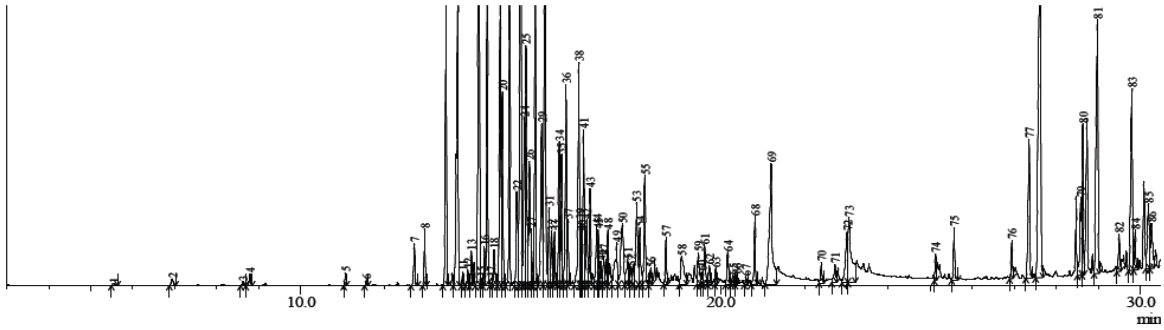


Figure 4.5: Chromatogram peak of chemical components in hexane extract of *Piper nigrum* spice

Table 4.4.6: Values of the Major Flavour Principles in Water Extract of *Piper nigrum*

Chemical compound identified	Molecular formula	Concentration %
Acetic acid	$C_2H_4O_2$	7.58
Diethyl phthalate	$C_{12}H_{14}O_4$	3.67
Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl] maleate	$C_{38}H_{56}O_6$	56.47

Table 4.4.6 present profile and content of major flavour principles in the water extract of *Piper nigrum*. The result includes the formula and concentration.

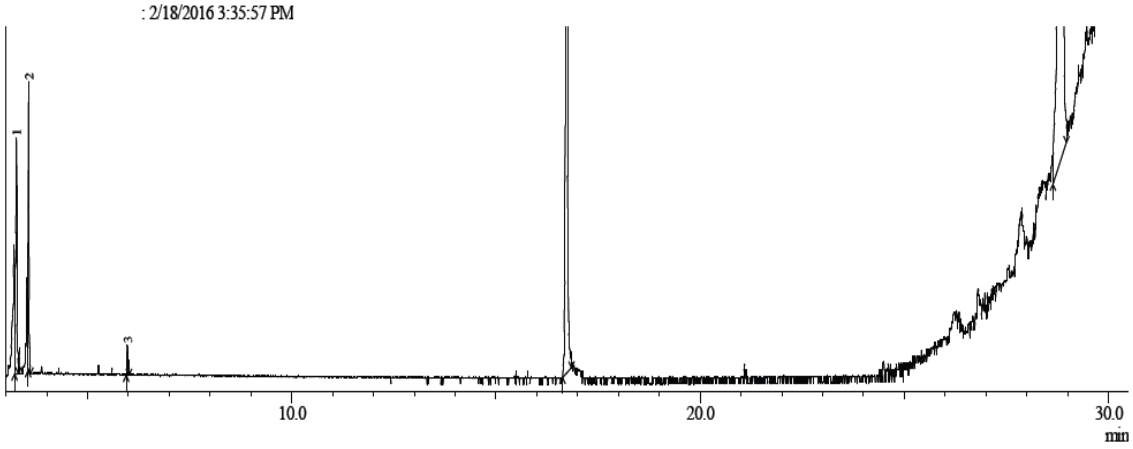


Figure 4.6: Chromatogram peaks of chemical components in water extract *Piper nigrum* spice

Table 4.5: List and Values (%) of common flavour principles identified in *Piper nigrum* extracts

Flavour chemical compounds	Absolute ethanol extract	40% ethanol extract	Methanol extract	Acetone extract	N-hexane extract	Water extract Control	LS D ($p<0.05$)
Cyclohexane-1-etheny-1-methyl- 2,4 Bis(1-methyl	$4.0^{c}\pm0.01$	$4.26^{a}\pm0.01$	Nil	$2.93^{d}\pm0.02$	$4.10^{b}\pm0.0$	Nil	0.014
Cis-beta-famescene	$6.39^{b}\pm0.01$	$6.55^{a}\pm0.02$	$1.08^{d}\pm0.02$	Nil	$2.99^{c}\pm0.0$	Nil	0.013
Humulene	$2.02^{b}\pm0.02$	$2.65^{a}\pm0.03$	Nil	$1.18^{d}\pm0.01$	$1.98^{c}\pm0.01$	Nil	0.018
Beta-Bisabolene	$6.81^{b}\pm0.01$	$7.22^{a}\pm0.02$	Nil	$2.50^{d}\pm0.01$	$3.54^{c}\pm0.03$	Nil	0.015
n-Hexadecanoic acid	$2.70^{a}\pm0.01$	$2.69^{a}\pm0.01$	Nil	Nil	$2.69^{a}\pm0.01$	Nil	0.008
Piperine	17.5^{a}+0.03	$15.42^{b}\pm0.01$	$6.38^{c}\pm0.02$	$5.05^{d}\pm0.01$	$2.43^{c}\pm0.01$	Nil	0.568

($\pm$) Standard deviation of triplicate sample, mean values with different superscript in the column are significantly different ($p<0.05$). LSD=Lease significant difference.

Table 4.6.1: Values of the Major Flavour Principles in Ethanol Extract of *Aframomum danielli*

Chemical compound identified	Molecular formula	Concentration %
Trimethylsilyl fluoride	C_3H_9FSi	7.36
Beta-pinene	$C_{10}H_{16}$	6.94
Eucalyptol	$C_{10}H_{18}O$	53.77
1-dodecanol	$C_{12}H_{26}O$	
Alpha-Terpineol	$C_{10}H_{18}O$	4.97
Oleic acid	$C_{18}H_{34}O_2$	16.89
Diethyl Phthalate	$C_{12}H_{14}O_4$	4.21
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	3.69

Table 4.6.1 present the profile and content of major flavour principles in absolute ethanol extract of *Aframomum danielli*. The result includes the formula and concentration.

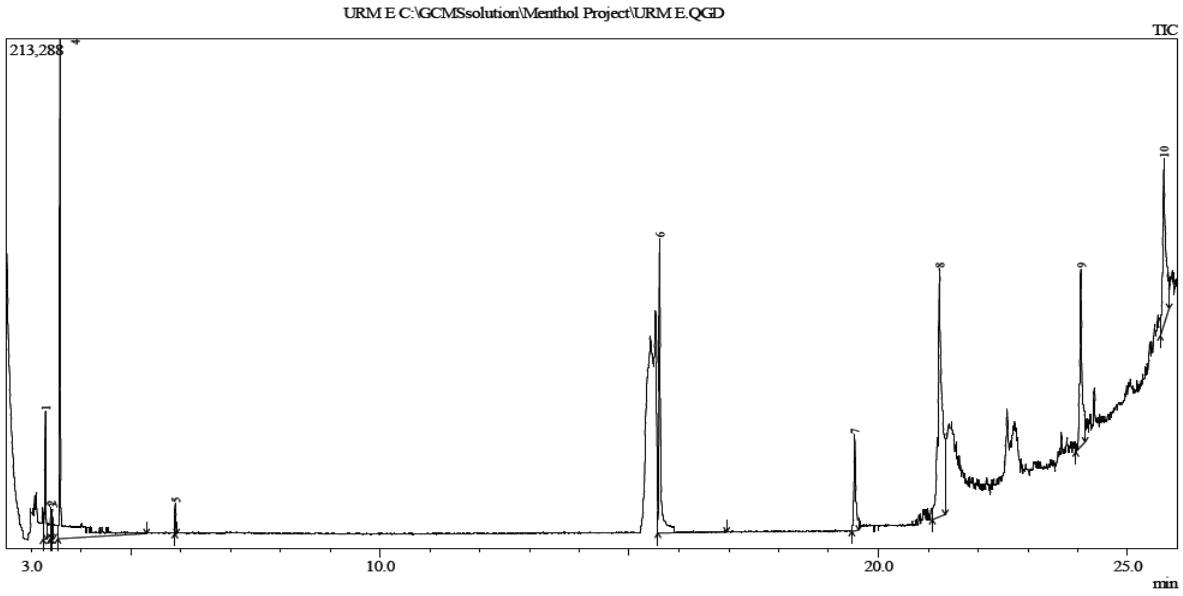


Figure 4.7: Chromatogram peaks of chemical components in absolute ethanol extract of *Aframomum danielli* spice.

Table 4.6.2: Values of the Major Flavour Principles in 40% Ethanol Extract of *Aframomum danielli*

Chemical compound identified	Molecular formula	Concentration %
(S)(-)1,2-propanediol	$C_3H_8O_2$	9.01
Beta -pinene	$C_{10}H_{16}$	4.37
Diethylphthalate	$C_{12}H_{14}0_4$	4.01
Eucalyptol	$C_{10}H_{18}O$	50.70
Alpha-Terpineol	$C_{10}H_{18}O$	3.93
Oleic acid	$C_{18}H_{34}O_2$	13.47
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	2.65

Table 4.6.2 present the profile and content of major flavour principles in 40% ethanol *Aframomum danielli*. The result includes the formula and concentration.

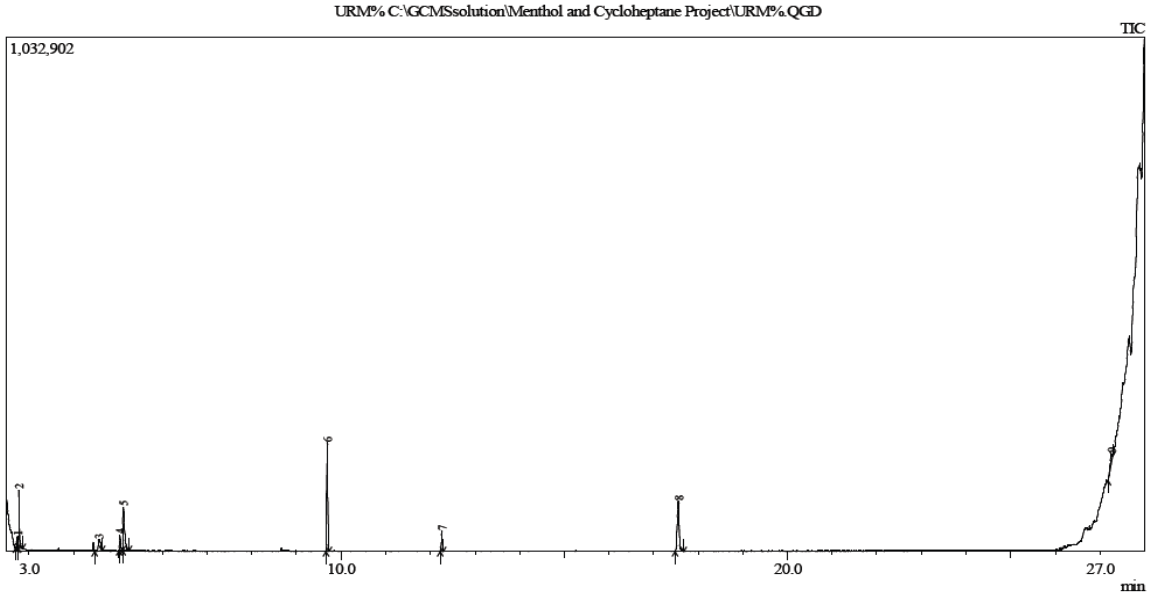


Figure 4.8: Chromatogram peaks of chemical components in 40% ethanol extract of *Aframomum danielli* spice.

Table 4.6.3: Values of the major flavour principles in methanol extract of *Aframomum danielli*

Chemical compound identified	Molecular formula	Concentration %
Eucalyptol	$C_{10}H_{18}O$	62.05
Alpha -Terpineol	$C_{10}H_{18}O$	4.23
Diethyl -phthalate	$C_{12}H_{14}O_4$	3.81
10,10-Dimethox-3,7-dimethyl-deca-2,6-dien-1-ol	$C_{14}H_{26}O_3$	2.18
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	1.65
Ergost-25-ene-3,5,6,,12tetro,(3-beta,5 alpha)	$C_{28}H_{48}O_4$	1.09

The profile and contents of major flavour principles in methanol extract *Aframomum danielli* is presented in Table 4.6.3. The result includes the formula and concentration.

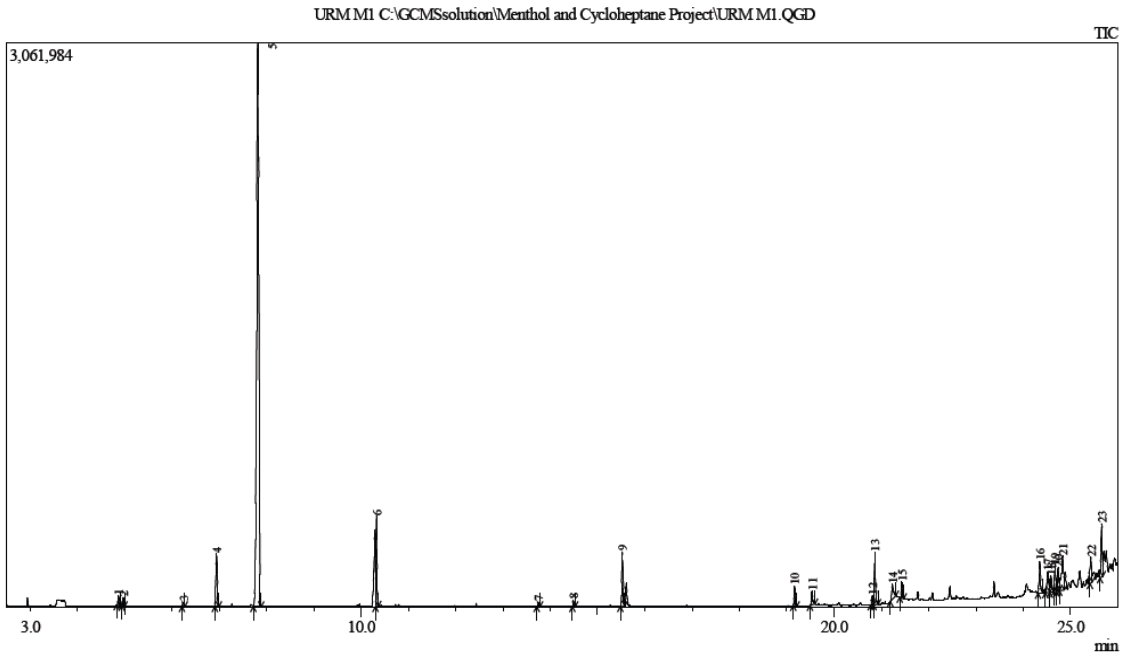


Figure 4.9: Chromatogram peaks of chemical components in methanol extract of *Aframomum danielli* spice.

Table 4.6.4: Values of the major flavour principles in acetone extract *Aframomum danielli*

Chemical compound identified	Molecular formula	Concentration %
2-Pentanonehychoxyl,4-methyl	$C_6H_{12}O_2$	7.44
Bicyclo(3,1,1)6,6dimethyl	$C_{10}H_{16}$	1.42
Eucalyptol	$C_{10}H_{18}O$	22.19
Alpha-terpineol	$C_{10}H_18O$	7.13
-1-Pentadecene	$C_{15}H_{30}$	1.20
n-Hexadonic acid	$C_{16}H_{32}O_2$	4.26
1-Eicosanol	$C_{20}H_{42}O$	1.14
Oleic acid	$C_{18}H_{34}O_2$	7.62
1-Heptadec-1-ynyl-cyclopentanol	$C_{22}H_{40}O$	2.98
Caryophyllene oxide	$C_{15}H_{24}O$	4.16
Alloaromadendiene oxide	$C_{15}H_{24}O$	2.86
Cholesta-3-ene	$C_{27}H_{46}O$	2.25
1-Naphthalenemethanol	$C_{15}H_{26}O$	5.55
Longipinane	$C_{15}H_{26}$	5.29
4,8,13-cyclotetradecatriene-1,3-diol,1,5,9-trine	$C_{20}H_{34}O_2$	15.79
6-epi-shyobunol	$C_{15}H_{26}O$	3.14

Table 4.6.4 shows the profile and contents of major flavour principles in acetone extract of *Aframomum danielli*. The result includes the formula and concentration.

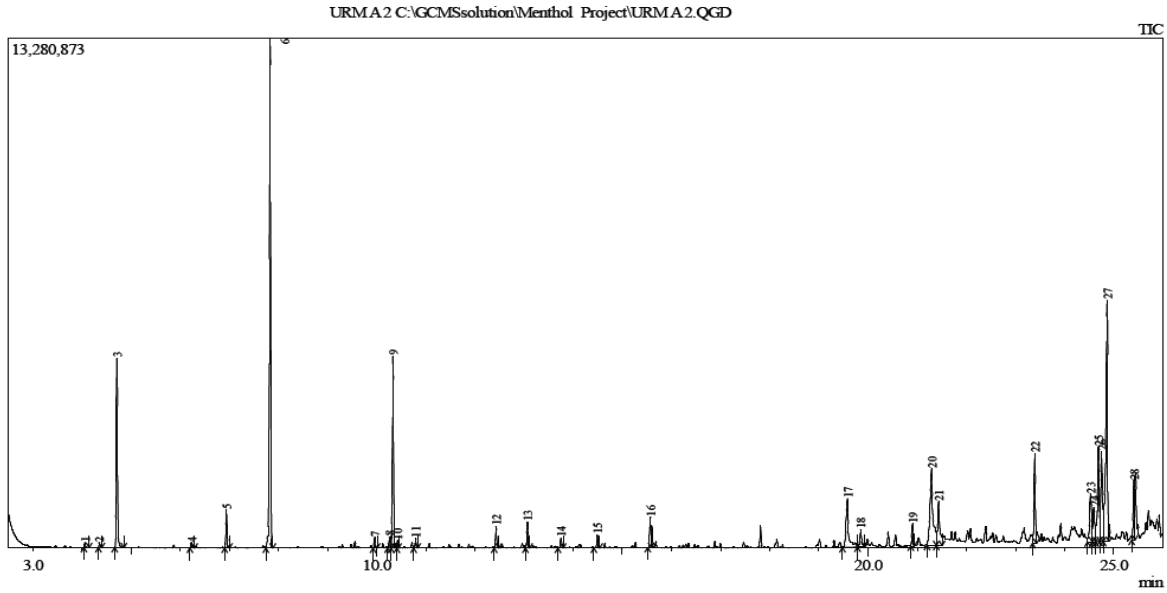


Figure 4.10: Chromatogram peaks of chemical components in acetone extract of *Aframomum danielli* spice.

Table 4.6.5: Values of the major flavour principles in n-hexane extract of *Aframomum danielli*

Chemical compound identified	Molecular formula	Concentration %
Eucalyptol	$C_{10}H_{18}O$	20.53
Alpha-Terpineol	$C_{10}H_{18}O$	6.53
Caryophyllene	$C_{15}H_{24}$	0.61
Bicyclo (3,1,1) hept-2-ene	$C_{15}H_{24}$	1.06
Tetradecanoic acid	$C_{14}H_{28}O_2$	1.07
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	12.11
9-octadecanoic acid methyl ester (E)	$C_{19}H_{36}O_2$	1.09
2,4A,8,8-tetramethyl decahydrocyclo-propa (d)	$C_{15}H_{26}$	1.34
Oleic acid	$C_{18}H_{34}O_2$	24.23
8-Hexadecenal, 14-mehtyl (Z)	$C_{17}H_{32}O$	1.04
Caryophyllene oxide	$C_{15}H_{24}O$	1.98
1-Heptatriacotanol	$C_{37}H_{76}O$	2.64

Table 4.6.5 shows the profile and contents of major flavour principles in n-Hexane extract *Aframomum danielli*. The result includes the formula and concentration

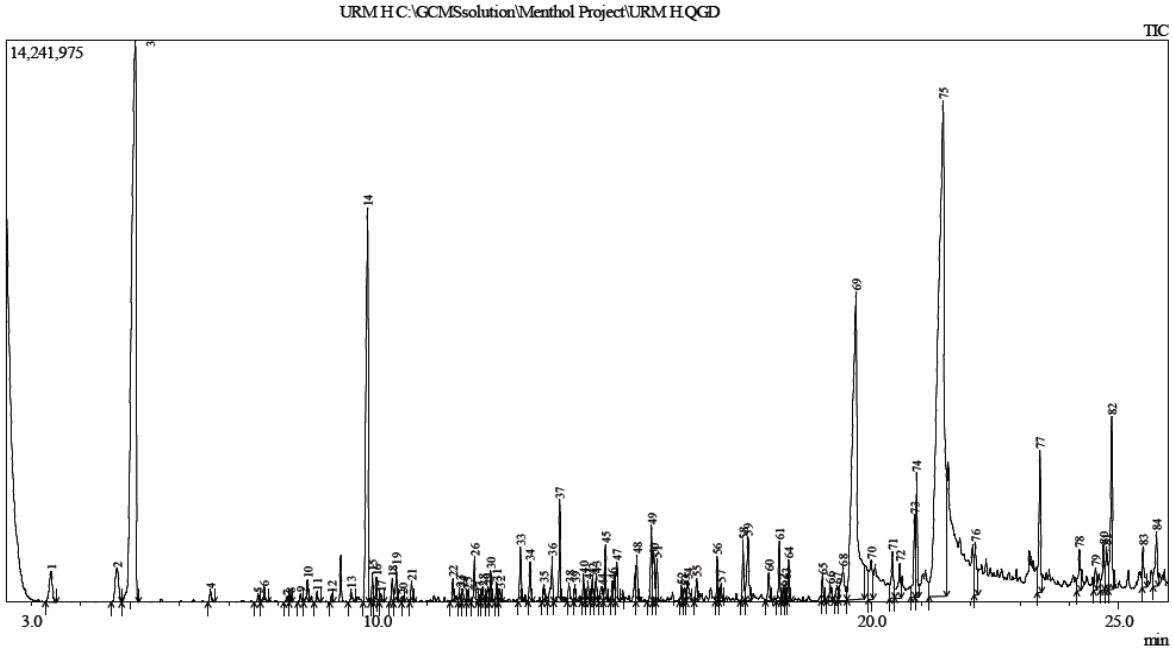


Figure 4.11: Chromatogram peaks of chemical components in n-hexane extract of *Aframomum danielli* spice.

Table 4.6.6: Value of the major flavour principles in water extract *Aframomum danielli*

Chemical compound identified	Molecular formula	Concentration %
Ethanol	C_2H_6O	84.72

Table 4.6.6 shows the profile and content of flavour principle in water extract of *Aframomum danielli*. The result includes the formula and concentration.

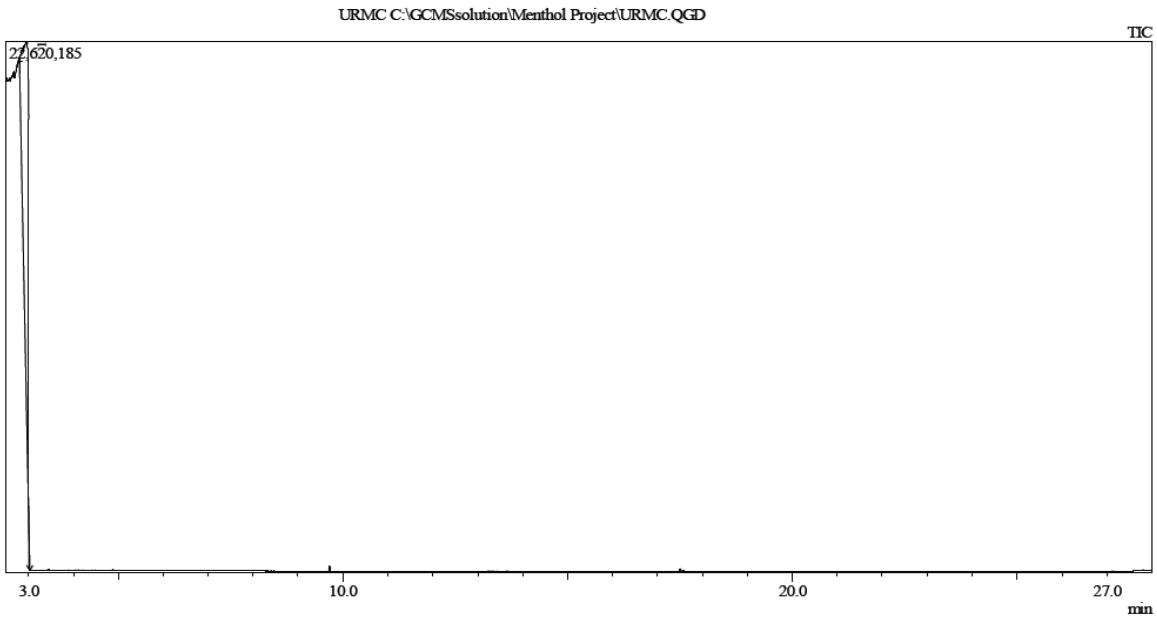


Figure 4.12: Chromatogram peaks of chemical components in water extract of *Aframomum danielli* spice.

Table 4.7: List and values of common flavour principles in solvent extracts of *Aframomum danielli*

Flavour chemical compounds	Absolute ethanol extract	40% ethanol extract	Methanol extract	Acetone extract	N-hexane extract	Water extract (Control)	LS D ($p<0.05$)
Eucalyptol	$53.77^{b}\pm0.0$	$50.70^{c}\pm0.05$	$62.05^{a}\pm0.0$	$22.19^{d}\pm0.0$	$20.53^{e}\pm0.01$	ND	2.754
Alpha-Terpineol	$4.97^{d}\pm0.01$	$12.26^{a}\pm0.03$	$4.23^{e}\pm0.01$	$7.13^{b}\pm0.01$	$6.53^{c}\pm0.02$	ND	0.055
n-Hexandonic acid	$7.36^{b}\pm0.03$	$9.01^{a}\pm0.01$	ND	$4.26^{c}\pm0.02$	ND	ND	0.867
Oleic acid	$16.39^{a}\pm0.01$	$13.47^{b}\pm0.01$	ND	ND	ND	ND	0.772
Beta-pinene	$6.94^{a}\pm0.02$	$4.37^{b}\pm0.01$	ND	ND	ND	ND	0.549
Ethyl iso-allocholate	$4.03^{a}\pm0.01$	$2.65^{b}\pm0.0$	$1.65^{c}\pm0.01$	ND	ND	ND	0.228
Diethyl phthalate	$4.21^{a}\pm0.01$	$4.01^{b}\pm0.02$	ND	ND	ND	ND	0.0664

($\pm$) Standard deviation of triplicate sample, mean values with different superscript in the column are significantly different ($p<0.05$). LSD=Lease significant difference, ND- Not Detected.

Table 4.8.1: Profiles of major flavour principles in *Piper nigrum* spice product samples

Spice product sample	Cyclohexane-1-etheny-1-2,4Bis-1-methyl	Cis-beta.famescene	Humulene	Beta-Bisabolene	n-Hexadecanoic acid	Piperine
Piper nigrum pulverized powder in bottle	4.8[a]	6.88[a]	3.88[a]	7.72[a]	3.22[a]	17.53[a]
Piper nigrum extract in bottle	4.0[b]	6.55[b]	2.65[b]	7.22[b]	2.69[b]	15.42[b]
Piper nigrum extract in rice grits coated at 1:2 ratio	2.87[c]	4.69[c]	1.74[c]	4.33[c]	1.99[c]	13.87[c]
Piper nigrum extract in rice grits coated at 1:3 ratio	2.32[d]	3.83[d]	1.64[d]	3.98[d]	1.62[e]	13.69[d]
Piper nigrum extract in potato grits coated at 1:2 ratio	2.76[c]	4.60[c]	1.73[c]	4.34[c]	1.78[d]	13.11[e]
Piper nigrum extract in potato grits coated at 1:3 ratio	2.30[d]	3.76[d]	1.59[d]	3.97[d]	1.59[e]	12.11[f]
LSD ($p<0.05$)	0.356	0.250	0.211	0.330	0.207	0.2280

Mean of duplicate sample, ($\pm$) = standard deviation. Mean values on the same column with different letter are significantly different $p<0.05$)

Table 4.8.2: Piperine profile in *Piper nigrum* spice product samples

Spice product sample	Spice product sample code	Concentration %	Percentage Retention (PR) of Piperine content of 40% ethanol extract in carrier materials %
Piper nigrum pulverized powder in bottle	PP-PB (control)	17.53[a]	-
Piper nigrum extract in bottle	PP-EB	15.42[b]	-
Piper nigrum extract in rice grits coated at 1:2 ratio	$PPERC_1$	13.87[c]	89.95
Piper nigrum extract in rice grits coated at 1:3 ratio	$PPERC_2$	13.69[d]	88.78
Piper nigrum extract in potato grits coated at 1:2 ratio	$PPEPC_1$	13.11[e]	85.02
Piper nigrum extract in potato grits coated at 1:3 ratio	$PPEPC_2$	12.11[f]	78.53
LSD ($p<0.05$)		0.2280	-

Ratio means 40% ethanol extract of *Piper nigrum* to carrier material, mean values with different letters in the same column are significantly different ($p<0.05$)

Table 4.9.1: Profiles of major flavour principles in *Aframomum danielli* spice product samples

Spice product sample	Eucalyptol %	α-Terpineol %	Oleic acid %	n-Hexadonic acid %	β-pinene %	Ethy iso-allocholate %	Diethy-phthalate %
AFD-PB (Control)	$65.28^{a}\pm0.02$	$6.87^{a}\pm0.01$	$16.89^{a}\pm0.0$	$9.0^{a}\pm0.0$	$6.88^{a}\pm0.0$	$2.95^{a}\pm0.0$	$7.86^{a}\pm0.02$
AFD-EB	$50.70^{b}\pm0.01$	$4.79^{b}\pm0.01$	$10.33^{b}\pm0.02$	$5.53^{b}\pm0.02$	$4.37^{b}\pm0.01$	$2.65^{b}\pm0.01$	$4.01^{b}\pm0.0$
$AFDERC_1$	$28.76^{c}\pm0.01$	$3.39^{c}\pm0.01$	$4.11^{c}\pm0.02$	$2.89^{c}\pm0.01$	$2.66^{c}\pm0.01$	$1.83^{d}\pm0.01$	$2.56^{c}\pm0.03$
$AFDERC_2$	$20.34^{e}\pm0.01$	$2.82^{e}\pm0.01$	$2.06^{f}\pm0.02$	$1.33^{e}\pm0.0$	$2.06^{e}\pm0.01$	$1.42^{e}\pm0.01$	$1.84^{e}\pm0.02$
$AFDEPC_1$	$27.65^{d}\pm0.02$	$3.06^{d}\pm0.02$	$4.0^{d}\pm0.0$	$2.63^{d}\pm0.0$	$2.58^{d}\pm0.02$	$2.01^{c}\pm0.01$	$2.62^{c}\pm0.01$
$AFDEPC_2$	$20.15^{f}\pm0.0$	$2.55^{f}\pm0.0$	$2.18^{e}\pm0.01$	$1.18^{f}\pm0.0$	$1.98^{f}\pm0.0$	$1.40^{e}\pm0.02$	$2.01^{d}\pm0.01$
LSD ($p<0.05$)	0.9568	0.0312	0.2812	0.4550	0.1138	0.0239	0.5768

Mean of duplicate sample, (±) = standard deviation. Mean values on the same column with different letter are significantly different $p<0.05$)

AFD-PB = pulverized sample of *Aframomum danielli* in amber bottle, AFD-EB = 40% ethanol extract of *Aframomum danielli* product sample in amber bottle, $AFDERC_1$ = 40% ethanol extract of *Aframomum danielli* in rice grits carrier at 1:2v/w, $AFDERC_2$ = 40% ethanol extract of *Aframomum danielli* in rice grits carrier at 1:3v/w, $AFDEPC_1$ = 40% ethanol extract of *Aframomum danielli* in potato grits carrier at 1:2v/w, $AFDEPC_2$ = 40% ethanol extract of *Aframomum danielli* in potato grits carrier at 1:3v/w.

Table 4.9.2: Eucalyptol profile in *Aframomum danielli* spice product samples

Spice product sample	Spice product sample code	Concentration %	Percentage Retention (PR) of Eucalyptol content of 40% ethanol extract in carrier materials %
Aframomum danielli pulverized powder in bottle	AFD-PB (Control)	65.28[a]	-
Aframomum danielli extract in bottle	AFD-EB	50.70[b]	-
Aframomum danielli extract in rice grits coated at 1:2 ratio	$AFDERC_1$	28.76[c]	56.73[a]
Aframomum danielli extract in rice grits coated at 1:3 ratio	$AFDERC_2$	20.34[d]	40.12[c]
Aframomum danielli extract in potato grits coated at 1:2 ratio	$AFDEPC_1$	27.65[c]	54.57[b]
Aframomum danielli extract in potato grits coated at 1:3 ratio	$AFDEPC_2$	20.15[d]	39.74[d]
LSD ($p<0.05$)	LSD ($p<0.05$)	0.4521	2.382

Ratio means 40% ethanol extract of *Aframomum danielli* to carrier material, mean values with different letters in the same column are significantly different ($p<0.05$

Table 4.10: Percentage (%) Retention of Piperine in *Piper nigrum* spice products at 6months storage

Spice product samples	Piperine content (%) of spice product samples under storage										
	At zero Hr	2weeks	4weeks	6weeks	2months	3months	4months	5months	6months	LSD ($p<0.05$)	Retention capacity %
Piper nigrum pulverized in bottle	17.54^{a}	17.53^{ab}	17.50^{b}	17.09^{c}	17.0^{d}	17.0^{d}	16.58^{e}	16.01^{f}	15.72^{g}	0.2081	89.67
Piper nigrum extract in bottle	15.42^{a}	15.38^{b}	15.31^{c}	15.18^{d}	15.10^{e}	15.09^{e}	14.86^{f}	14.81^{g}	14.08^{h}	0.2851	91.31
Piper nigrum extract in rice grits coated at 1:2 ratio	13.87^{a}	13.86^{b}	13.79^{c}	13.28^{d}	12.69^{e}	12.08^{f}	12.01^{g}	11.76^{h}	10.96^{i}	0.2122	79.02
Piper nigrum extract in rice grits coated at 1:3 ratio	13.69^{a}	13.28^{b}	13.12^{c}	12.76^{d}	11.82^{e}	11.14^{f}	11.06^{g}	10.58^{h}	9.16^{i}	0.0133	66.91
Piper nigrum extract in potato grits coated at 1:2 ratio	13.11^{a}	12.93^{b}	12.56^{c}	11.42^{d}	11.06^{e}	10.77^{f}	10.70^{g}	9.84^{h}	7.58^{i}	0.0850	57.82
Piper nigrum extract in potato grits coated at 1:3 ratio	12.11^{a}	11.66^{b}	11.18^{c}	10.98^{d}	10.22^{e}	8.66^{f}	8.43^{g}	8.18^{h}	7.06^{i}	0.01414	56.65

Ratio means 40% ethanol extract of *Piper nigrum* to carrier material, mean values on the same row with different letter are significantly different $p<0.05$)

Table 4.11: Percentage (%) Retention of Eucalyptol in *Aframomum danielli* spice product samples at 6months storage

Spice product samples	Eucalyptol content (%) of spice product samples under storage										
	At zero Hr	**2weeks**	**4weeks**	**6weeks**	**2months**	**3months**	**4months**	**5months**	**6months**	**LSD ($p<0.05$)**	**Retention capacity %**
Aframomum danielli pulverized in bottle	65.28^{a}	65.20^{b}	63.44^{c}	62.08^{d}	60.26^{e}	59.16^{f}	56.06^{g}	55.82^{h}	54.48^{i}	0.3659	83.46
Aframomum danielli extract in bottle	50.70^{a}	50.0^{b}	48.06^{c}	48.0^{d}	45.22^{e}	44.56^{f}	41.09^{g}	40.0^{h}	39.19^{i}	0.8441	77.30
Aframomum danielli extract in rice grits coated at 1:2 ratio	28.76^{a}	28.06^{b}	27.83^{c}	27.25^{d}	25.86^{e}	24.55^{f}	24.01^{g}	22.96^{h}	22.06^{i}	0.996	76.70
Aframomum danielli extract in rice grits coated at 1:3 ratio	20.34^{a}	20.08^{b}	20.0^{c}	18.66^{d}	18.08^{e}	16.98^{f}	15.0^{g}	14.28^{h}	12.06^{i}	0.4195	59.29
Aframomum danielli extract in potato grits coated at 1:2 ratio	27.65^{a}	$27\text{-}58^{b}$	26.98^{c}	26.12^{d}	23.62^{e}	22.82^{f}	20.97^{g}	19.33^{h}	18.08^{i}	0.6255	65.39
Aframomum danielli extract in potato grits coated at 1:3 ratio	20.15^{a}	19.92^{b}	19.39^{c}	19.0^{d}	18.28^{e}	16.43^{f}	13.60^{g}	12.0^{h}	11.44^{i}	4.658	56.77

Ratio means 40% ethanol extract of *Aframomum danielli* to carrier material, mean of duplicate sample, Mean values on the same column with different letter are significantly different $p<0.05$)

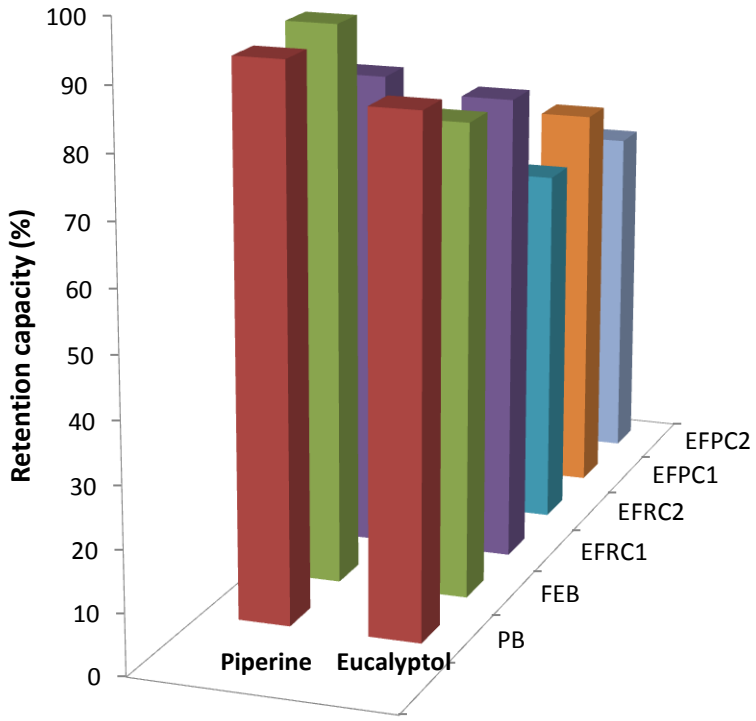


Figure 4.13: Percentage (%) Retention of piperine and eucalyptol in *Piper nigrum* and *Aframomum danielli* spice products at 6monhs storage

Key:

PB = Pulverized in bottle, EB = 40% ethanol spice extract, $EFRC_1$ = 40% ethanol spice extract in rice grits at ratio 1:2v/w, $EFRC_2$ = 40% ethanol spice extract in rice grits at ratio1:3v/w, $EFPC_1$ = 40% ethanol spice extract in potato grits at ratio 1:2v/w, $EFPC_2$ = 40% ethanol spice extract in potato grits at ratio 1:3v/w.

Table 4.12.1: Descriptive mean scores for the sensory notes detected in *Piper nigrum* spice products samples

Spice product samples	Colour	Pungency	Sweetness	Minty flavour	Taste	Bitterness	Harsh-in-taste	Hot-in-taste	After-taste	Overall acceptability
Piper nigrum pulverized in bottle	$6.29^{a}\pm0.4$	$6.71^{a}\pm0.4$	$3.57^{a}\pm0.5$	$2.86^{a}\pm0.3$	$2.0^{c}\pm0.8$	$4.29^{b}\pm0.4$	$2.43^{a}\pm0.5$	$5.43^{a}\pm0.5$	$1.86^{a}\pm0.5$	$4.29^{f}\pm0.6$
Piper nigrum extract in bottle	$6.29^{a}\pm0.7$	$5.71^{b}\pm0.4$	$3.43^{b}\pm0.5$	$2.43^{b}\pm0.5$	$2.29^{b}\pm0.7$	$5.0^{a}\pm0.8$	$2.0^{b}\pm0.7$	$4.71^{a}\pm0.5$	$1.57^{b}\pm0.7$	$4.57^{e}\pm0.5$
Piper nigrum extract in rice grits coated at 1:2 ratio	$4.57^{b}\pm0.5$	$5.50^{c}\pm1.0$	$2.71^{c}\pm0.7$	$2.0^{d}\pm0.8$	$1.71^{d}\pm0.7$	$2.7^{c}1\pm1.4$	$1.57^{c}\pm0.7$	$3.14^{b}\pm0.5$	$1.57^{b}\pm1.3$	$5.43^{b}\pm0.5$
Piper nigrum extract in rice grits coated at 1:3 ratio	$4.33^{c}\pm1.0$	$3.86^{f}\pm0.8$	$2.50^{e}\pm0.5$	$1.83^{e}\pm0.7$	$2.33^{a}\pm0.5$	$2.0^{d}\pm0.6$	$1.17^{d}\pm0.9$	$1.83^{c}\pm0.4$	$1.50^{c}\pm0.4$	$5.17^{d}\pm0.5$
Piper nigrum extract in potato grits coated at 1:2 ratio	$4.0^{d}\pm0.5$	$4.86^{d}\pm0.6$	$2.71^{c}\pm0.7$	$2.13^{c}\pm0.6$	$1.50^{f}\pm0.5$	$1.63^{f}\pm0.5$	$1.05^{f}\pm0.5$	$1.63^{c}\pm0.7$	$1.38^{d}\pm0.5$	$5.25^{c}\pm0.5$
Piper nigrum extract in potato grits coated at 1:3 ratio	$3.75^{e}\pm0.8$	$4.63^{e}\pm1.0$	$2.60^{d}\pm0.9$	$1.43^{f}\pm0.5$	$1.57^{e}\pm0.5$	$1.71^{e}\pm0.4$	$1.14^{e}\pm0.5$	$1.43^{c}\pm0.6$	$1.0^{e}\pm0.5$	$5.57^{a}\pm0.0$
LSD ($p<0.05$)	0.39244	0.44047	0.37827	0.34687	0.35529	0.45578	0.33585	0.41904	0.27690	0.32003

Ratio means 40% ethanol extract of *Piper nigrum* to carrier material, mean of seven panelist, (±) =standard deviation. Mean scores on the same column with different letters are significantly different ($p<0.05$)

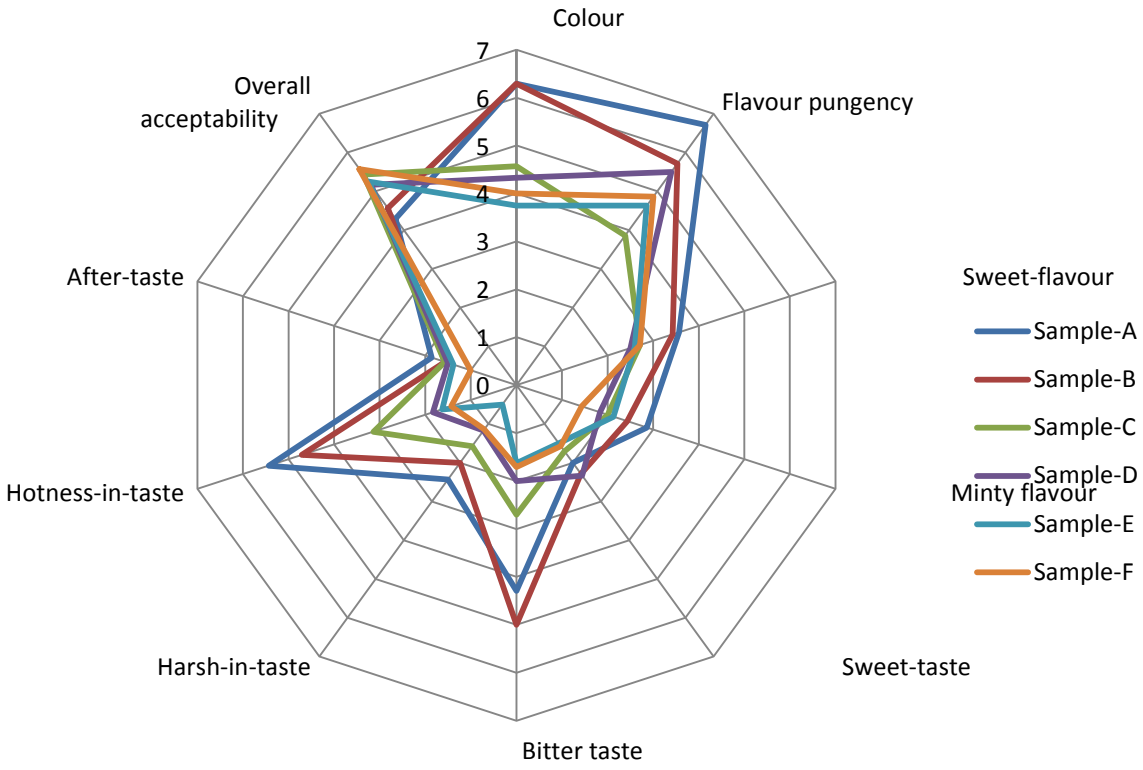


Figure 4.14: Descriptive sensory notes chart for black pepper spice products

Key:
Sample-A= pulverized powder of *Piper nigrum* spice product
Sample-B= solvent flavour extract from *Piper nigrum* (at ratio 1:1w/v) flavour product
Sample-C= 1:2 (v/w) ratio of 40% ethanol extract of *Piper nigrum* in rice grits carrier
Sample-D= 1:3 (v/w) ratio of 40% ethanol extract of *Piper nigrum* in rice grits carrier
Sample-E= 1:2 (v/w) ratio of 40% ethanol extract of *Piper nigrum* in potato grits carrier
Sample-F= 1:3 (v/w) ratio of 40% ethanol extract of *Piper nigrum* in potato grits carrier

Table 4.12.2: *Mean scores of consumers' preference for major sensory attributes of *Piper nigrum* spice products

Spice product sample	Colour	Pungency	Aroma	Overall acceptability
Piper nigrum pulverized in bottle	$6.20^{a}\pm0.6$	$6.80^{a}\pm0.4$	$6.75^{a}\pm0.4$	$6.70^{a}\pm0.4$
Piper nigrum extract in bottle	$5.95^{b}\pm0.9$	$6.30^{b}\pm0.4$	$6.15^{b}\pm0.5$	$6.15^{b}\pm0.3$
Piper nigrum extract in rice grits coated at 1:2 ratio	$5.60^{c}\pm0.6$	$5.55^{c}\pm0.6$	$5.35^{d}\pm0.4$	$5.80^{c}\pm0.4$
Piper nigrum extract in rice grits coated at 1:3 ratio	$5.50^{d}\pm0.7$	$4.30^{d}\pm0.4$	$4.45^{e}\pm0.5$	$5.10^{d}\pm0.3$
Piper nigrum extract in potato grits coated at 1:2 ratio	$5.50^{d}\pm0.6$	$4.65^{d}\pm0.7$	$4.45^{e}\pm0.5$	$4.90^{e}\pm0.3$
Piper nigrum extract in potato grits coated at 1:3 ratio	$5.45^{e}\pm0.5$	$4.65^{d}\pm0.4$	$5.40^{c}\pm0.5$	$4.40^{f}\pm0.5$
LSD ($p<0.05$)	0.22537	0.17181	0.16100	0.12687

Ratio means 40% ethanol extract of *Piper nigrum* to carrier material, mean of twenty panelist, ($\pm$) =standard deviation. Mean score on the same column with different letter are significantly different ($p<0.05$)

Table 4.12.3: Nonparametric correlations test on sensory attributes of *Piper nigrum* product samples

Pearson Correlations test on *Piper nigrum* product samples					
Sensory attributes		**Color**	**Pungency**	**Aroma**	**Overall acceptability**
color	Pearson Correlation	1			
	N	6			
Flavour pungency	Pearson Correlation	.870*	1		
	N	6	6		
aroma	Pearson Correlation	.829*	.932**		
	N	6	6		
Overall acceptability	Pearson Correlation	.850*	.927**	.895*	1
	N	6	6	6	6

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

N= Number of spice product sample = 6

Table 4.13.1: Descriptive mean scores for some sensory notes detected in *Aframomum danielli* flavour products

Spice product samples	Colour	Pungency	Sweetness	Minty flavour	Taste	Bitterness	Harsh-in-taste	Hot-in-taste	After-taste	Overall acceptability
AFD-PB	5.57^{b}±1.5	5.71^{a}±1.1	5.0^{c}±0.8	5.57^{a}±1.1	4.43^{c}±0.9	3.14^{a}±1.3	2.86^{b}±1.7	5.71^{a}±1.2	5.86^{a}±0.6	6.86^{a}±0.3
AFD-EB	6.29^{a}±1.1	5.14^{ab}±1.3	5.0^{c}±1.1	5.14^{b}±1.2	4.43^{c}±0.5	3.0^{b}±1.4	5.14^{a}±1.5	4.86^{b}±1.4	5.86^{a}±1.3	6.57^{b}±0.7
AFD-ERC_1	4.29^{e}±0.5	4.71^{bc}±0.7	5.57^{a}±1.5	5.14^{b}±0.8	5.86^{a}±1.2	2.0^{c}±1.5	2.43^{c}±1.3	4.43^{c}±1.2	5.14^{b}±1.4	5.14^{c}±1.0
AFD-ERC_2	4.14^{f}±0.6	4.71^{bc}±1.1	5.0^{c}±1.5	4.86^{c}±1.2	5.0^{b}±1.2	1.86^{d}±1.4	1.57^{e}1.1	3.57^{e}±1.2	5.0^{c}±1.1	4.57^{f}±0.8
AFD-EPC_1	4.86^{c}±0.7	4.42^{c}±0.9	5.29^{b}±0.9	4.71^{c}±1.2	4.43^{c}±1.7	1.71^{e}±1.2	1.86^{d}±1.5	4.0^{d}(±0.8	4.0^{d}±0.8	4.86^{d}±0.7
AFD-EPC_2	4.42^{d}±1.3	4.14^{c}±1.5	4.71^{d}±0.7	4.71^{c}±0.7	4.0^{d}±1.9	1.43^{f}±1.8	1.43^{f}±1.9	3.57^{e}±1.2	3.57^{e}±0.7	4.71^{e}±0.7
LSD (p=0.05)	0.54917	0.62814	0.79682	0.58515	0.79682	1.23718	1.27153	0.80390	0.57931	0.43121

*Mean of seven panelist, (±) = standard deviation. Mean scores on the same column with different letters are significantly different ($p<0.05$)

Key:

Sample-A= powder *Aframomum danielli* spice product
Sample-B= solvent flavour extract from *Aframomum danielli* (at ratio 1:1w/v) flavour product
Sample-C= 1:2 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in rice grits carrier
Sample-D= 1:3 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in rice grits carrier
Sample-E= 1:2 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in potato grits carrier
Sample-F= 1:3 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in potato grits carrier

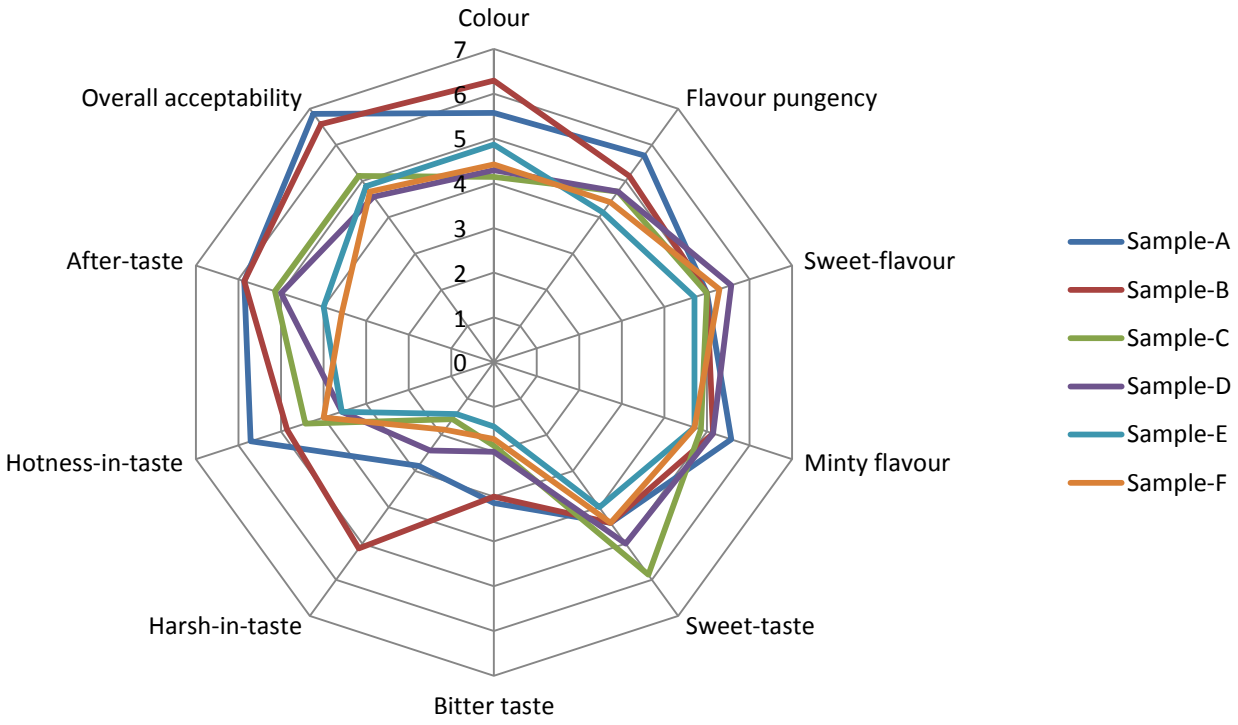


Figure 4.15: Descriptive sensory notes chart for *Aframomum danielli* spice product samples

Key:

Sample-A= powder *Aframomum danielli* spice product
Sample-B= solvent flavour extract from *Aframomum danielli* (at ratio 1:1w/v) flavour product
Sample-C= 1:2 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in rice grits carrier
Sample-D= 1:3 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in rice grits carrier
Sample-E= 1:2 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in potato grits carrier
Sample-F= 1:3 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in potato grits carrier

Table 4.13.2: *Mean scores of consumers' preference for major sensory attributes of *Aframomum danielli* spice products

Spice product sample	Colour	Pungency	Aroma	Overall acceptability
Aframomum danielli pulverized in bottle	$5.45^{b}\pm1.1$	$6.35^{a}\pm0.6$	$5.55^{a}\pm0.8$	$6.15^{a}\pm0.8$
Aframomum danielli extract in bottle	$6.30^{a}\pm0.9$	$5.40^{b}\pm0.8$	$5.05^{b}\pm1.0$	$5.45^{c}\pm0.9$
Aframomum danielli extract in rice grits coated at 1:2 ratio	$4.95^{e}\pm1.0$	$5.0^{c}\pm0.9$	$4.90^{d}\pm0.9$	$4.85^{d}\pm0.9$
Aframomum danielli extract in rice grits coated at 1:3 ratio	$4.30^{f}\pm0.7$	$4.90^{d}\pm0.7$	$4.80^{e}\pm0.9$	$4.45^{e}\pm0.7$
Aframomum danielli extract in potato grits coated at 1:2 ratio	$5.30^{c}\pm1.0$	$5.40^{b}\pm0.5$	$5.0^{a}\pm1.2$	$6.10^{b}\pm1.7$
Aframomum danielli extract in potato grits coated at 1:3 ratio	$5.0^{d}\pm1.1$	$4.40^{e}\pm0.8$	$4.75^{a}\pm1.0$	$4.45^{e}\pm0.7$
LSD ($p<0.05$)	0.12512	0.25262	0.10261	01.15309

Ratio means 40% ethanol extract of *Aframomum danielli* to carrier material, mean of twenty panelist, (±) =standard deviation. Mean scores on the same column with different letters are significantly different ($p<0.05$)

Table 4.13.3: Nonparametric correlations test on sensory properties of *Aframomum danielli* flavour products

Pearson Correlations test on *Aframomum danielli* flavour product samples					
Sensory properties		**Color**	**Flavour pungency**	**Aroma**	**Overall acceptability**
Color	Pearson Correlation	1			
	N	6			
Flavour pungency	Pearson Correlation	.431	1		
	N	6	6		
Aroma	Pearson Correlation	.399	.969**	1	
	N	6	6	6	
Overall acceptability	Pearson Correlation	.456	.671	.563	1
	N	6	6	6	6

**. Correlation is significant at the 0.01 level (1-tailed).

Key:

Sample-A= powder *Aframomum danielli* spice product
Sample-B= solvent flavour extract from *Aframomum danielli* (at ratio 1:1w/v) flavour product
Sample-C= 1:2 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in rice grits carrier
Sample-D= 1:3 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in rice grits carrier
Sample-E= 1:2 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in potato grits carrier
Sample-F= 1:3 (v/w) ratio of 40% ethanol extract of *Aframomum danielli* in potato grits carrier

Table 4.14.1: *Percentage (%) recommended food for spice product samples from *Piper nigrum* and *Aframomum danielli*

List of suggested food	Pulverized *Piper nigrum*	40% ethanol extract of *Piper nigrum*	*Piper nigrum* extract coated in rice grits at 1:2ratio	Pulverized *Aframomum danielli*	40% ethanol extract of *Aframomum danielli*	*Aframomum danielli* extract coated in rice grits at 1:2ratio
Jollof rice	0	0	0	0	0	0
Boiled meat	4.5	2	4.5	8.1	4.0	5.7
Porridge	8.7	0	6.7	0	4	0
Cooked beans	0	0	0	0	0	0
Ewedu draw soup	0	0	0	0	0	7.0
Okro soup	0	0	0	4.0	2	4.0
Banga soup	6.3	1.6	2.3	15.4	14	15.0
Edikaigan soup	6.0	4.5	6.0	10.3	12	12.3
Egusi soup	0	0	0	5.5	0	0
Vegetable soup	0	0	0	0	0	0
Sauce/stew	2.8	1.0	2.8	0	0	0
Ofe-Owerri soup	0	0	0	5.4	6.1	7.0
Ofe-olubu	3	2	3	5.6	0	5.0
Pepper-soup	68.7	61.4	71.7	34	30.4	32
Bread	0	0	0	5	0	5
Biscuit	0	0	0	7	0	7
Cake	0	0	0	0	0	0
Ice cream	0	0	0	0	6.2	0
Yoghurt	0	0	0	0	5.0	0
Tea	0	4	0	0	6.0	0
Fruit juice/drink	0	3	0	0	3.0	0
Alcoholic drink	0	20.5	0	0	7.2	0
Akamu/Ogi	0	0	0	0	0	0

Ratio mean 40% ethanol spice extract to carrier material.

Table 4.14.2: *Mean sensory scores of 'Pepper-soup' seasoned with *Piper nigrum* and *Aframomum danielli* spice product sample

Sensory property	Pepper-soup samples seasoned with *Piper nigrum* products			Pepper-soup samples seasoned with *Aframomum danielli* products			LSD ($p<0.05$)
	Sample-A	Sample-B	Sample-C	Sample-D	Sample-E	Sample-F	
Color	$7.6^{a}\pm1.5$	$7.3^{a}\pm1.1$	$8.2^{a}\pm0.9$	$7.4^{a}\pm1.8$	$6.8^{a}\pm1.5$	$8.5^{a}\pm1.2$	0.634
Flavour pungency	$6.6^{a}\pm1.17$	$7.1^{a}\pm1.4$	$7.9^{a}\pm1.10$	$7.8^{a}\pm0.6$	$6.6^{a}\pm1.3$	$7.8^{a}\pm1.3$	0.544
Taste	$6.7^{a}\pm1.5$	$7.0^{a}\pm1.5$	$7.3^{a}\pm0.94$	$7.2^{a}\pm1.8$	$7.1^{a}\pm1.6$	$7.7^{a}\pm1.4$	0.679
Mouth-feel	$6.7^{a}\pm1.6$	$7.2^{a}\pm1.3$	$7.6^{a}\pm1.3$	$6.9^{a}\pm1.4$	$6.7^{a}\pm1.5$	$7.8^{a}\pm1.5$	0.663
Overall acceptability	$7.5^{a}\pm1.5$	$7.5^{a}\pm1.5$	$7.3^{a}\pm1.5$	$7.3^{a}\pm1.4$	$6.8^{a}\pm1.6$	$6.8^{a}\pm1.6$	0.716

*****Mean of twenty panelists, ($\pm$) = standard deviation; mean scores across rows having different superscript letter are significantly different ($p<0.05$)

Key

Sample-A = Pepper-soup seasoned with *Piper nigrum* spice extract in rice grits coated at 1:2ratio
Sample-B = Pepper-soup seasoned with 40% ethanol extract of *Piper nigrum*
Sample-C = Pepper-soup seasoned with pulverized *Piper nigrum* spice sample
Sample-D = Pepper-soup seasoned with *Aframomum danielli* spice extract in potato grits coated at 1:2ratio
Sample-E = Pepper-soup seasoned with 40% ethanol extract of *Aframomum danielli*
Sample-F = Pepper-soup seasoned with pulverized *Aframomum danielli* spice sample

Table 4.15.1: Total counts and characteristics of fungal isolates from *Piper nigrum* and *Aframomum danielli*

Sample code	Total colony count (cfu/g)	Colony code	Colonial characteristics	Microscopic characteristics	Identity of isolates
Whole *Aframomum danielli* Urima Raw (A)	9.2×10^8	A_1	Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
		A_2	Dull and dry serrated cream colonies	Gram positive ellipsoidal budding cells	*Saccharomyces ellipsoideus*
Whole *Piper nigrum* Uziza Raw (B)	$1.61. \times 10^9$	B_1	Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
		B_2	Dirty green spores enclosed in short hyphae	Hyphae septate,conidia arranged like mop head	*Penicillium notatum*
		B_3	Black to brown powdery spores attached on short white hyphae	Hyphae septate ,conidia globosed and attached to vesicle via serigma	*Aspergillus sp*
Aframomum danielli 'Urima' pulverized powder (C)	1.12×10^3	C_1	Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
		C_2	Dull and dry serrated cream colonies	Gram positive ellipsoidal budding cells	*Saccharomyces ellipsoideus*
		C_3	Tall white filamentous hyphae	Non septate hyphae. spores enclosed in a sporangium Non septate hyphae. Spores enclosed in a sporangium	*Rhizopus sp*
Piper nigrum 'Uziza' pulverized powder (D)	0.45×10^2	D_1	Cream mucoid circular colonies	Gram positive spherical budding cells	*Saccharomyces cerevisiae*
		D_2	Dirty green spores enclosed in short hyphae		*Penicillium notatum*
		D_3	Tall white filamentous hyphae	Non septate hyphae. Spores enclosed in a sporangium	*Rhizopus sp*

Table 4.15.2: Total counts and colonial characteristics of bacteria isolates on nutrient agar

Sample code	Total count (cfu/g)	Colony code	Colonial characteristics	Microscopic characteristics	Probable identity
Whole *Aframomum danielli* 'Urima'Raw (A)	7.8×10^8	A_1	Moist and shiny smooth golden yellow colonies	Gram positive cocci in clusters, few in pairs and tetrads	*Staphylococcus* sp
		A_2	Gram positive short rods with central spores Small circular yellow colonies	Gram positive cocci in chains	*Enterococcus* sp
		A_3	Dull and dry flat serrated cream colonies	Gram positive cocci predominantly in tetrads and few in clusters	*Micrococcus* sp
		A_4	Dull and dry flat serrated cream colonies	Gram positive short rods with central spores	*Bacillus* sp
Whole *Piper nigrum* Uziza Raw (B)	1.21×10^9	B_1	Dull and dry flat serrated cream colonies	Gram positive short rods with central spores	*Bacillus* sp
		B_2	Bluish green pigments	Gram negative single rods	*Pseudomonas* sp
		B_3	Gram positive short rods with central spores	Gram positive cocci in chains	*Enterococcus* sp
Aframomum danielli 'Urima' pulverized (C)	1.8×10^3	C_1	Dull and dry flat serrated cream colonies	Gram positive short rods with central spores	*Bacillus* sp
		C_2	Small circular yellow colonies	Gram positive cocci predominantly in tetrads and few in clusters	*Micrococcus* sp
		C_3	Gram positive short rods with central spores	Gram positive cocci in chains	*Enterococcus* sp
		C_4	Moist and shiny smooth golden yellow colonies	Gram positive cocci in clusters, few in pairs and tetrads	*Staphylococcus* sp
Piper nigrum r 'Uziza' pulverized powder (D)	0.9×10^3	D_1	Dull and dry flat serrated cream colonies	Gram positive short rods with central spores	*Bacillus* sp
		D_2	Gram positive short rods with central spores	Gram positive cocci in chains	*Enterococcus* sp

Table 4.15.3: Total counts and colonial characteristics of bacteria on MacConkey agar

Sample code	Total plate counts Cfu/g	Colony code	Colonial characteristics	Microscopic characteristics	Probable identity
Whole *Aframomum danielli* 'Urima'Raw (A)	7.0×10^8	A_1	Dull and dry finger like projections	Gram positive short rods with central spores	*Bacillus* sp
		A_2	Mucoid pinkish colonies	Gram positive cocci in clusters	*Staphylococcus* sp
Whole *Piper nigrum* 'Uziza' Raw (B)	1.22×10^9	B_1	Small circular moist and shiny pink colonies	Short gram negative rods predominantly in singles	*Escherichia coli*
		B_2	Dull and dry finger like projections	Gram positive short rods with central spores	*Bacillus* sp
Aframomum danielli 'Urima' pulverized powder (C)	NG	C_1	NG	NG	NG
		C_2			
		C_3			
		C_4			
Piper nigrum 'Uziza' pulverized powder (D)	NG	D_1	NG	NG	NG
		D_2			

NG= No Growth

Table 4.15.4: Total counts and colonial characteristics of bacteria on Brain Heart Infusion Agar (BHIA)

Sample code	Total plate count		Colonial characteristics	Microscopic characteristics	Probable identity
Whole *Aframomum danielli* 'Urima'Raw (A)	1.0×10^5	A_1	Small circular golden yellow colonies	Cocci in long chains	*Streptococcus* sp
		A_2	Dull and dry serrated flat colonies	Slender rods in chains	*Lactobacillus* sp
		A_3	Mucoid and slimy cream colonies	Large gram positive rods with central spores	*Bacillus* sp
Whole *Piper nigrum* Uziza Raw (B)	2.1×10^6	B_1	Dull and dry serrated flat colonies	Slender rods in chains	*Lactobacillus* sp
		B_2	Golden yellow colonies	Cocci in long chains	*Streptococcus* sp
		B_3	Dull and dry serrated flat colonies	Slender rods in chains	*Lactobacillus* sp
Aframomum danielli 'Urima' pulverized powder (C)	NG	-	-	-	-
Piper nigrum 'Uziza' pulverized powder (D)	NG	-	-	-	-

NG= No Growth

Table 4.15.5: Biochemical and Carbohydrate fermentation reactions of bacterial isolates

Colony code	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
A_1	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	-	+	*Staphylococcus aureus*
A_2	-	-	-	-	+	-	+	-	+	+	+	+	-	+	-	-	-	*Enterococcus faecalis*
B_1	+	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	*Escherichia coli*
B_2	+	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	+	*Bacillus cereus*
C_1	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	*Bacillus subtilis*
C_2	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	*Lactobacillus* sp
C_3	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	+	+	*Streptococcus* sp
D_1	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	*Micrococcus luteus*
D_2	+	+	-	-	+	-	+	+	+	+	-	-	-	+	+	+	-	*Pseudomonas aeruginosa*

+ = positive reaction, - = negative reaction. Colony code: A= *Aframomum danielli raw*, B= Raw *Piper nigrum*, C= Pulverized *Aframomum danielli*, D = Pulverized *Piper nigrum.*

4.2 DISCUSSION OF RESULTS

4.2.1Moisture content of pulverized and encapsulated spice samples and specific gravity of solvents flavour extracts of spice product samples

Moisture content of pulverized powder and encapsulated flavour products from *Piper nigrum* and *Aframomum danielli* were presented in Table 4.1. There was significant difference ($p<0.05$) on the moisture content of the spice flavour products. Moisture content 8.22% in pulverized *Piper nigrum* powder and 7.01% in pulverized *Aframomum danielli* were higher than moisture content in encapsulated flavour extract samples in corresponding spices. However, the percentage moisture content in pulverized spice powder samples were below 12.0% maximum limit for moisture of dried and grounded spices (International Organization of Spice Trade Associations (IOSTA) 2013; International Organization of the Flavour Industry (IOFI) (2012); European Spice Association (ESA) (2011).

Moisture content of pulverized spice samples in this study was closely agreed with the report of earlier works in literature (Olalekan *et al.,* 2003; Peter, 2001). The moisture content of spice coated product samples (encapsulated) varied slightly. In rice grits coated spice extract samples (RG-C_1 and RG-C_2), the moisture content reduced from 6.88% to 6.08% in *Piper nigrum* product samples and 6.45% to 6.12% in *Aframomum danielli* product samples when the proportion of carrier or coating material increased from 1:2v/w ratio to 1:3v/w ratios. Similarly in potato grits coating material samples (PG-C_1 and PG-C_2), moisture content of the coated spice products samples decreased from 7.11% to 6.32% in *Piper nigrum* product samples and 6.96% to 6.01% in *Aframomum danielli* product when the proportion of potato grits carrier increased from 1:2v/w ratio to 1:3v/w ratio. The reduction on moisture content could be simply due to the increase in

proportion of the carrier as the proportion of the extract is decreases. Since the carrier is dry, the moisture must be reduced as it increases. This result and observation was in agreement with Prince *et al.,* (2014) who reported decreased moisture content level of encapsulated or coated nutmeg oleoresin when blending proportion of gum arabic increased from 0% to 60%. The slight reduction on moisture content of encapsulated samples could be due to effect of chemical property carrier materials (carbohydrate in rice and potato grits) which is known to have high absorption capacity for solvents used for flavour extract and as well as increased ratio or proportion in the blends (Kanakdande *et al.,* 2007; Shaikh *et al.,* 2006; Krishman *et al.,* 2005a, Krishman *et al.* 2005b). However, the low moisture content of pulverized spice flavour powder and encapsulated flavour samples is an indication of the fact that these spice flavour products can be stored for a longer period without deterioration in quality (Agomuo *et al.,* 2011; Ogunka-Nnoka and Mepba, 2008). The implication of high moisture content in food is that it support growth and proliferation of micro-organisms under normal storage condition (Agomuo *et al.,* 2011; Ogunka-Nnoka and Mepba, 2008).

4.2.2 Specific gravity of *Piper nigrum* and *Aframomum danielli* flavour extracts

Specific gravity of solvents and spice extracts samples in *Piper nigrum* and *Aframomum danielli* spices (Table 4.2) were significantly varied ($p<0.05$) among the samples. The increased specific gravity in absolute ethanol extracts from *Piper nigrum* and *Aframomum danielli* spices showed 0.784 and 0.612 highest values respectively. Specific gravity is an important physical constant and is specific for each liquid. It is a criterion which indicates the quality and purity of liquid and oil extracts (Kumar, 2014). Specific gravity of the ethanol spice flavour extracts from both spices was higher than other solvents extract which indicates that the extracted spice flavour is highly pure than other solvents used for spice extracts. Following in this order of specific gravity was

0.750 in *Piper nigrum* and 0.632 in *Aframomum danielli* spice flavour extract samples with methanol. In addition, 40% ethanol extracts in both spices had 0.626 and 0.570 specific gravity. Acetone spice flavour extract in *Piper nigrum* and *Aframomum danielli* showed specific gravity of 0.576 and 0.632 respectively. N-hexane (non-polar solvent) extracts in both spices studied had 0.320 and 0.228 specific gravity while water extracts (control) in each spice shown specific gravity of 0.435 and 0.320. The specific gravity found on the solvents spice flavour extracts in this work was slightly lower compared to 0.860-0.884 specific gravity of black pepper flavour oil (Meghwal and Goswami, 2012). In this it could be said that the polarity of the solvent influence the specific gravity which determine the density of flavour extracts in each spices used. Aremu, *et al*., (2015) stated that increased molecular mass of organic matter in the plant and as well as nature of the polarity of the components therein increased the viscosity of the plant extract. It could be said from this work that ethanol solvent may be more suitable for the extraction of bioactive flavour components in spices.

4.2.3 Phyto-chemical properties of spice flavour extracts

The phyto-chemical screening and quantitative determination of the percentage yields of phyto-chemical constituents of *Piper nigrum* and *Aframomum danielli* (Table 4.3) showed that the seeds of the spices contained alkaloids, flavonoids, phytic acid, saponin, tannins and oxalate. These phyto-chemicals identified and quantified in this work were similar to those found in 70% ethanol extracts of *Aframomum melegueta* and *Piper guineense* spice varieties (Ekpo *et al.,* 2013). These phyto-chemicals exhibit a wide range of bioactive effect naturally in plant where they exist and responsible for spice flavour pungencies and antioxidant properties in food (Okwu, 2005).

A number of solvents and their mixtures like heptane, acetone, ethanol-water azeotrope, methyl-pentane, isohexane, petroleum-ether, trichloroethane, chlorinated hydrocarbons, alcohols etc. for extraction of bioactive compounds in plant materials (such as cotton seeds) have been reported (Gandhi *et al*., 2003; Maria *et al*., 2008; Kuk. *et al*., 2005; Sepidar *et al*., 2009). Junfung (2010) has reported simultaneous extraction of oil and other chemical components using mixture of methanol and hexane. The residual hexane in extracted product is not within the acceptable limits, but, Bhowmick (2003) have advocated the use of isopropanol due to the higher solvency for extraction. In the alcohol series, ethanol is the safest solvent as it is obtained from biological sources by the fermentation process and is placed in the category of GRAS (generally recognized as safe) (Bhowmick, 2003).

4.2.3.1 Alkaloid content of spice flavour extracts

The alkaloid content in *Piper nigrum* and *Aframomum danielli* spice flavour extracts (appendix II Figure 1) showed significant difference ($p<0.05$). *Piper nigrum* and *Aframomum danielli* had 7.51% and 8.89% total alkaloid content respectively. *Piper nigrum* and *Aframomum danielli* absolute ethanol extract contained 6.54% and 7.08% alkaloid respectively. The proportion of alkaloid in 40% ethanol solvent extracts is 3.91% in *Piper nigrum* and 4.12% in *Aframomum danielli.* In methanol spice flavour extracts recorded 2.08% in extract of *Piper nigrum* and 3.72% in extract of *Aframomum danielli*.

In addition, the least alkaloid content 1.04 % in n-hexane extract of *Piper nigrum* and 2.83% in n-hexane extract of *Aframomum danielli* was discovered. The control spice extract samples (water extract) showed 2.11% in *Piper nigrum* and 3.10% in *Aframomum danielli* extracts. The bite and pungency in Piper species (black pepper and white pepper) has been associated with the non-volatile alkaloids (piperine and chavicin), and their ratio varies in different pepper, thus,

giving rise to different pungencies (Raghavan, 2006). The alkaloid content found in absolute ethanol flavour and 40% ethanol was higher than 1.67% in *Piper guineensis* and 2.17% in *Aframomum melegueta* 70% ethanol (Ekpo, 2013). It was noted that the percentage yield of alkaloid in absolute ethanol and in 40% ethanol extracts were higher significantly ($p<0.05$) compared to other solvents used for extraction as observed in both *Piper nigrum* and *Aframomum danielli.*

The variation found in alkaloid content could be due to the effect of the polarity of the solvents used. The polarity and less hazardous nature of the solvent are of utmost importance in the extraction of bioactive compounds of herbal and spice products (Tanko *et al*., 2005; Luthria *et al.,* 2006; Tsimogiannis *et al.,* 2006). Chemical components in spices and other plant materials have been known to be polar or non-polar in nature, this becomes important factor to be considered in choice of solvent and methods of spice flavour extraction (Pellegrini *et al.,* 2007). Differences in the structure of phyto-chemical compounds in plant materials (such spice and herbs) also determine their solubility in solvents of different polarity.

Therefore type of extraction solvent as well as the isolation procedures may have a significant effect on the yield of extractable phyto-chemicals, and anti-oxidants from plant material. There are some reports concerning optimization of extraction conditions of anti-oxidant compounds content and antioxidant activities of some plant foods but as some researches indicated optimal procedure is usually different for different plant matrices (Rababah *et al*., 2010; Pellegrini *et al.,* 2007).

4.2.3.2 Flavonoid content of spice extracts samples

Flavonoid content analyzed in this work is among the phenolic compounds in herb and spice plant materials reported by Boskou (2006a). Flavonoid content (appendix II Figure 2) in solvents

flavour extracts from *Piper nigrum* and *Aframomum danielli* spices varied. Flavonoid values of 5.66% and 5.56% in ethanol (absolute and 40%) *Piper nigrum* extracts and 6.45% and 5.10% in ethanol (absolute and 40%) *Aframomum danielli* extracts respectively were significantly ($p<0.05$) higher than flavonoid content in other solvents (methanol, acetone, hexane and distil water) within each spice. In addition, flavonoid yields of 5.18% and 4.90% were found in acetone and methanol extract of *Piper nigrum*.

Flavonoid content of methanol extract of *Aframomum danielli* was 3.27% and 3.22% in acetone extract. Hexane flavour extract of *Piper nigrum* recorded the least value (2.05%) followed by the distil water (2.09%). Ekpo *et al.,* (2013) reported 1.92% and 2.03% flavonoid in 70% aqueous ethanol extracts from *Piper guineesis* and *Aframomum melegueta*.

4.2.3.3 Phytic acid content of spice extracts samples

Similar trends were recorded for phytic acid content (appendix II Figure 3) of solvent extracts of *Piper nigrum* and *Aframomum danielli* samples. Phytic acid content 7.72% and 5.91% in *Piper nigrum* ethanol extract samples (at absolute and 40% concentration) and 4.19% and 3.11% in *Aframomum danielli* ethanol extract samples (at absolute and 40% concentration) were significantly ($p<0.05$) greater than phytic acid content in other solvent extracts used for extraction in both spices. However polar solvents (ethanol, methanol, acetone) used in this work are similar to solvents (either pure or aqueous mixture) reported for the extraction of active components in plant materials (Zlotek *et al.,* 2016; Alothman *et al*., 2009; Lafka *et al*., 2007; Boskou, 2006a). Also, the level of phenolic compounds discovered in this work was in line with 5mg/100g to10mg/100g in Olive and Olive oil (Boskou, 2006a); basil leaves (Zlotek *et al.,* 2016). But not agreed with 37.10% and 35.40% phenol found in 70% ethanol extracts from *Piper guineesis* and *Aframomum melegueta* (Ekpo *et al.* 2013).

But variation observed on phenolic compounds in *Piper nigrum* and *Aframomum danielli* flavour extracts and those reported in literatures could be associated with the isolation of antioxidants from a plant material using different techniques and solvents because of diversity of chemical nature of these compounds and often unique distribution of these compounds in the plant matrix (Sultana *et al.,* 2009; Antolovich *et al*. 2000). Solvent extraction is the most frequently used technique for recovery of the plant antioxidants, phyto-chemical and other bioactive components (Shabbir *et al*. 2011; Sultana *et al*. 2009).

However, the yields and antioxidant efficacy of the resulting extracts is strongly affected by the polarity of the solvent as well as the chemical nature of the isolated compounds (Shabbir *et al*. 2011; Sultana *et al*. 2009). Pandey and Rizvi (2009) stated that plant phenolic compounds play an important role in the shaping of the biological properties of the plant including antioxidant properties and protective mechanism against attack by insect. Flavonoid has applications in food industry as well as cut flower industry. Flavonoids are generally responsible for color, taste, prevention of fat oxidation and protection of vitamins and enzymes in foods (Yao, 2004).

4.2.3.4 Saponin contents of spice flavour extracts

The percentage yield of saponin in *Piper nigrum* and *Aframomum danielli* spice extracts using different solvents were presented in Table 4.7. The percentage saponin (appendix II Figure 4) found in solvent extracts of *Piper nigrum* were varied significantly ($p<0.05$) and were all below 1%. Percentage yields of saponin in 40% ethanol and methanol extracts of *Piper nigrum* were 0.64%, and 0.40% respectively. Acetone extract had 0.06% least saponin content in solvents extract samples. But the saponin in solvent extracts of *Aframomum danielli* ranged from 0.07% least value in hexane extract to 0.82% highest value found in absolute ethanol and 40% ethanol extracts. Bernhoft (2010) stated that saponin is one of the categories of glycoside (with foaming

characteristic) a secondary metabolite produced in plant. It is associated with bitter taste and have significant bioactivities like anti-inflammatory (Wang *et al.,* 2008), anti-tumor (Jung *et al.,* 2004) roles.

However, the percentage yields of saponin from *Piper nigrum* and *Aframomum danielli* spices were very low in all the solvents extract samples (polar and non-polar). This suggests that the biosynthesis of saponin (unlike tannin and oxalate) in both spices studied in this work may be minimal compared to yields of alkaloid, flavonoid and phytic acid in the same spices. Gurib-Fakim, (2005), Heirich *et al.*, (2004) and Bruneton (1999) in separate reports stated that glycosides in herbs and spices made-up two components glycone (carbohydrate component) and aglycone (a non-carbohydrate component). The solubility of glycosides such as saponin depends on the nature of the aglycone and the number and type of sugar molecules linked to the aglycone (Starmans and Nijhuis, 1996). Aglycones tend to be soluble in organic solvents and sugar part in aqueous solvents. In general, glycosides can be extracted with acetone, ethanol or an aqueous/ethanol mixture (Jones and Kinghorn, 2005).

4.2.3.5 Tannin contents of spice flavour extracts

Tannin content (appendix II Figure 5) of *Piper nigrum* and *Aframomum danielli* solvent extract samples varied significantly ($p<0.05$). In both spices studied in this work, tannin content of 1.33% in absolute ethanol extract in *Piper nigrum* spice and 0.46% in *Aframomum danielli* were higher than values of tannin found in other solvents used for extraction. In all solvent extract samples of *Piper nigrum*, hexane extract recorded 0.46% (least) tannin yield while 0.22% was the least yield found in solvent extracts of *Aframomum danielli*. Variation on the percentage yields of tannin discovered in different solvents used in both spices in this study was in line with

the report of Baldosano *et al.,* (2015) who discovered tannin content of *Spondias purpurea* fruit ranged from 2.76% to 19.19% in aqueous ethanol at 50-95% v/v at different extraction time. Also Downey and Hanlin (2010) reported that percentage yield of condensed tannin from grape skin varied with type of solvent (acetone and ethanol) and concentration of the solvent used.

However, different yields of tannin discovered in solvent extracts in each spice could be attributed to a number of factors which may include the nature and chemical composition in plant material, interaction between tannin and extracting solvent, polarity and concentration of extracting solvent, effect of particle size and extraction time (Baldosano *et al.,* 2015).

In spite of the report that tannin is highly soluble in water (Baldosano *et al.,* 2015), the result found in this work suggests that ethanol was an effective extraction solvent for tannin just as revealed for other phyto-chemicals. This could be due to it being an organic polar solvent. The polarity of ethanol makes it able to have strong interactions with polar substances such as tannin. In food, tannin possesses several beneficial characteristics such as being astringent, an antioxidant, and anti-bacterial (Sher, 2004) and also has a wide range of uses in industries such as in wine production.

4.2.3.6 Oxalate contents of spice flavour extracts

Oxalate content of solvents extracts of *Piper nigrum* and *Aframomum danielli* (appendix II Figure 6) showed that there was significant difference ($p<0.05$) in oxalate content of the spice extracts. Ethanol extracts of *Piper nigrum* and *Aframomum danielli* recorded 2.51% and 0.98% respectively. These values were higher compared to 2.43% and 0.36% in 40% aqueous ethanol extracts from *Piper nigrum* and *Aframomum danielli* spices. The highest oxalate content 2.51%

in ethanol extract of *Piper nigrum* and its total oxalate 3.67% were higher than 0.55mg/100g in leaf and seeds of *Piper guineensis* (Ojinnaka *et al.*, 2016).

However, the difference in oxalate content in solvent extracts of *Piper nigrum* and *Aframomum danielli* could be attributed to the nature and chemical composition of the spices, while the efficiency and polarity of the solvents used may have caused different levels of oxalate to be discovered on flavour extracts in each spice. Oxalates in foodstuffs are known to sequester and precipitate some useful metallic elements, thus making them unavailable for absorption in human system (Ojinnaka *et al.*, 2016; Groff *et al.*, 1995). Therefore adequate extraction or processing methods has been advocated as means of preventing their lethality in human being.

The choice of solvent for extraction bio-active content of plant materials depends mainly on the polarity and the solubility of the bioactive compounds of interest. Although water is usually applied as a solvent in many traditional protocols, organic solvents of varying polarities are often used (either alone or in different combinations) in modern methods of extraction in order to exploit the various solubility of herbal and spice ingredients (Handa *et al*., 2008; Lapornik *et al.*, 2005). The polarity and chemical profiles of most of the common extraction solvents have been determined (Eloff, 2001; Cowan, 1999; Ayaffor *et al*., 1994). In this study, ethanol (within the volume used) was most favorable organic solvent for extraction of phyto-chemicals responsible for the flavour pungency in *Piper nigrum* and *Aframomum danielli.*

4.2.4 Major flavour components in *Piper nigrum* spice flavour extract

Gas chromatography-mass spectrometry (GC/MS) technique identified various chromatogram spectrums, molecular formula and as well as percentage concentration areas of flavour principles in *Piper nigrum* solvents extracts (Tables 4.4.1). Figure 4.1 showed that fifty eight (56) peaks

were identified with 98% ethanol extract of *Piper nigrum* and 18 of these peaks were above 1% and referred to as aroma active components (AACs) or major flavour principles (MFPs) (Table 4.3.1). These chemical components include beta-pinene 2.57%, copaene 2.07%, Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- $$ Cyclohexane, 2,4-diisopropenyl-1-methyl-1-vinyl-, 4.01%, bicycle (7,2,0) undec,4,1111-trimethy-8 5.22%, cis-beta-famescence 6.39%, humulene 2.02%, naphthalene decahydro-4a-methyl-1-methylene 1.34%, beta-bisabolene 6.81%, n-hexadecanoic 2.70%, 9,12-octadecadienoic acid 1.15%, 3-Adamantan-1-yl-3-oxo-propionitrile 2.15%, kauran-18-al, 17-(acetyloxy-4-beta) 1.30%, ethyl piperroncyano acetrate 14.47%, G-eicosyne 2.66%, Methanone, (2-amino-6,7-dihydro-5H-pyrrolo[1,2-a]imidazol-3-yl)(2,4-dimethoxyphenyl)- 8.38%, pyrrolodine (5-1,3-benzodioxol-5-yl-1-oxo) 11.05%, piperine 17.53% and 9,12-octadecadienoyl chloride (Z,Z).

Furthermore, sixty peaks of chemical compounds were eluted from 40% ethanol extract of *Piper nigrum* sample (Figure 4.2) and Table 4.4.2 present the twenty five (25) major flavour principles (MFPs) were identified. Among the MFPs are copaene 1.84, cyclohexane, 1ethenyl-1methyl-2,4-bis (1-methyl) 4.26%, bicycle (7,2,0) undec-4-ene,4,11,11-rimethyl-8 was 5.09%, gamma-elemene 1.29%,beta-famescene 6.55%, humulene 2.65%, beta-tlangene 3.63%, Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-1.52%, Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)- was 1.51%, beta-bisabolene 7.22%, cycohexane, 3-(1,5-dimethyl-4-hexenyl) 1.92%, apiol 2.33%, n-hexadecanoic acid 2.96%, hexadecanoic acid ethyl ester 1.63%. Also, chemical compounds in this category are 9,12-octadecadienoic acid ethy ester 1.14%, ethyl oleate 1.22%, benzene (3-iodo-1-methyoxy-1-methylpropyl) 2.66%, 9-eicosyne 3.91%, benzaminde, (-2-hydroxypheny)-2,-dimethrox 5.33%, methanone, (2-amino-6,7-dihydro-5H-pyrrolo[1,2-a]imidazol-3-yl)(2,4-dimethoxyphenyl)-

11.72%, hexadeca-2,6,10,14-tetraen-1-ol,3,7,11,16-tetra 1.35%, pyrroidine-1-(-5)1,3-benzodioxol-5-yl-oxo 1.67%, piperine 15.42%, 9,12-octadecadienoyl chloride (Z,Z) and n-octadeca-6,9,12,15-tetraenoylpyrrolized was 1.25%. Flavour components identified in *Piper nigrum* flavour extract samples with ethanol and mixture of water and ethanol solvents were similar to aroma active compounds (AACs) found in *Piper nigrum* berries (Jirovetza *et al.*, 2002; Meghwal and Goswami 2002). Vasavirama and Upender (2014) stated that the major two components in *Piper nigrum* are essential oil and piperine which contributes to the flavour pungency.

The peaks profile of chemical components in methanol extract of *Piper nigrum* sample showed 42 peaks (Figure 4.3) and twenty (20) major flavour principles (PFPs) were identified (Table 4.4.3). The MFPs in methanol solvent spice extract are bicycle (3,1,1) heptain, 6,6-dimethy-2-methylene (IS) 1.65%, alpha-guaiene 1.92%, 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-was 3.21%, beta-bisadolene 2.14%, Cyclohexane,3-(1,5-dimethyl-4-hexenyl)6-methylene, [S-(R*,S*)] 5.24%, diethyl phthalate 10.92%, 2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane 6.13%,1-Dodecanone, 2-(imidazol-1-yl)-1-(4-methoxyphenyl)-1.40%, 2-hexanone, 3-cyclohexylidene-4-ethyl 1.50%, Tricyclo[3.3.1.1(3,7)]decane-1-carboxylic acid, 2,2,3,3,4,4,5,5,6,6,7,7,-dodecafluoroheptyl ester 1.99%, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one, 9-[[(1,3-benzodioxol-5-ylmethyl)amino]methyl]octahydro-2,5a-dimethyl- 7.96%, piperine 6.38%, cholest-22-ene-21-ol-3,5-dehydro-6-methyoxyl, pivalate 8.21%, 5aH-3a,12-Methano-1H-ycloprop[5',6']cyclodeca[1',2':1,5]cyclopenta[1,2-d][1,3]dioxol-13-one, 1a,2,3,9,12,12a-hexahydro-9-hydroxy-10-(hydroxymethyl)- 9.02%, 2-Benzofuranmethanol, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-, cis- 6.15%, Silane, [[(3.beta.)-gorgost-5-en-3-yl]oxy]trimethyl-

4.65%. Also included are 22, 26-oxido-4, 17-cholestedian-3-beta, 1-alpha 4.52%, piperine 5.52% and 1.42% of ethyl iso-allocholate.

Seventy eight chromatogram peaks were shown in acetone extract of *Piper nigrum* sample (Figure 4.4). Based on concentration above 1% twenty six chemicals were selected as major flavour principles (Table 4.4.4). These MFPs are Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-1.44%, alfa-copaene 2.38%, 1-pentadecene 1.59 Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-2.93%, caryophyllene 4.11%, 1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)- 1.44%, beta-famescene 1.43%, humulene 1.18% alpha-guaiene 2.28%, 1,6cyclodecadiene, 1-methyl-5-methylene 3.79%, cyclohexane, 1,3-disopropenyl-6-methyl 2.26%, beta-bisabolene 2.50%, cyclohexene-3-(1,5-dimethyl-4-hexenyl) 5.80%, bromoacetic acid, pentadecyl ester 4.05%, 1-nonadeaene 1.55%, 3-Adamantan-1-yl-3-oxo-propionitrite 1.19%, 1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose 3.59%, beta-sitosterol 1.63%, 3-amino-4-piperronyl-5-pyrazolene8.41%, 5aH-3a,12-Methano-1H-cyclopropa[5',6']cyclodeca[1',2':1,5]cyclopenta[1,2-d][1,3]dioxol-13-one, 1a,2,3,9,12,12a-hexahydro-9-hydroxy-10-(hydroxymethyl)- 2.18%, piperine 6.01%, 2,2,6-trimethyl-bicyclo (4,1,0) hept-1-yl 1.83%, 3,4-dimethoxbenzoicanhydride 3.88%, isoxaben 5.29% and alpha.-Santonin 1.36%. The MFPs found in methanol and acetone spice extract samples in this study were in agreement with reports in literature (Tomsone *et al.,* 2012; Aziz *et al.,* 2012; Jirovetza *et al.,* 2002; Meghwal and Goswami 2002).

From results obtained in this study it could be suggested that ethanol solvent (within the volume used in this work) was most suitable for food flavour extraction from *Piper nigrum* coupled with the safety of using ethanol compared to methanol and acetone (International Organization of the

Flavour Industry (IOFI) (2012). This observation was in line with Zhou *et al.,* (2004) who stated that plant extracts obtained using high polarity solvents (ethanol and methanol) were considerably more effective radical scavengers than using less polarity solvents (acetone and chloroform). With change in solvent polarity its ability to dissolve special group of antioxidant compounds alters and influences the antioxidant activity estimation. Shams *et al*., (2015) reported classic techniques for solvent extraction of active constituents from spices and herbs matrices are based on the choice of solvent coupled with the use of heat or agitation. Therefore various types and concentration on spectrums of chemical compounds found in different solvents used for extraction of flavour from *Piper nigrum* may not be due only to polarity of the compounds and solvent alone but also the nature of the spice matrices, particle size, temperature and time of extraction as well as the degree of heat or agitation.

Thirty (30) major flavour principles (MFPs) (Table 4.4.5) out of total eighty six peaks of chemical constituents in n-hexane flavour extract of *Piper nigrum* (Figure 4.5) was found. MFPs found in this sample were similar to those listed in other solvent extract samples but are different in concentration. These are alpha-copaene 2.90%, cyclohexan-1-ethenyl-1-methyl-2,4-bis 4.10%, caryophyllene 5.10%, cis-beta-farmescene 2.99%, humulene 1.98%, alpha guaiene 3.05%, gamma-murolene 1.24%, beta-copaene 5.17%, Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]- 1.58%, 1,3-benzodioxide, 4-methyoxy-6-2-propenyl 2.76%, beta-humulene 1.52%, beta-bisabolene 3.54%, 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- 2.15%, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- 6.08%, Benzene, 1,2,3-trimethoxy-5-(2-propenyl) 1.29%, cyclohexanemethanol,4-ethenyl-apha 1.21%, 1,6,10-didecatrien-3-ol 1.93%, 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene 2.47%, caryophyllene oxide 1.64%, 9-Isopropyl-1-methyl-2-methylene-5-

oxatricyclo[5.4.0.0(3,8)]undecane 1.27%, alpha-bisabolol 1.22%, beta-santalol 1.28%, n-hexadecanoic acid 2.69%, 9,12-octadecadienoic acid 1.64%, 1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose 1.79%, 3-Ethyl-5-hydroxy-5-trifluoromethyl-2524,5-dihydropyrazol-1-yl)-(3-hydroxy-2-m332ethylphenyl)methanone 6.78%, (R)-(-)-28514-Methyl-8-hexadecyn-1-ol 1.39%, Isoxaben 3.87%, piperine 2.43% and 1.06% of (R)(-)-14-methy-8-hexadecyn-1-ol. Indication showed that the concentration of the MFPs in n-hexane flavour extract were lower compared to the concentration in polar solvents (ethanol, methanol and acetone) but higher than compounds in water flavour extract (control sample). Figure 4.6 showed that five (5) chromatogram spectrums were found in water spice extract sample. Three (3) of these spectrums were presented in Table 4.4.6 as major flavour principles (MFPs). MFPs in water spice extract sample include acetic acid 7.58%, diethyl phthalate 3.67% and Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl) propyl] maleate 56.47%. Piperine was found insoluble in aqueous water flavour extract. This result was in line with Smita *et al.,* (2011) who reported that piperine was soluble in petroleum ether, chloroform and methanol but insoluble in water. It is noteworthy to recall that piperine was identified in this work and other reports in literature as the major aroma active compound responsible for flavour pungency in Piper species 'black pepper' (Aziz *et al.,* 2012; Jirovetza *et al.,* 2002; Meghwal and Goswami, 2002). This thereby suggested that water as solvent cannot be used for flavour extraction from black pepper.

Table 4.5 present the common major flavour principles (MFPs) among the solvent flavour extract samples from *Piper nigrum* seeds. Common MFPs found all the flavour extracts except water are cyclohexane-1-ethenyl-1-methyl-2, 4-bis (1-methyl) 2.93% to 4.26%, cis-beta-famescene 1.08% to 6.55%, humulene 1.18% to 2.65%, beta-famescene 2.50% to 7.22%, n-

hexadecanoic acid 2.69% to 2.70% and piperine2.43% to 17.50%. These common MFPs were not found in water extract samples, also, there was significant different ($p<0.05$) on quantity of MFPs of the flavour extract samples. Also, indication from result showed that piperine was insoluble in water extract samples; this was in agreement with Smita *et al.,* (2011).

4.2.5 Major flavour components in *Aframomum danielli* flavour extract

The chemical composition of flavour extracts from the seeds of *Aframomum danielli* by solvents extraction shown different total peaks of chromatogram spectrums in different solvents. Figure 4.7 showed ten (10) peaks in absolute ethanol extract and eight of these compounds had percentage concentration above 1% and referred as major flavour principles (MFPs) or aroma-active compounds (AACs) (Table 4.6.1). The MFPs identified are trimethylsilyl fluoride 3.88%, beta-pinene 6.94%, eucalyptol 53.77%, alpha-terpineol 4.97%, oleic acid, 16.89%, diethyl-phthalate 4.21%, 1-dodecanol 4.03% and ethyl iso-allocholate 3.69%.

Also, nine chromatogram peaks were found in 40% ethanol extract of *Aframomum danielli* flavour extract (Figure 4.8) and seven MFPs of the compounds was presented in Table 4.6.2. The list of MFPs in 40% ethanol flavour extract sample was similar to MFPs in absolute ethanol flavour extract but differed in percentage area concentration. This includes (S)(-)-1,2-propanediol 9.01%, beta-pinene 4.37%, diethyl phthalate 4.01%, eucalyptol 50.70%, alpha-terpineol 3.93%, oleic acid 13.47% and 2.65% of ethyl iso-allocholate. The essential oil and flavour extract from the seeds of *Aframomum* species have been reported to consist some of the chemical compounds found in this study (Owokotomo *et al.,* 2014; Adegoke *et al.,* 1998). However, difference in concentration could be due to the polarity and concentration of ethanol solvent in the extraction processes.

In addition spice extract samples in other polar solvents (methanol and acetone) showed similar compounds with ethanol flavour extract samples. Figure 4.9 showed 22 peaks of chemical compounds in methanol extract sample and six of the compounds were presented in Table 4.6.3 as major flavour principles (MFPs). The MFPs identified are eucalyptol 62.05%, alpha-terpineol 4.23%, diethyl-phthalate 3.81%, also, included are 2.18% of 10,'10-dimethox-3, 7-diethyl-dec-2,6-dien-1-ol, while ethyl iso-allocholate was 1.65% and 1.09% ergost-25-ene-3,5,6,12 tetro (beta, 5 alpha).

A total of 28 peaks of compounds (Figure 4.10) were found in acetone flavour extract sample and of which 15 MFPs were identified (Table 4.6.4). The MFPs in acetone flavour extract were 2-pentanonehychoxyl-4-methyl 7.44%, bicycle (3,1,1) 6,6-dimethyl 1.4%, eucalyptol 22.19%, alpha-terpineol 7.13%, -1-pentadecene 1.20%, n-hexadecanoic acid 4.26% and1-eicosanol 1.14%. Also included are oleic acid 7.62%,1-heptadec-1-ynyl-cyclopentanol 2.98%, caryophyllene oxide 4.16%, alloaromadendiene oxide 2.86%, Cholesta-3-ene 2.25%, 1-Naphthalenemethanol 5.55%, longipinane 5.29%, 4,8,13-cyclotetradecatriene-1,3-diol,1,5,9-trine 15.79%, 6-epi-shyobunol 3.14%. Most of the major flavour principles (MFPs) found in hexane flavour extract are similar in all the solvent flavour extracts samples except in water extract (as control), this could be due to the poor solubility of these compounds in water (Tomsone *et al.*, 2002).

Highest number of chromatogram peaks of compounds was found in Hexane solvent flavour extract of *Aframomum danielli* sample. A total of 84peaks were found (Figure 4.11), whilst twelve (12) MFPs were presented in Table 4.6.5. Hexane flavour extract contained eucalyptol 20.53%, alpha-terpineol 6.53%, caryophyllene 0.61%, bicycle (3,1,1)hep-2-ene,2,6-dimethyl-6-(-4) 1.06%, tetradecanoic acid 1.07%, n-hexadecanoic acid 12.11%, 9-octadecanoic acid methyl

ester (E) 1.09%, 2,4a,8,8-tetramethyl dechydrocyclo-propa (d) 1.34%, oleic acid 24.23%, 8-hxadecenal,14-methyl (Z) 1.04%, caryophyllene oxide 1.98% and 2.64% of 1-heptatriacotanol. The result obtained in this study corroborates the findings of Ajaiyeoba and Ekundayo, (1999) on the chemical compounds in *Aframomum melegueta* (Roscoe) K. Schum. seeds extracts. There was no peak of volatile flavour compound (Figure 4.12) discovered in water extract sample. So, no flavour compound detected apart from ethanol solvent used for sample preparation prior to injection into GC-MS analyzer. This could be due to less affinity of the matrices of compounds in the seeds *Aframomum danielli* in water.

Therefore, success in the identification of bioactive compounds from a wide variety of plants is highly dependent on the type of solvent used in the extraction process. Furthermore, the choice of solvent depends on the specific nature of the bioactive flavour compound that is being targeted (Ncube *et al*., 2008; Hughes (2002). However, it could be suggested that ethanol at volumes used in this work was more suitable for the extraction of flavour from *Aframomum danielli* spice especially for application in food; it is safer in food coupled high evaporation at low heat as observed during flavour concentration and low binding affinity to an extract so as to avoid the formation of new complex substances and preservative action (Hughes, 2002).

Table 4.7 present the list of common major flavour principles (MFPs) identified among the solvents used in flavour extraction. The MFPs in *Aframomum danielli* flavour extracts are eucalyptol which concentration extracted ranged from 20.53% in hexane flavour extract to 62.05% in methanol flavour extract. Alpha-terpineol was ranged from 4.23% in methanol flavour extract to 12.26% in 40% ethanol flavour extract. These two compounds were the only MFPs found in all the solvent extracts apart from water extract. Like other MFPs, the concentration of MFPs were significantly ($p<0.05$) varied among the flavour extract samples.

Other MFPs are n-hexadonic acid which valued between 0.84% in methanol flavour and 9.01% in aqueous 40% ethanol, but not found in hexane and water flavour extract samples. Also oleic acid, beta-pinene and diethyl phthalate were common in ethanol extracted flavour samples. Their concentration was ranged from 13.47% to 16.39%, 4.37% to 6.94% and 4.01% and 4.21% respectively. Ethyl iso-allocholate was common MFPs in absolute ethanol, aqueous 40% ethanol and methanol flavour extracts and its concentration was ranged between 1.65% in methanol and 4.03% in ethanol (absolute) flavour extract. The indication from these results suggested that polarity nature and molecular weight of the flavour chemical component matrices in spice coupled with their affinity with the solvent polarity could affect the extraction and percentage area concentration of MFPs from spices. Furthermore, it could be seen that eucalyptol has the highest concentration and referred to as most MFPs responsible for pungency of flavour in *Aframomum danielli* spice. The eucalyptol concentration in methanol extract (62.05%) was higher compared to that in ethanol flavour extract samples (53.77% and 50.70%). The findings of this study are in agreement with the report of Owokotomo *et al.,* (2014), Ajaiyeoba and Ekundayo, (1999) and Adegoke *et al.* (1998).

4.2.6 Evaluation of retention of core volatile flavour compounds in spice products samples

4.2.6.1 Major flavour principles and percentage retention of 'Piperine' in *Piper nigrum* spice products samples

The concentration of major flavour compounds in *Piper nigrum* products was presented in Table 4.8.1. Result showed that Cyclohexane-1-etheny-1-2,4Bis-1-methyl, Cis-beta.famesce, humulene, Beta-Bisabolene, n-hexadecanoic acid and piperine was found in the spice products at varied concentration and significant ($p<0.05$). Concentration of 4.8% of Cyclo-hexane-1-etheny-

1-2,4Bis-1-methyl was highest found on pulverized *Piper nigrum* sample, followed by 4.0% of Cyclo-hexane-1-etheny-1-2,4Bis-1-methyl on 40% extract of *Piper nigrum* sample. The concentration of Cyclo-hexane-1-etheny-1-2, 4Bis-1-methyl was reduced to 2.87% and 2.32% for rice grits coated of 40% extract of *Piper nigrum* samples at 1:2w/v and 1:3w/v respectively. Similarly the concentration of Cyclo-hexane-1-etheny-1-2,4Bis-1-methyl was reduced to 2.76% and 2.30% for potato grits coated of 40% extract of Piper nigrum samples at ratio 1:2w/v and 1:3w/v.

Concentration 6.88% of Cis-Beta.famesce found in pulverized *Piper nigrum* was higher than 6.55% on 40% extract of *Piper nigrum*. The concentration of Cis-Beta.famesce was further reduced to 4.69% and 3.83% on rice grits coated of 40% extract of *Piper nigrum* at ratio 1:2w/v and 1:3w/v samples. Also, on potato grits of 40% extract of *Piper nigrum* the concentration of Cis-Beta.famesce was reduced to 4.60% and 3.76% respectively. Similar pattern was found on the concentration of Humulene, Beta-Bisabolene, n-hexadecanoic acid and Piperine.

Humulene concentration of 3.88% and 2.65% were found on pulverized and 40% extract of *Piper nigrum* samples. Other coated samples had 1.74% and 1.64% on rice grits coated of 40% extract of *Piper nigrum* at ratio1:2w/v and 1:3w/v samples. And humulene concentration of 1.73% and 1.59% were found on potato grits coated *Piper nigrum* product samples. For Beta-Bisabolene compound, pulverized Piper nigrum product sample had 7.72% and 7.22% was found on 40% extract of *Piper nigrum* product sample. Coated 40% extract of *Piper nigrum* product samples have 4.33% and 3.98% for rice grits coated samples at ratio 1:2w/v and 1:3w/v. Also, 4.34% and 3.97% were found on potato grits coated of 40% extract of *Piper nigrum* product samples.

Pulverized and 40% extract of *Piper nigrum* product samples have 3.22% and 2.69% of n-hexadecanoic acid. These values were higher than 1.99% and 1.62% found on rice grits coated of 40% extract of *Piper nigrum* product samples and as well as 1.78% and 1.59% found on potato grits coated of 40% extract of *Piper nigrum* product samples. Piperine concentration also showed similar pattern where pulverized and 40% extract of *Piper nigrum* product samples have 17.53% and 15.42%. For coated 40% extract of *Piper nigrum* product samples the concentration reduced to 13.87% and 13.69% in rice grits coated at ration 1:2w/v and 1:3w/v samples. Also reduced to 13.11% and 12.11 % in potato grits coated at ratio 1:2w/v and 1:3w/v respectively.

The percentage retention of core volatile compound 'Piperine' in *Piper nigrum* spice flavour products is shown in Table 4.8.2. Volatilization or degradation processes can cause loss of biological activity of essential oils, labile flavour or bioactive ingredients in spices (Ayala-Zavala *et al.,* 2008). The encapsulation of essential oil and flavour ingredients is among the most important applications in the food industry to entrap sensitive ingredients, such as volatile and labile flavour, into solid carriers to increase their protection, reduce evaporation, promote easier handling, and control their release during storage and application (Baranauskieneù *et al*., 2006; Gouin, 2004; Bylaite *et al*., 2001; Kim and Morr, 1996). Choosing the best materials and encapsulation technique are important steps in food encapsulation (Hussein *et al.*, 2016).

In this research work 17.53% of 'Piperine' was the core material in 40% ethanol extract of *Piper nigrum* (Table 4.8) out of the sixty peaks eluted (Figure 4.2). Figure 4.13 showed the percentage retention (PR) in pulverized spice flavour powder, 40% ethanol extract and encapsulated core volatile flavour compound from 40% ethanol in rice and potato grits wall matrix respectively. There was variation on the percentage retention of core volatile 'piperine' in different food carriers studied. Concentration 15.42% of piperine was found in 40% ethanol extract of *Piper*

nigrum, after encapsulation process of this initial concentration, 89.95% retention of piperine was found in rice grits carrier at 1:2v/w ratio (PP-ERC$_1$), followed by 88.78% retention of piperine in rice grits carrier at 1:3v/w ratio (PP-ERC$_2$).

In potato grits carrier of the 40% ethanol extract of *Piper nigrum* spice products samples, the percentage retention of piperine was 85.02% at 1:2v/w ratio of encapsulation (PP-EPC$_1$) and 78.53% in 1:3v/w ratio (PP-EPC$_2$). These percentages were slightly lower compared to retention of piperine in rice grits at these same ratios after lyophilization and encapsulation processes. The variation on percentage retention of core volatile compound 'Piperine' in different carrier materials was in agreement with the findings of Hussein *et al.,* (2016) who reported that chemical composition and antioxidant activity of cumin and fennel essential oils in different wall materials as carriers were not the same. The changes in the chemical composition of volatile chemical compound(s) in flavour and aroma compounds of spices and other labile flavour compounds after encapsulation may be due to different chemical structures, i.e. the properties of wall materials carrier, the physicochemical properties of bioactive components and binding capacity of the carrier materials with aroma components (Hussein *et al.,* 2016).

Retention of volatile flavour compound(s) (encapsulation efficiency) and the release of these compound(s) depend on the nature and concentration of volatile compounds present in the food, as well as their availability for perception as a result of interactions between the major components and the aroma compounds in foods (Bakker *et al.,* 1996). Flavour-matrix interactions in food products have been widely investigated with respect to influences on flavour release and perception products which allow for the effective use of flavour materials (Schober and Peterson, 2004).

Food matrix components can bind, entrap or encapsulate volatile and nonvolatile flavour compounds if the "binding sites" of food components are still available. As a result, the interactions reduce the rate of flavour release and also affect the flavour intensity and quality of foods (Naknean and Meenune 2010). This influences the consumer overall acceptance. However four types of interactions that can exist between predominant volatile 'piperine' and other components in *Piper nigrum* liquid flavour extract and wall material of the carrier during encapsulation process include covalent bonding, hydrogen bonding, hydrophobic bond and physical binding (Naknean and Meenune 2010; McGorrin, 1996; Le Thanh *et al.,* 1992). Naknean and Meenune (2010) said that the type of chemical interactions depend on the physicochemical properties of flavour compounds, food components such as carbohydrate and its concentration in encapsulate. This statement could support why higher retention capacity in 1:2v/w ratio in rice and potato grits carrier samples (PP-ERC_1 and PP-EPC_1) than in 1:3v/w ratio of these same carrier or coating material used. There are three mechanisms of binding that can exist between flavour compounds and food matrices. These include binding, partitioning and distribution of flavour compounds between phase such as the oil, water and gas phases which affect release of flavour compounds from the bulk foods into the gas phase for sensory perception (McGorrin, (1996).

However, it could be suggested that rice grits carrier at 1:2 ratio v/w having 89.95% highest piperine retention capacity may be chosen for commercial production of novel spice products. This process prevents volatilization or oxidation during food production and storage, and at the same time it facilitates the production of foodstuffs with novel properties. Other advantages connected with the encapsulation of Piperine in *Piper nigrum* spice flavour product may include their easier introduction to solid products, controlled release of encapsulated substances,

improved taste, and extended shelf-life of the product (Adamiec and Kalemba 2006; Janiszewska and Witrowa-Rajchert 2006). Encapsulation of core volatile chemical compound(s) in *Piper nigrum* spice flavour product may provide an effective method to protect core flavour compound Piperine from degradation, oxidation and migration from food. Atmane *et al.*, (2006) commercially available food flavour in liquid forms are difficult to handle or incorporate into food systems.

4.2.6.2 Major flavour principles and percentage retention of core volatile 'Eucalyptol' in *Aframomum danielli* spice products samples

Table 4.9.1 showed the common major flavour principles 'MFPs' in *Aframomum danielli* products samples. In 40% ethanol extract of *Aframomum danielli* product sample major flavour principles include 50.70% eucalyptol, 4.79% alpha terpineol, 10.33% oleic acid, 5.53% n-hexadonic acid, 4.37% β-pinene, 2.65% ethy iso-allocholate and 4.01% diethyl-phthalate. These volatile compounds were found at varied concentration in wall materials (rice and potato grits) used for encapsulated *Aframomum danielli* spice products.

In rice grits carrier at 1:2v/w and 1:3v/w ratios the major flavour principles ranged from 20.34-28.76% eucalyptol, 2.82-3.39% alpha-terpineol, 2.06-4.11% oleic acid, 1.33-2.89% n-hexadonic acid, 2.06-2.66% β-pinene, 1.42-1.83% ethy iso-allochalate and 1.84-2.56% diethyl-phthalate were found. The concentration of these volatile compounds in wall material 'rice grits' were slightly varied when compared to 20-27.65% eucalyptol, 2.55-3.06% alpha-terpineol, 2.18-4.0% oleic acid, 1.18-2.63% n-hexadonic acid, 1.98-2.58% β-pinene, 1.40-2.01% ethy iso-allocholate and 2.01-2.62% diethyl-phthalate in potato grits carrier material at these same ratios. The variations in quantity of volatile compounds in encapsulated spice flavour products could be due

to the type and ratios (1:2ratio and 1:3 ratios v/w) of 40% ethanol extract of *Aframomum danielli* to carrier materials used, chemical interaction between flavour volatile compounds as well as effect of encapsulation processes.

Several physicochemical characteristics of the volatile compound could partly explain these differences such as molecular weight, chemical group and polarity (Naknean and Meenune 2010; Bhandari *et al.,* 2001). Generally, high molecular weight flavour compounds will retain in carbohydrate matrix more than low molecular flavour compound. Chain length of flavour compounds is another factor that affects retention and release of flavour compounds. Long chain length molecules will be retained more than short chain molecules (Bhandari *et al.,* 2001). Many studies have shown that polysaccharides influence the rate and intensity of flavour release in foods. The influence on retention and release of flavour compounds is related to types of carbohydrates and polysaccharides. Interaction between flavour compounds and polysaccharides play an important role in the flavour perception of food products and consequently their acceptability to consumers. Thus, a fundamental understanding of aroma-starch interactions is useful to improve food flavouring and to develop new carriers for flavour encapsulation (Bhandari *et al.,* 2001).

This result and observation found in this work was similar to the reports in literature (Hussein *et al.,* 2016; Prince *et al.,* 2014; Dhall, 2013; Rojas-Crau *et al.,* 2009) where difference in the encapsulation efficiency could be due to the physicochemical properties of essential oils or labile flavour components or bioactive compounds which are determined by its composition and how the size of the molecules fit into the wall of carrier materials at different blending ratios or proportion.

Table 4.9.2 present the percentage retention of core volatile material 'Eucalyptol' out of 9 peaks eluted in 40% ethanol extract of *Aframomum danielli* (Figure 4.8). However Figure 4.13 showed the percentage retention of core volatile compound 'eucalyptol' from 40% ethanol extract of *Aframomum danielli* in rice and potato grits wall carrier materials. Percentage retention of 56.73% eucalyptol in rice carrier at 1:2v/w was higher than 40.12% in 1:3 ratio v/w rice grits, 54.57% in potato 1:2ratio v/w and 39.74% in potato at 1:3 ratio v/w. Lower concentrations of core volatile compound(s) found in encapsulated *Aframomum danielli* flavour products samples could be attributed to interaction with the carbohydrate wall materials (rice and potato grits) used in encapsulation. This was in agreement with Naknean and Meenune (2010) who stated that carbohydrates change the volatility of flavour compounds relative to water, but the effect depends on the interaction between the particular flavour and carbohydrate molecules (Naknean and Meenune, 2010). Complex carbohydrates offer many more possibilities for chemical interaction than the simple sugar due to the diversity of functional groups available. In model systems, polysaccharides generally induce a reduction in aroma release caused by an increase in viscosity and/or by molecular interactions with flavour compounds. For polysaccharides especially, starch is the most commonly matrix used to entrap flavour due to its structure (Goubet *et al.,* 1998).

Rice and potato grits used in this work as wall carrier materials for encapsulation were classified as A-type (cereal crop) and B-type (tuber crop) respectively based on diffraction pattern of starch in the system (Jouquand *et al.* 2006). Therefore, A- type starch can bind water or ligand less than B-type starch. The types of carbohydrate materials used in this work was in line with Arvisenet *et al.*, (2002) who studied retention of flavour compounds in different starches including corn starch, potato starch, waxy corn starch, amylase rich corn starch and cross-linked corn starch.

4.2.7 Retention rate of core flavour compounds in *Piper nigrum* and *Aframomum danielli* spice products samples at 6months storage

4.2.7.1 Piperine in *Piper nigrum* flavour products samples at 6months storage

The profile of piperine and other peaks (appendix III, Figure 1-9) in pulverized spice flavour, 40% ethanol extract and encapsulated *Piper nigrum* flavour products (Table 4.10) was varied during 6months storage. Significant difference ($p<0.05$) in piperine content of the *Piper nigrum* spice flavour products was noticed in the samples during six months storage under ambient condition ($30\pm3^{o}C$). In pulverized spice flavour of *Piper nigrum* sample in bottle (PP-PB) had 17.53% piperine at zero hour of storage and this value (17.53%) declined gradually without any significant difference ($p>0.05$) within 4weeks of storage to 17.50%. Significant change ($p<0.05$) of piperine content began from 16.58% at 4^{th} month of storage till 15.72% piperine found at 6^{th} month of pulverized *Piper nigrum* storage.

In 40% ethanol extract of *Piper nigrum* sample in bottle piperine (PP-EB) piperine value of 15.42% was found at zero hour, this value reduced slightly without any significant difference to 15.31% at 4^{th} week of storage. Then significant reduction began from 15.18% piperine at 6^{th} week to 14.08% piperine at 6^{th} month under ambient condition ($30\pm3^{o}C$). There was no significant difference ($p>0.05$) observed on the piperine content 13.87% to 13.79% of encapsulated rice grits *Piper nigrum* flavour extract sample (at 1:2 ratio v/w coating) sample within first 4weeks of storage. Piperine content changed significantly ($p<0.05$) from 12.69% at 2months to 10.96% at 6^{th} month of storage. In encapsulated flavour in rice grits wall carrier material sample PP-ERC_2 (at 1:3 ratio v/w coating) shows changes in piperine form initial value of 13.69% to 13.28% at 2^{nd} week of storage. These changes continued significantly till 9.16%

piperine was found at 6th month of storage. The observation in piperine content in sample PP-ERC_1 was not the same compared with stability and changes in piperine content of sample PP-ERC_2. This may be attributed to ratios (v/w) of 40% ethanol extract of *Piper nigrum* and wall material of carrier.

The stability of Piperine in potato grits flavour coating showed gradual reduction during storage for 6months. Piperine content 13.11% in potato grit flavour (1:2ratio v/w) PP-EPC_1 sample at zero hour reduced to 12.56% at 4nd week of storage without any significant difference ($p>0.05$). But in 1:3ratio v/w PP-EPC_2 piperine reduced significantly from 12.11% at zero hour to 11.66% at 2nd week of storage. Piperine 7.58% and 7.06% were found in samples PP-EPC_1 and PP-EPC_2 respectively at 6th month of storage. The retention of piperine content rate varied during storage in spice products. Liquid *Piper nigrum* flavour (PP-EB) had 91.31% highest retention of Piperine compared to 89.67% in pulverized spice flavour (PP-PB), 79.02% in rice grits encapsulated flavour at 1:2ratio v/w (PP-ERC_1), 66.91% in sample PP-ERC_2 (rice grits encapsulated flavour at 1:3ratio v/w), 57.82% in sample PP-EPC_1 and 56.65% in sample PP-EPC_2.

The variation observed major volatile 'Piperine in this was in line with Fadel *et al.*, (2014) who discovered reduction of volatile compounds identified in headspace of the encapsulated beef-like process flavouring during storage for 6 months. The variations in behavior between the chemical classes of the volatiles in the encapsulated *Piper nigrum* flavour compounds during 6months storage may be correlated to the fact that; release of the volatiles from encapsulated samples is affected by independent variables. Several mechanisms can take place such as component/component interaction and component/capsule wall material interaction. Furthermore molecular weight and volatility of the compounds can affect their migration from the wall carrier

material of the capsule (Hussein *et al.,* 2016; Fadel *et al.,* 2014; Prince *et al.,* 2014, Dhall, 2013, Rojas-Crau *et al.,* 2009).

4.2.7.2 Core volatile 'Eucalyptol' compound in *Aframomum danielli* products samples at 6months storage

Table 4.11 presents the percentage retention of peaks of core volatile 'eucalyptol' and appendix IV Figure 10-18 showed the peaks of chemical compounds in spice flavour products samples at 6months storage. Percentage retention of eucalyptol in *Aframomum danielli* spice products was presented in Figure 4.13. The eucalyptol content in pulverized powder of *Aframomum danielli* sample 'AFD-PB' changed value from 65.28% at zero hour (control) to 65.20% 2^{nd} week of storage without any significant difference ($p>0.05$). Also in 40% ethanol extract of *Aframomum danielli* product 'AFD-EB' reduced from 50.70% at zero hour to 50.0% at 2^{nd} week.

Significant ($p<0.05$) reduction of eucalyptol began from 63.44% and 48.06% in AFD-PB and AFD-EB samples respectively at 4^{th} week to 54.48% and 39.19% at 6^{th} month of storage. Similar change in eucalyptol content in rice grits encapsulated spice product samples was found. Samples made from 40% ethanol extract of *Aframomum danielli* in rice grits carrier at ratio 1:2v/w (AFD-ERC_1) and in 1:3v/w (AFD-ERC_2) had 28.76% and 20.34% respectively at zero hour of storage. These values changed slightly to 28.06% and 20.08% at 2^{nd} week of storage respectively without any significant difference ($p>0.05$). At 4^{th} week the reduction in eucalyptol became obvious and was significant. In sample AFD-ERC_1 eucalyptol reduced from 27.83% at 4^{th} week to 22.06% at 6^{th} months, also, in sample AFD-ERC_2 20.0% eucalyptol at 4^{th} week changed to 12.06% at sixth month of storage. But in potato grits encapsulated samples at 1:2v/w

(AFD-EPC_1) and 1:3v/w (AFD-EPC_2) significant reduction in eucalyptol was not found until sixth week of storage. At zero hour 27.65% retention in 1:2v/w ratio of 40% ethanol extract of spice to potato grits carrier encapsulated sample 'AFD-EPC_1' and 20.15% in 1:3v/w ratio of 40% ethanol extract of spice to potato grits carrier encapsulated sample 'AFD-EPC_2' were recorded. Noticeable changes started from 23.62% and 18.28% eucalyptol in both samples from fourth week to 19.33% in sample AFD-EPC_1 and 11.44% in sample AFD-EPC_2 at sixth month of storage.

The indication from this result suggested that the interaction between component/component (volatile compound and wall material) may be responsible for this observation (Fadel *et al.*, 2014). Figure 4.41 showed that pulverized *Aframomum danielli* product AFD-PB had 83.46% retention rate/capacity, followed by 77.30% in sample AFD-EB, 76.70% in sample AFD-$ERC_{1,}$ 65.39% in sample AFD-$EPC_{1,}$ 59.29% in sample AFD-$ERC_{2,}$ and 56.77% in sample AFD-$EPC_{2.}$

4.2.8 Sensory evaluation of spice flavour products samples

4.2.8.1 Sensory attributes of *Piper nigrum* flavour product samples

Sensory attributes of *Piper nigrum* spice products were presented in Table 4.12.1 and Figure 4.14. The sensory notes of *Piper nigrum* products shows that appearance, pungency, bitter-taste, hot-in-taste and overall acceptability were sensory attributes identified. These sensory attributes were rated above 4.0 (neither like nor dis-like) in a 7-point hedonic scale. However, there was significant difference ($p<0.05$) found on the mean scores of the sensory attributes of the spice flavour products evaluated.

For colour attribute of pulverized powder of *Piper nigrum* products sample (PP-PB) and 40% ethanol extract of *Piper nigrum* sample (PP-EB) had 6.29 mean score each. These mean score (6.29) was higher than 4.57 and 4.33 mean scores of 40% ethanol extract of *Piper nigrum* encapsulated in rice grits carrier at 1:2v/w ratio (PP-ERC_1) and 1:3v/w ratio (PP-ERC_2). Also higher than 4.0 and 3.75 mean scores of 40% ethanol extract of *Piper nigrum* encapsulated in potato grits carrier at 1:2v/w ratio samples PP-EPC_1 and 1:3v/w ratio sample PP-EPC_2.

The mean score of 6.71 in pulverized of *Piper nigrum* sample PP-PB and 5.71 of 40% ethanol extract of *Piper nigrum* sample PP-EB are higher in pungency than 5.50 in 40% ethanol extract coated in rice grits at ratio 1:2v/w sample PP-ERC_1, 3.86 in 40% ethanol extract coated in rice grits at ratio 1:3v/w sample PP-ERC_2 and 4.86 and 4.63 mean scores in 40% ethanol extract coated in potato grits at ratio 1:2v/w sample PP-EPC_1 and 40% ethanol extract coated in potato grits at ratio 1:3v/w sample PP-$EPC_{2.}$

The indication from this result suggested that the major volatile compound(s) responsible for the flavour perception is higher in pulverized and 40% ethanol extract of *Piper nigrum* flavour products from *Piper nigrum*. This further supported result piperine being core volatile flavour compound in 98% and 40% ethanol extracts samples of *Piper nigrum* products samples (Table 4.3.1 and Table 4.3.2). Furthermore, the perception of the pungency could be attributed to the sum of the identified major attributes of the flavour products taken in the mouth, perceived principally by the senses of taste and smell, and also interpreted by brain (International Organization of the Flavour Industry (2012). However, lower mean scores of the pungency in 40% ethanol extract of *Piper nigrum* encapsulated in rice and potato grits carrier products samples (PP-ERC_1, PP-$ERC_{2,}$ PP-EPC_1, and PP-EPC_2) could be due to the presence of non-

flavouring food ingredients (carrier materials) used for the production (International Organization of the Flavour Industry, 2012).

Bitter taste sensory attribute with mean scores of 4.29 and 5.0 in pulverized spice flavour sample PP-PB and 40% ethanol extract of *Piper nigrum* sample PP-EB respectively were discovered compared to other encapsulated *Piper nigrum* flavour product samples that their mean scores were below 4.0 of 7-point hedonic scale. This effect could be due to component/component interactions of the wall material of non-flavouring material (grits from rice and potato) (Jouquand *et al.* 2006).

Hot-in-taste sensory attribute was found in the pulverized spice flavour and 40% ethanol extract spice flavour extract samples. Mean score of 5.43 for pulverized of *Piper nigrum* spice product sample and 4.71 for 40% ethanol extract *Piper nigrum* product sample were found among the *Piper nigrum* spice products samples. Generally, the mean scores 5.17 and 5.57 for 40% ethanol extract of *Piper nigrum* coated in rice grits at 1:2v/w ratio and potato grits at 1:2v/w ratio samples respectively were higher than 4.57 for 40% ethanol extract of *Piper nigrum* product sample and 4.29 for pulverized *Piper nigrum* spice product sample. The method used in this work was in agreement with Chambers and Kadri (2013) who stated that descriptive sensory tests are highly reliable and consistent and obviously identify the sensory attributes of human perception of spice flavour products.

Table 4.12.2 presents the major sensory attributes of *Piper nigrum* spice products samples. There were significant differences ($p<0.05$) on sensory attributes of product samples from *Piper nigrum*. The mean scores of 6.20 colour, 6.80 pungency, 6.75 aroma and 6.70 overall acceptability was found in pulverized of *Piper nigrum* product sample. The mean scores found in

pulverized spice flavour sample were higher than mean scores obtained for 40% ethanol extract of *Piper nigrum* sample and encapsulated spice product samples. Table 4.12.3 shows the non-parametric correlations of sensory attributes of *Piper nigrum* products samples. Pungency of the products showed highest 0.927 significant correlations ($p<0.01$) to overall acceptability, followed by 0.895 correlations ($p<0.05$) aroma and the least 0.850 correlations was found on colour attribute of the spice products. This result suggests that the overall judgment or perception of the flavour products may depend more on the pungency than aroma and color attributes.

4.2.8.2 Sensory attributes of *Aframomum danielli* products samples

The mean scores of sensory attributes of *Aframomum danielli* was presented in Table 4.13.1. Eight sensory attributes of the spice products identified are colour, pungency, sweet-flavour, minty flavour, sweet taste, hot-in-taste, after-taste and overall acceptability (Figure 4.17). Mean scores of these identified attributes were significantly different ($p<0.05$) among the *Aframomum danielli* products samples. Mean score 6.29 for colour found on 40% ethanol extract of *Aframomum danielli* product sample (AFD-EB) was higher than 5.57 for the pulverized of *Aframomum danielli* sample (AFD-PB), also higher than 4.14 to 4.86 mean scores found in 40% ethanol extract of *Aframomum danielli* encapsulated in rice and potato grits at 1:2v/w and 1:3v/w ratios (AFD-ERC_{1-2} and AFD-EPC_{1-2}). The variation observed on colour of *Aframomum danielli* products could be due to effect of product formulation.

The degree of perception or judgment of other identified descriptive sensory attributes (such as pungency, sweetness, minty flavour, taste, hot-in-taste, after-taste and overall acceptability) in pulverized of *Aframomum danielli* spice product sample (AFD-PB) and 40% ethanol extract of *Aframomum danielli* (AFD-EB) samples were higher than the mean scores in encapsulated

flavour product samples. Among the encapsulated flavour samples the mean scores for pungency ranged from 4.14 for 40% ethanol extract of *Aframomum danielli* coated in potato grits at 1:3v/w (AFD-EPC_2) to 4.71 for 40% ethanol of *Aframomum danielli* coated in rice grits at 1:2v/w or 1:3v/w ratios (AFD-ERC_2), 4.71 to 5.57 sweetness, 4.71 to 5.14 minty-flavour, 4.0 to 5.86 taste, 3.57 to 4.43 hot-in-taste, 3.57 to 5.14 after-taste and 4.57 to 5.14 overall acceptability.

Furthermore, mean scores of sensory attributes found in 1:2ratio of 40% ethanol extract of *Aframomum danielli* coated rice grits or potato grits encapsulated product samples (AFD-ERC_1 and AFD-EPC_1) were higher than mean scores found in encapsulated spice product in the same carrier materials at 1:3 ratio samples (AFD-ERC_2 and AFD-EPC_2). This result could be attributed to component/component interaction at varied blending proportion (Bakker et al., 1996). The indication thereby suggested that the degree of perception and judgment could be affected by production process. Generally pulverized and 40% ethanol extract of *Aframomum danielli* products samples with mean scores of 6.86 and 6.57 were shown to have higher mean scores of perception of descriptive sensory attributes. The concentration of major flavour principle(s) such as eucalyptol, alpha-terpineol, oleic acid, β-pinene, ethyl iso-allocholate and diethyl-phthalate (Table 4.9.1) could responsible for this observation.

Consumers' preference result on identified sensory attributes (Table 4.13.2) showed that there was significant difference ($p<0.05$) on mean scores of sensory attributes of *Aframomum danielli* spice products samples. The 40% ethanol extract and pulverized *Aframomum danielli* products samples had 6.30 and 5.45 mean scores for colour respectively which were higher compared to 4.30 to 5.30 mean scores of encapsulated *Aframomum danielli* products samples. Also mean scores 6.35 and 5.40 for pungency for the pulverized and 40% ethanol extract *Aframomum danielli* product samples were higher than 4.40 to 5.40 mean scores for encapsulated

Aframomum danielli samples. The mean scores 5.55 and 5.05 preference for aroma and overall acceptability of pulverized and 40% ethanol extract *Aframomum danielli* samples were higher than 4.75 to 5.0 and 4.45 to 6.10 in encapsulated *Aframomum danielli* product samples. Non-parametric correlations test on sensory attributes (Table 4.13.3) indicated that pungency had correlation of 0.671 followed by 0.563 of aroma to 1.0 of overall acceptability. Colour had the least 0.456 correlations to 1.0 overall acceptability. Therefore, the result suggested that pungency had highest correlations to overall acceptance of the flavour products than aroma and color.

4.2.8.3 List of recommended foods and sensory attributes of pepper-soup seasoned with *Piper nigrum* and *Aframomum danielli* flavour products

The list of recommended foods for the spice product samples were presented in Table 4.14.1. In *Piper nigrum* products samples ten (10) food were recommended for the product samples. These foods include seasoned boiled meat (4.5%), porridge (8.7%), Banga soup (6.3%), sauce (2.8%), Ofe-olubu (3%), tea (4%), fruit juice (3%), alcoholic drink (20.5%) and pepper-soup had 68.7% highest recommendation. Fourteen foods were recommended for *Aframomum danielli* spice product samples. These lists includes seasoned boiled meat (5.7%), ewedu draw soup (7%), okro soup (4%), Banga soup (15%), edikaikan soup (12.3%), sauce (7%), ofe-owerri (5%), pepper-soup (32%), spiced bread (5%), biscuit (7%), ice cream (6.2%), yoghurt (5%), tea (6%), fruit juice (3%) and alcoholic drink (7.2%)

Table 4.14.2 presents the sensory scores for pepper-soup seasoned with spice products samples from *Piper nigrum* and *Aframomum danielli* spices. There was no significant difference ($p>0.05$) found on pepper-soup samples seasoned with spice products from *Piper nigrum* and *Aframomum danielli* respectively. In *Piper nigrum* products seasoned pepper-soup, pulverized *Piper nigrum*

spice product (sample-C) showed highest 8.2 colour, 7.9 aroma, 7.3 taste and 7.6 mouth-feel compared to 7.3-7.6 color, 6.6-7.1 aroma, 6.7-7.0 taste and 6.7-7.2 mouth-feel of pepper-soup seasoned with encapsulated *Piper nigrum* product sample (sample A) and pepper-soup seasoned with 40% ethanol extract of *Piper nigrum* product sample (sample B).

Similar result was obtained in pepper-soup seasoned with *Aframomum danielli* spice product samples. Pulverized *Aframomum danielli* seasoned pepper soup (sample-F) had 8.5 colour, 7.8 aroma, 7.7 taste and 7.8 mouth-feel. These mean scores were higher compared to 6.8-7.4 colour, 6.6-7.8 of aroma, 7.1-7.2 taste, and 6.7-6.9 mouth-feel in pepper-soup samples seasoned with 40% ethanol extract of *Aframomum danielli* coated in potato grits at 1:2v/w ratio (samples D) and 40% ethanol extract of *Aframomum danielli* spice product (samples E).

The result shows that the forms (pulverized, 40% ethanol extract spice flavour or encapsulated spice flavour products samples) at which the spice products exist or produced in this work may not necessarily cause any significant difference ($p>0.05$) in consumers' preference of seasoned foods recommended. Adedeji and Ade-Omowaye (2013) observed significant difference ($p<0.05$) in fried bean cake samples seasoned with varied concentration of spice extract from *Aframomum danielli* and *Zingiber officinale*. The use of spice flavour in food formulation and production has become a common trend in food industry. Therefore the result obtained in this work shows the potential of spice products samples developed from *Piper nigrum* and *Aframomum danielli*.

4.2.9.1 Total count and characteristics of fungi isolate

Total fungi count in raw and pulverized *Piper nigrum* and *Aframomum danielli* spices product samples was presented in Table 4.15.1. Initial total fungi count load of $1.61x10^9$cfu/g in raw *Piper nigrum* and $9.20x10^8$cfu/g in raw *Aframomum danielli* was found. For the pulverized spice samples $0.45x10^2$cfu/g and $1.12x10^3$cfu/g was found in *Piper nigrum* and *Aframomum danielli* respectively. The result found in this work was similar to earlier research works reported in literature. Salari *et al.,* (2013) reported $1.23x10^5$ to $9.35x10^3$cfu/g of moulds and yeast in Iranian red pepper samples, $5.56x10^3$cfu/g in un-parked red pepper in Bangladeshi (Parveen *et al.*, 2013), 1.2×10^5 cfu/g of yeast and mould count in powdered red pepper (Elmali *et al.* 2005) and $5x10^2$cfu/g (Filiz, 2001). The International Microbiological Standard recommended limits 10^1 to 10^3 cfu/g for yeast and mould contaminants in spices (Awe *et al.*, 2009).

Total fungi count (cfu/g) discovered on raw spices could be attributed to indigenous micro flora of plants, and gross contamination from air, dust, using contaminated water and animal/human excreta, pre- and post-harvest procedure including processing, storage and distribution may be the sources of microbial contamination of spices. Therefore, spices in raw form when added to foods without further processing or eaten raw may pose health problems and portrait health risk (Colak *et al*., 2006). Progressive reduction of fungi load in pulverized spice samples could be due to post- harvest phyto-sanitary handling treatment and hygienic procedures (Nair, 2004) employed. Colak *et al*., (2006) stated that in cleaning and processing procedure of spices, there is progressive reduction in the number and types of microorganisms; those remaining are usually aerobic spore-forming bacteria and common moulds.

In raw spices used in this work suspected fungi through colonial and microscopic characteristics include *Saccharomyces cerevisiae, Saccharomyces ellipsoideus, Pencillium notatum, Aspergillus*

spp, and *Rhizopus spp.* In spices and spice products production moulds are used as Process Control Limit (PCL) as well as specification in spice trade. This is because moulds reduce the quality of food, and creates a potential risk for human health with the production of toxic metabolites known as mycotoxins (such as Aflatoxins and ochratoxin) which are resistance to heat treatment (Galvano *et al*., 2005; Jay *et al*., 2005; Martins *et al*., 2001). Control of microbial contamination relies on the application of good hygiene practice in production and harvesting and post-harvest processing including storage (International Commission on Microbiological Specifications for Food (ICMSF), 2005).

4.2.9.2 Total count (TC) and characteristics of bacterial isolate in spices

Table 4.15.2, Table 4.15.3 and Table 4.15.4 present the total aerobic bacterial plate count on nutrient agar (NA), MacConkey agar (MA) and brain heart infusion (BHIA) respectively. Whole spice materials of *Piper nigrum* and *Aframomum danielli* shows $1.21x10^{9}$cfu/g and $7.8x10^{8}$cfu/g total plate count on nutrient agar. These values were reduced to $0.9x10^{3}$cfu/g in pulverized *Piper nigrum* and $1.8x10^{3}$cfu/g in pulverized *Aframomum danielli* flavour spice. Total plate counts on MacConkey agar differed slightly when compared with colony forming units (cfu) per gram in nutrient agar and brain heart infusion agar. Whole *Aframomum danielli* seeds had $7.0x10^{8}$cfu/g higher load compared to $1.22x10^{9}$cfu/g in fresh *Piper nigrum* berries. There was no growth of bacterial isolates from pulverized spice samples. Suspected bacterial through colonial and microscopic characteristics include *Bacillus spp, Staphylococcus,* and *Escherichia coli* on harvested whole spices.

Total plate counts of spore-former isolates on brain heart infusion agar (BHIA) showed $2.1x10^{6}$cfu/g and $1.0x10^{5}$cfu/g were recorded from fresh whole *Piper nigrum* and *Aframomum danielli* respectively. Pulverized spice samples did not show any colonial growth. Among the

organisms suspected in BHIA medium through colonial and microscopic characteristics are *Streptococcus spp, Lactobacillus spp,* and *Bacillus spp.* The level of total plate counts and suspected bacterial isolates contaminants especially on raw spice materials in this work was similar to results of earlier reports in literature (Hassan and Altalhi, 2013; Al-Juraifani, 2011; Hashem and Alamri, 2010; Casano *et al*., 2009; Cho *et al*., 2008; Bokhari, 2007; Galvano *et al*., 2005; Jay *et al*., 2005; Martins *et al*., 2001).

Furthermore total aerobic plate counts of bacterial isolates found in different media in this work was in agreement with Awe *et al.,* (2013) who stated that microbiological standard recommended limits for bacteria contaminants in spices are in the range of 10^1 to 10^3 cfu/g for coliform, 10^1 to 10^5 cfu/g total microbial plate count, 0/20 g for *Staphylococcus aureus* and 0/20 g for *Escherichia coli* (Awe *et al.,* 2009). However, the indigenous micro flora of plants and gross contamination from air, dust, using contaminated water and animal/human excreta, pre- and post-harvest procedure may be the sources of microbial contamination of spices (Colak *et al*., 2006). The indication from these results suggests that microorganisms detected in raw spices have the potential to cause health problems when added to food (Casano *et al*., 2009; Cho *et al*., 2008).

4.2.9.3 Biochemical characteristics and carbohydrate fermentation of bacterial isolates

The result of biochemical characteristics of identified bacterial isolates was presented in Table 4.15.5. Bacteria identified include *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Bacillus cereus, Bacillus subtilis, Lactobacillus spp, Streptococcus spp, Micrococcus luteus* and *Pseodomonas aeruginosa.* The bacteria identified on spice materials in this work was similar to those reported in earlier works on spices and herbs in literature (Vitullo *et al*., 2011; Abou Donia, 2008; Garcia *et al*., 2001).

ICMSF (2005) and Banerjee and Sarkar, (2003) in separate reports stated that spices and herbs are not major contributors to food-borne disease they occasionally contain pathogenic microorganisms which may pose a risk to public health, especially when added to meals without further treatments. Of particular significance are *Salmonella* spp., *E. coli*, *S. aureus*, *L. monocytogenes*, *B. cereus* and *Cl. perfringens*. The results of this study indicated that no sample was found to be contaminated by *Salmonella* spp., and *L. monocytogenes* (ICMSF, 2005; Banerjee and Sarkar, 2003).

However, low levels of contamination by *S. aureus*, *Enterococcus faecalis* and *E. coli* may occur as shown in the result of total plate counts of bacterial isolates (Table 4.15.2, Table 4.15.3 and Table 4.15.4). These organisms found may be attributed to the natural occurrence in the soil environment of plants and fecal contamination of the spices during pre and post-harvest of the spices. This finding further supported results of other published studies in which these food-borne pathogens were uncommon in spices and herbs (Vitullo *et al*., 2011; Abou Donia, 2008; Garcia *et al*., 2001) or could be at low prevalence (Sospedra *et al*., 2010; Hampikyan *et al*., 2009; Sago *et al*., 2009; ICMSF, 2005; Little *et al*., 2003). Escherichia coli found in the samples may indicate faecal contamination by humans, livestock and wildlife or the poor hygiene of the workers during pre-harvesting (Elviss *et al*. 2009; Leifert *et al*. 2008). While Phytosanitary procedure used during post-harvest treatment of the spices could be responsible for eradication of these organisms in pulverized spice samples.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The results obtained in this research work revealed the potential of under-utilized spices *Piper nigrum* and *Aframomum danielli* in production of spice products samples. The overall evaluation of this concludes that there are number of aroma active flavour compounds in spices studied. Major flavour principles in solvents extracts of *Piper nigrum* indentified include piperine, cyclohexane-1-etheny-1-methyl, cis-beta-famescene, humulene, beta-bisabolene, and n-hexadecanoic acid. Chemical compounds identified as major flavour principles in solvents extract of *Aframomum danielli* samples were eucalyptol, alpha-terpineol, n-hexandonic, oleic, beta-pinene,ethyl iso-allocholate and diethyl phthalate.

On the basis of the percentage area concentration of chemical components in solvent extracts some were identified as major flavour principles (MFPs) or core volatile flavour compound were responsible for the flavour pungency in *Piper nigrum* and *Aframomum danielli*. Piperine was core volatile flavour compound in *Piper nigrum* spice products samples and eucalyptol as core volatile flavour compound in *Aframomum danielli* spice products samples. The core flavour compound in both spice products samples were found insoluble in water but readily soluble in most other polar solvents and non-polar solvent such as ethanol, methanol, acetone and n-hexane for commercial solvent extraction processing. However ethanol was most suitable and safe solvent for extraction of spice flavour compounds due to its less toxic and food compatible characteristics.

Piperine and eucalyptol retention was higher in pulverized of *Piper nigrum* and *Aframomum danielli* product samples than in 40% ethanol extract of the spice product samples and encapsulated flavour product in both spices. It was also observed that increased ratio of wall material of coating agent rice grits or potato grits reduced the percentage retention of core volatile compound in encapsulated spice product samples. Furthermore, retention capacity of core flavour principles (MPFPs) during storage varies in spice products samples. Highest percentage retention of core flavour principles after six month storage was found in pulverized spice product samples of *Piper nigrum* and *Aframomum danielli* product samples.

Five sensory attributes such as color, pungency, bitter taste, hot-in-taste and overall acceptability were characterized in *Piper nigrum* products samples while eight sensory attributes which include color, pungency, sweet-flavour, minty-flavour, sweet-taste, hotness-in-taste, after-taste and overall acceptability in *Aframomum danielli* products samples. However, pungency sensory attribute was identified as important sensory attribute of the spice products samples and have the highest Pearson correlation to overall acceptability. This suggests that indigenous spices have place in industrial utilization for food spice flavour production that can compete favorably in use with foreign spice flavour products.

5.2 RECOMMENDATIONS

Based on the results, observation and conclusion, the following recommendation are suggested:

- Retention capacity or encapsulation efficiency of other coating or carrier materials such as protein and syrup from starch component of other plant material should be examined.
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- There is need to examine the potentials of spice active flavour compounds such as piperine, humulene, bet-bisabolene and cis-beta-famescene on *Piper nigrum* and eucalyptol, alpa-terpineol, beta-pinene, ethyl iso-allocholate and diethyl-phthalate for antioxidants and anti-microbial in food preservation, pharmaceuticals and medicinal roles.
- Potential of natural food flavour from the indigenous spices is high, agricultural policy should be geared toward increased indigenous production of spices that will meet industrial need for food flavour production.
- There is need to examine the effect of different packaging material on the retention capacity of major flavour principles of *Piper nigrum* and *Aframomum danielli* spice products. Investigation on the effect of storage temperatures on shelf-stability of most important quality parameters such as core and major flavour principles is also required.

5.3 CONTRIBUTIONS TO KNOWLEDGE

This study has introduced encapsulation as a processing method on local spices and has revealed that rice grits was the best carrier material for flavour retention.

For the first time, Humulene, Beta-pinene, Beta-bisabolene, Beta-famescene and Copaene compounds were identified in *Piper nigrum* L. and Eucalyptol, diethylphthalate, Beta-pinene, Ethyl iso-allocholate and Alpha-Terpineol were also identified for the first time in *Aframomum danielli*.

Findings in this work revealed for the first time that the total number of chromatogram spectrum (GC-MS) of flavour compounds eluted and as well as concentration in different solvents used were not the same both in *Piper nigrum* L. and Aframomum danielli. In *Piper nigrum* L. 56 GC-

MS compounds spectrum, 59 GC-MS compounds spectrum, 42 GC-MS compounds spectrum, 78 GC-MS compounds spectrum, 86 GC-MS compounds spectrum and 5 GC-MS compounds spectrum were identified in 98% ethanol, 40% ethanol, methanol, acetone, n-hexane and water solvent spice extracts respectively. However, in *Aframomum danielli* solvent extracts, 10 GC-MS compounds spectrum, 9 GC-MS compounds spectrum, 22 GC-MS compounds spectrum, 28 GC-MS compounds spectrum, 84 GC-MS compounds spectrum were identified in % ethanol, 40% ethanol, methanol, acetone, n-hexane and water solvent spice extracts but none was eluted in water extract.

Indigenous spices made in different product samples such as pulverized powder, aqueous solvent extract and encapsulated spice product samples which create value additional to varieties compared to whole or grounded spice form used traditionally in soup preparation.

This study also established on the two indigenous spices studied that percentage retention (PR) of core volatile flavour principles in extract coated in suitable carriers in both spices reduced when the ratios of rice and potato grits increased. Percentage retention of 89.67%, 91.31% and 79.02% for core aromatic flavour compound 'Piperine' in *Piper nigrum* flavour samples and 83.46%, 77.30%, 76.70% for 'eucalyptol' in *Aframomum danielli* spice flavour samples were found for pulverized powder, 40% ethanol extracts of spice and encapsulated or coated 40% ethanol extracts at 6months storage.

Sensory evaluation identified 5 and 8 sensory attributes of *Piper nigrum* and *Aframomum danielli* spice flavour product samples respectively. This study showed that flavour pungency among other sensory attributes identified was most specific attribute contributed to consumers preference for overall acceptability of spice product samples in Piper nigrum and as well as in *Aframomum danielli*.

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APPENDICES

APPENDIX I
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-flavour aroma						
Taste						
Bitterness						
Harse-taste						
Hot-in-taste						
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 indigenous soup ☐

Peppe-soup ☐ Ugba ☐ Stew ☐ Igbo indigenous soup ☐

SAUCE ☐ Tea ☐ Akamu/pap ☐ Hausa indigenous soup ☐

Baked products ☐ Ice cream ☐ Yoghurt drink ☐ Non-alcoholic beverage ☐

Alcoholic beverage ☐

Comment: ……

APPNDIX II

PHYTO-CHEMICAL CONTENT AND PROFILE OF MAJOR FLAVOUR COMPOUNDS OF *Piper nigrum* AND *Aframomum danielli* SAMPLES

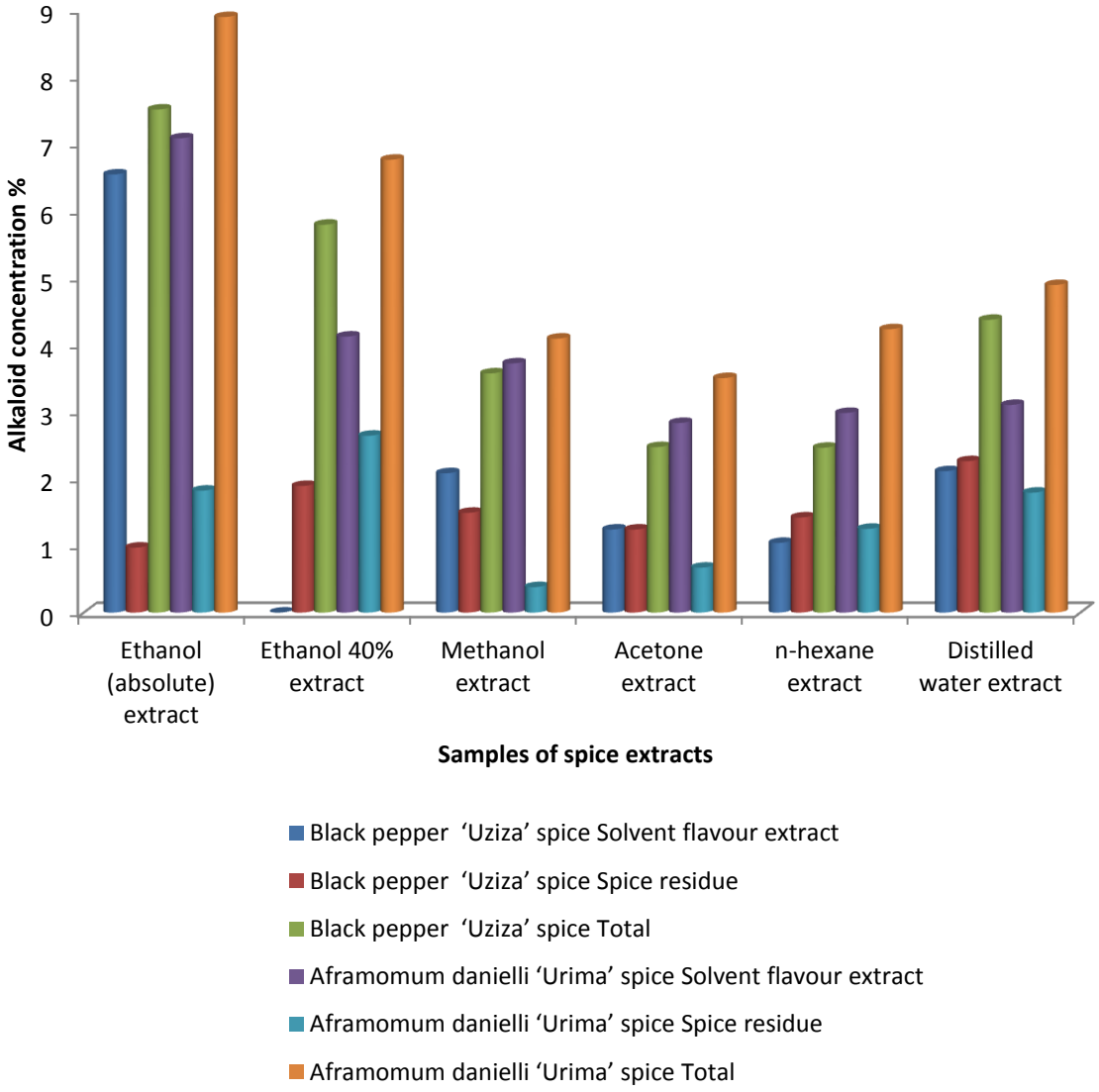

Figure 1: Alkaloid content of solvents spice extracts

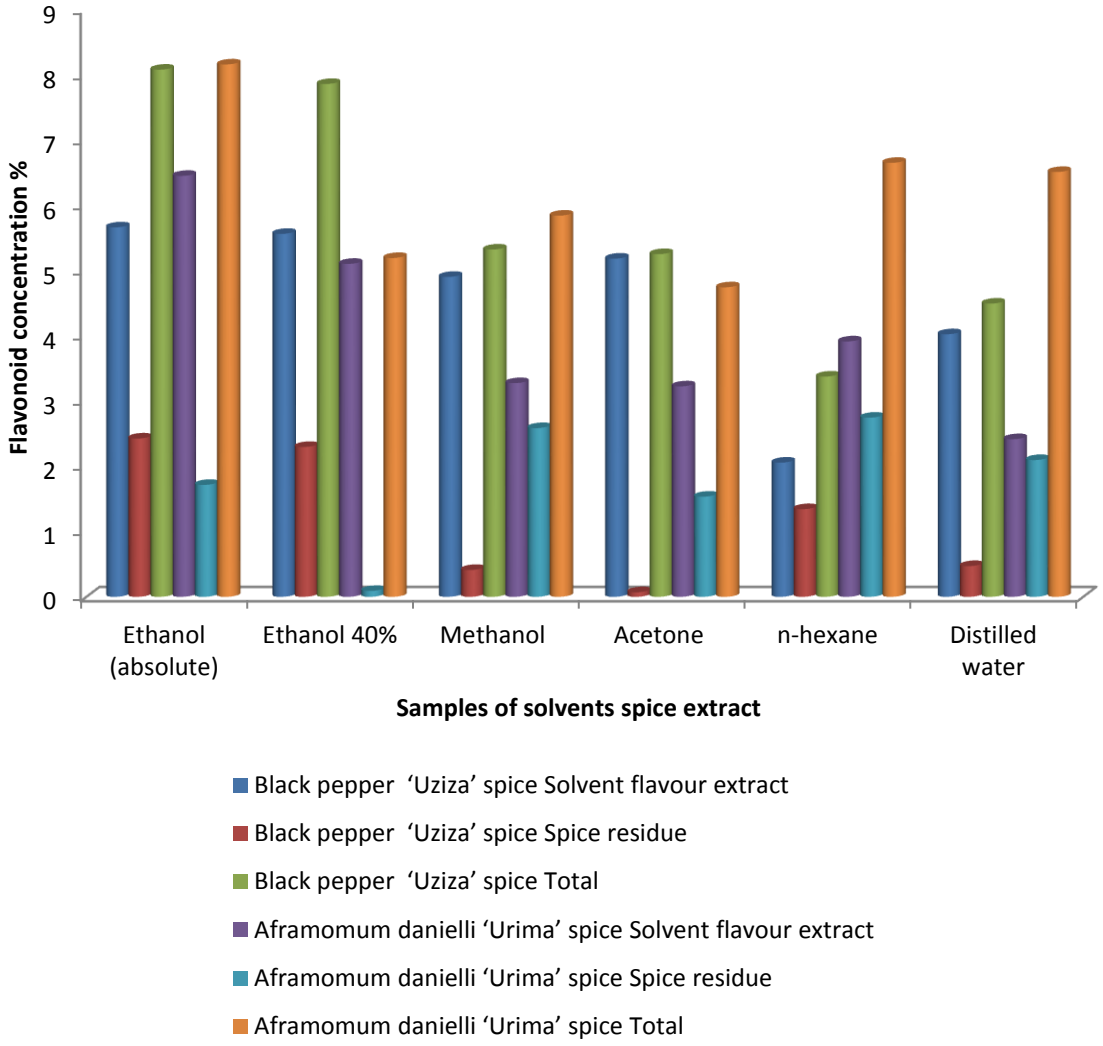

Figure 2: Flavonoid (%) content of solvents spice extracts

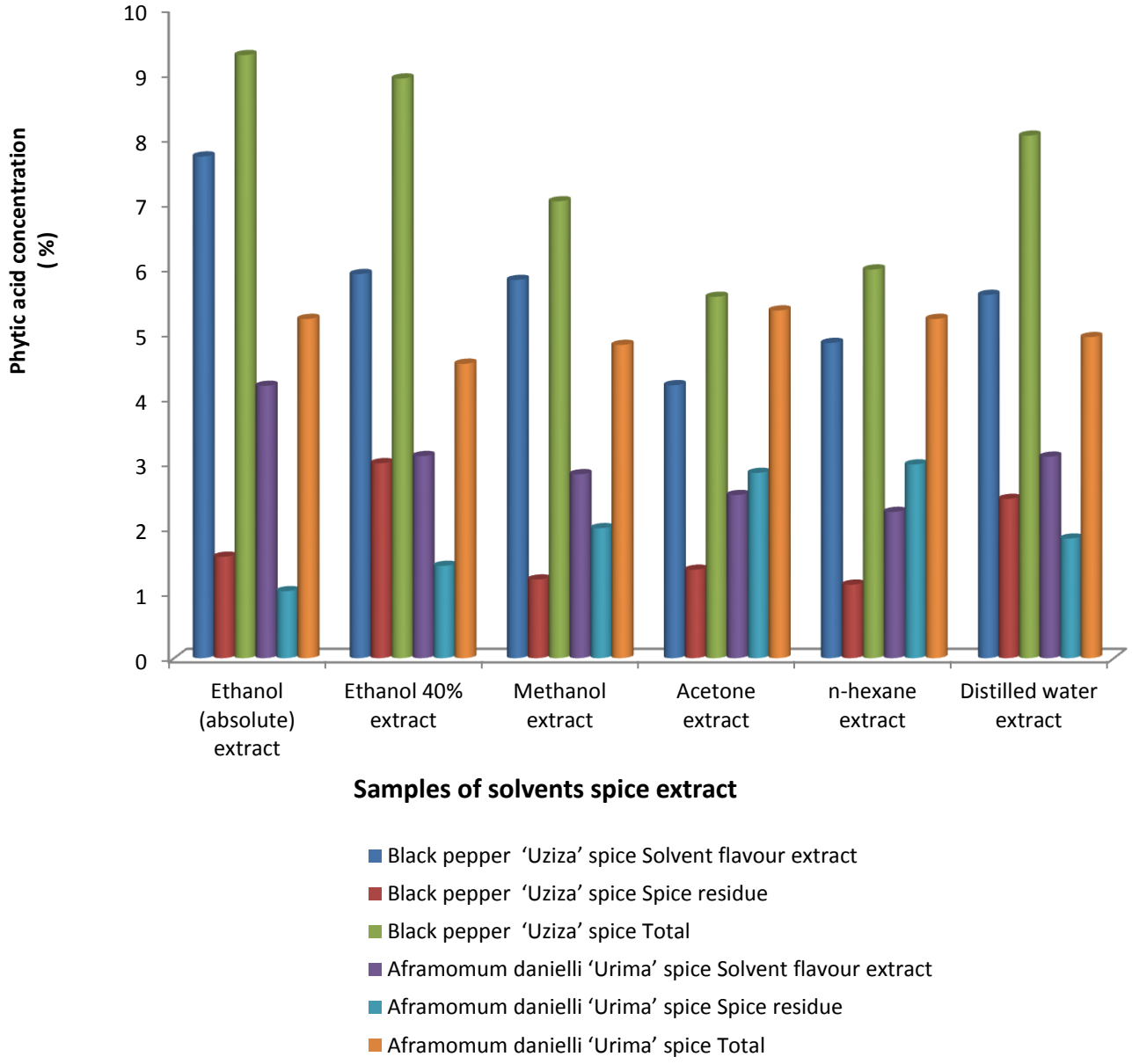

Figure 3: Phytic acid (%) content of solvents spice extracts

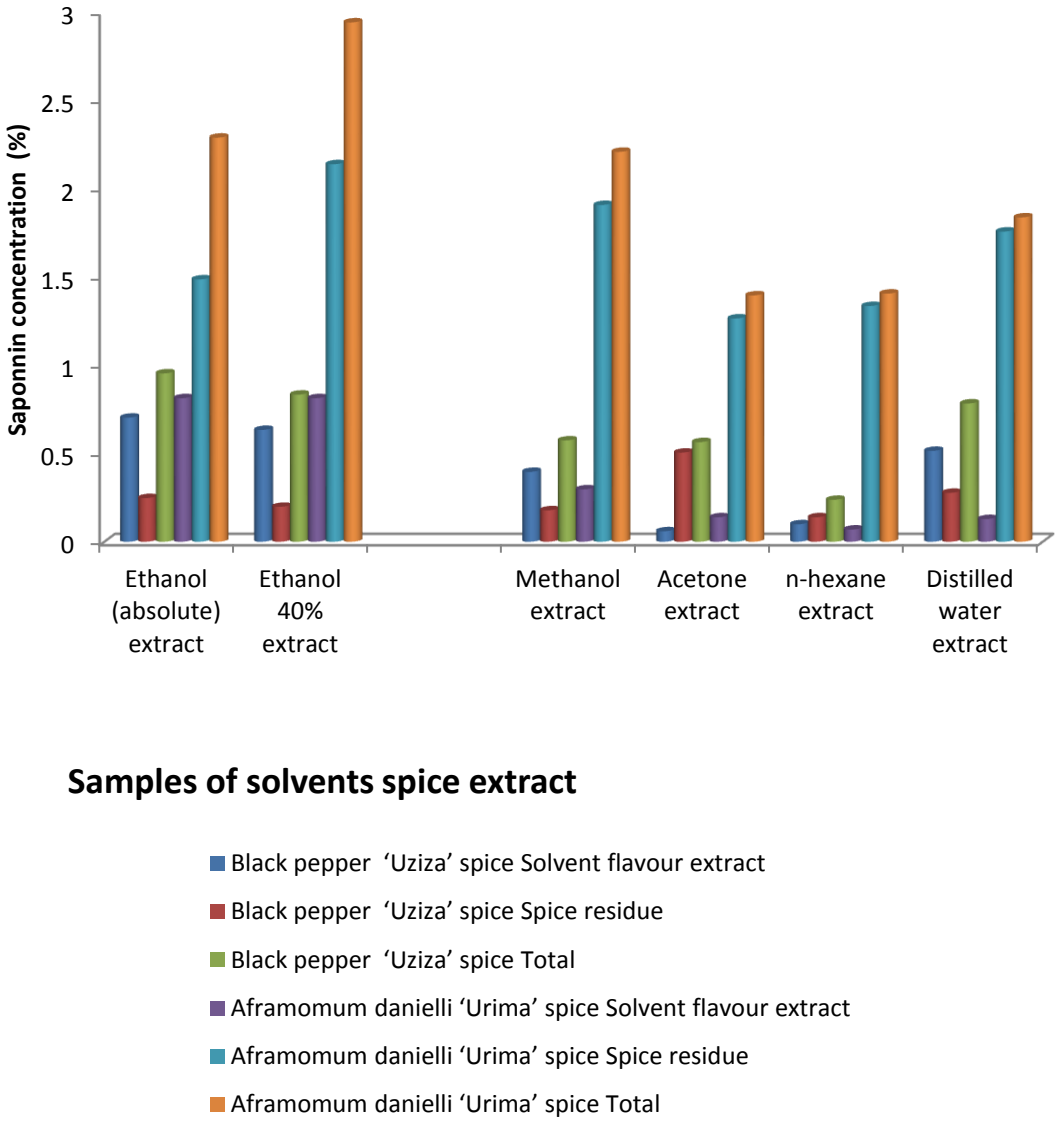

Figure 4: Saponin (%) content of solvents spice extracts

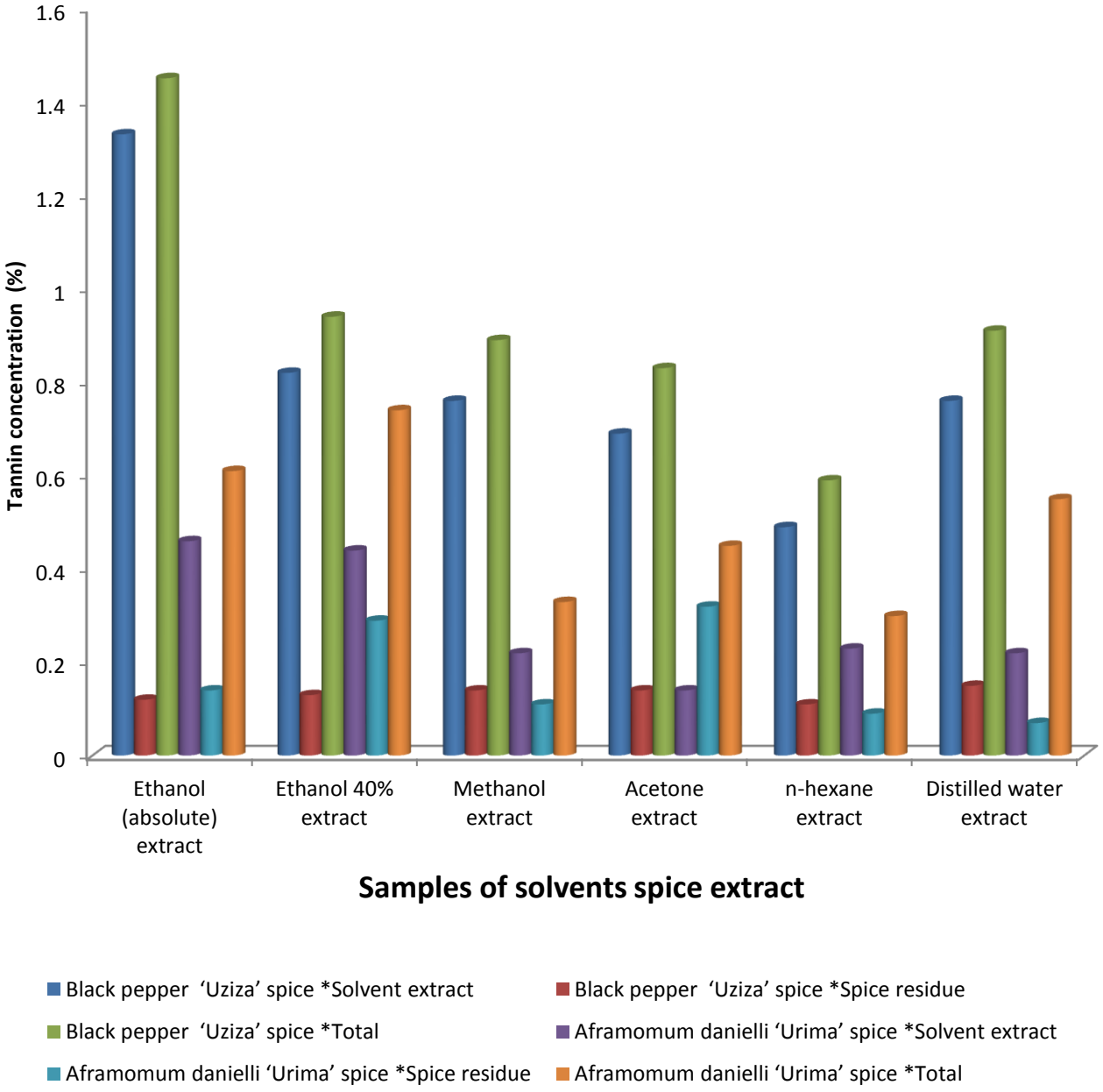

Figure 5: Tannin (%) content of solvents spice extracts

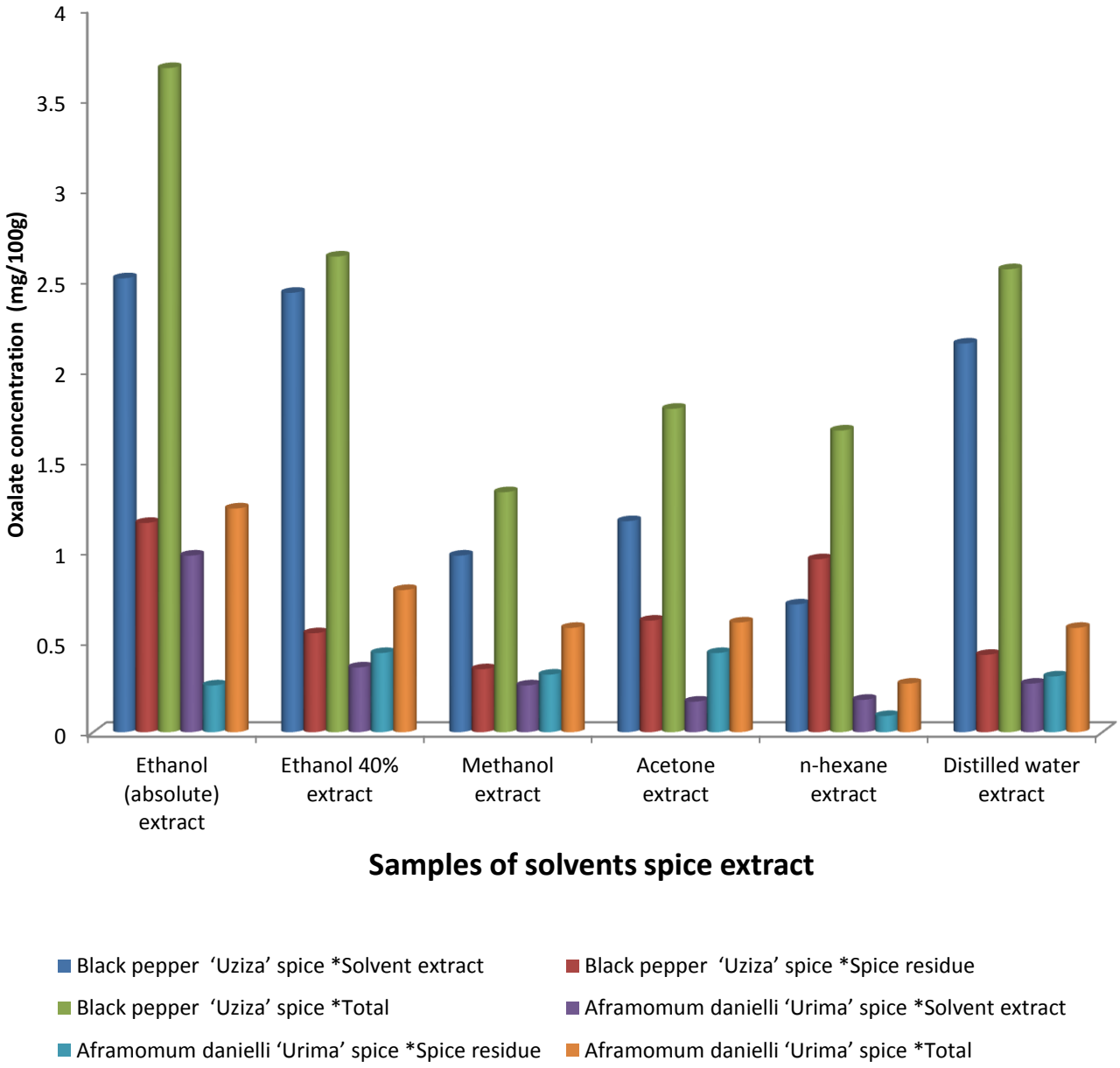

Figure 6: Oxalate (mg/100g) content of solvents spice extracts

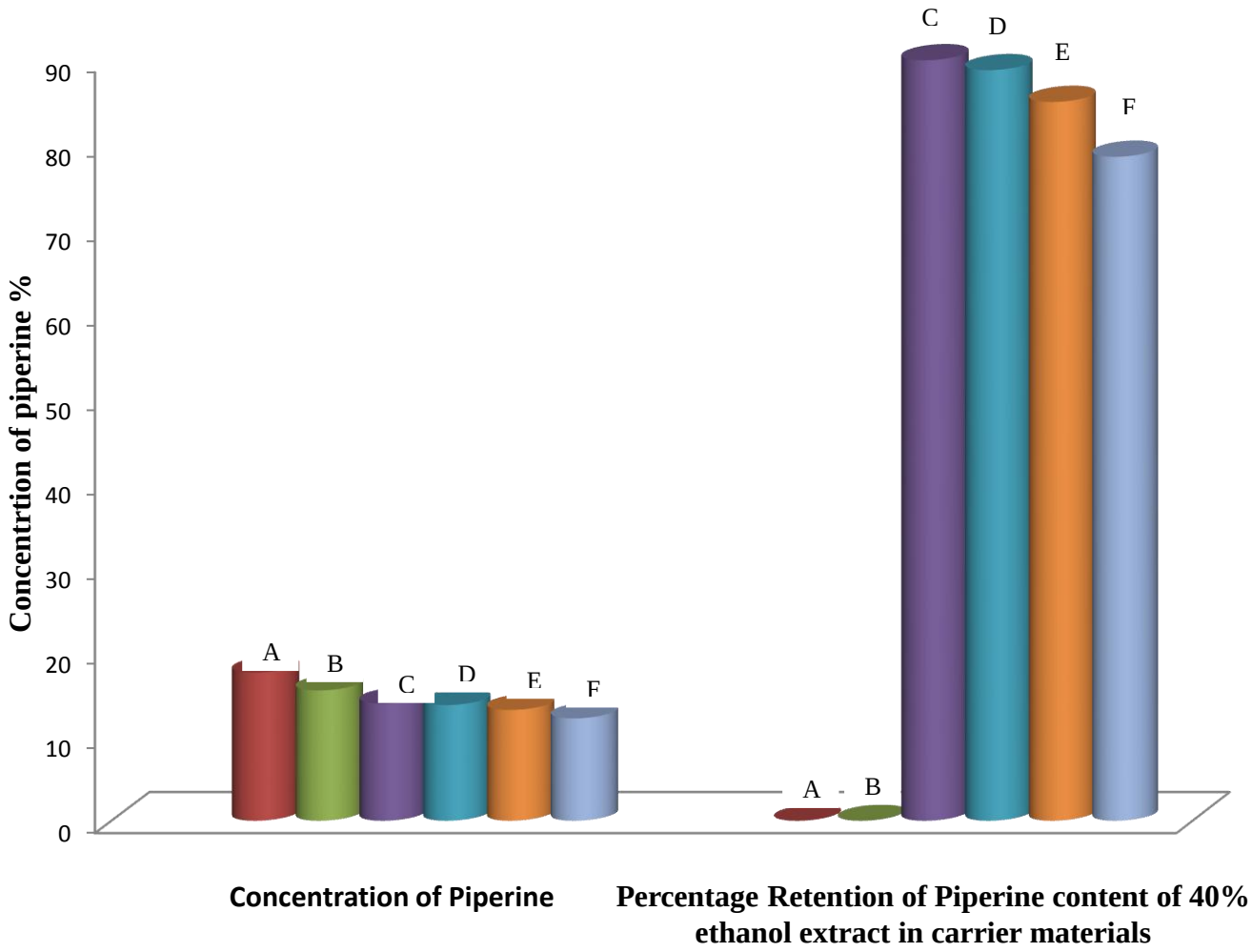

Figure 4.7: Piperine profile in *Piper nigrum* flavour products

PP-PB **(A)** = *Piper nigrum* pulverized sample in amber coloured bottle, PP-EB **(B)** = 40% ethanol extract of *Piper nigrum* product sample in amber bottle, $PPERC_1$**(C)** = 40% ethanol extract of *Piper nigrum* in rice grits carrier at ratio1:2v/w, $PPERC_2$ **(D)** = 40% ethanol extract of *Piper nigrum* in rice grits carrier at ratio1:3v/w, $PPEPC_1$ **(E)** = 40% ethanol extract of *Piper nigrum* in potato grits carrier at ratio1:2v/w, $PPEPC_2$ **(F)**= 40% ethanol extract of *Piper nigrum* in potato grits carrier at ratio1:3v/w.

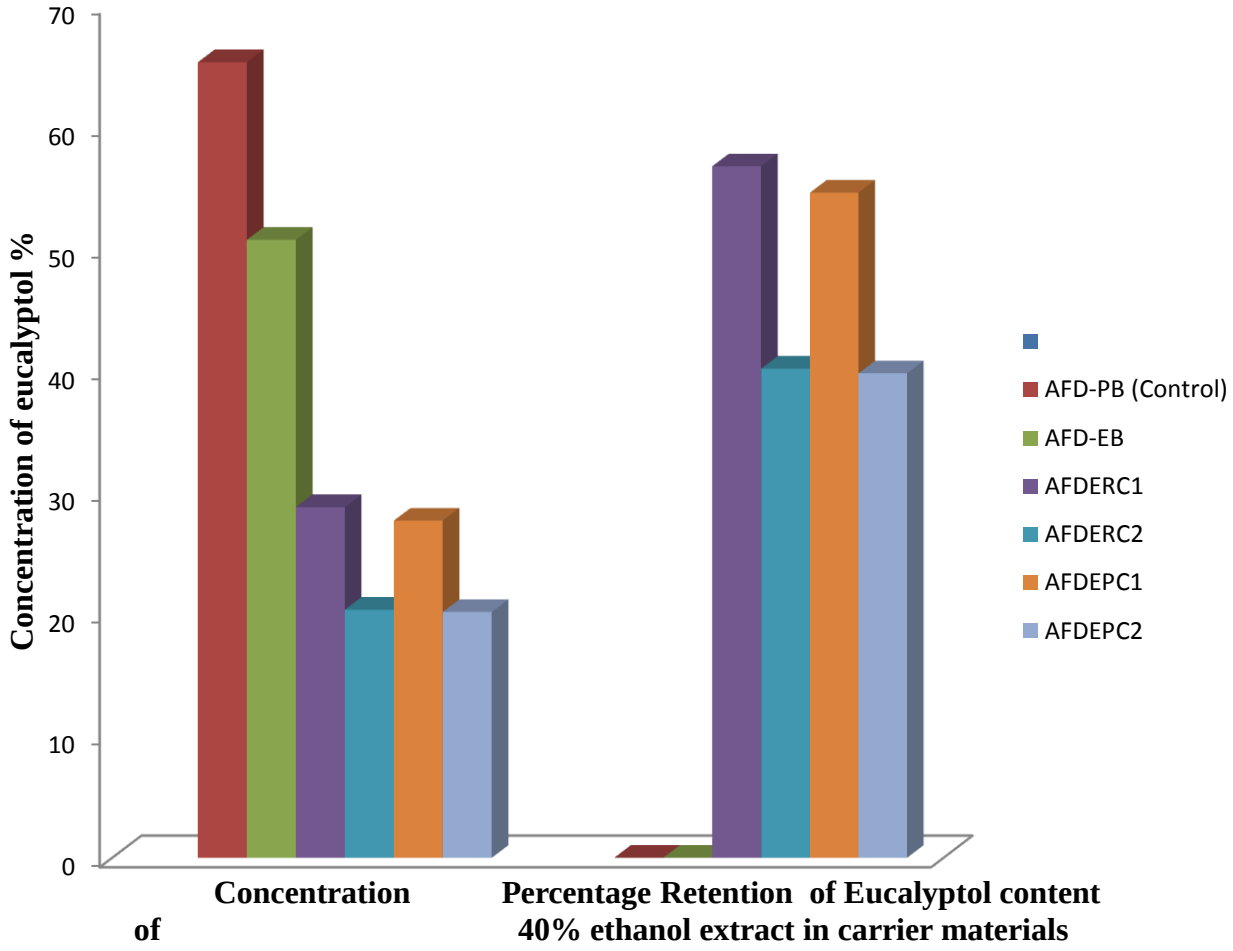

Figure 4.8: Eucalyptol profile in *Aframomum danielli* flavour products

AFD-PB = pulverized sample of *Aframomum danielli* in amber bottle, AFD-EB = 40% ethanol extract of *Aframomum danielli* product sample in amber bottle, $AFDERC_1$ = 40% ethanol extract of *Aframomum danielli* in rice grits carrier at 1:2v/w, $AFDERC_2$ = 40% ethanol extract of *Aframomum danielli* in rice grits carrier at 1:3v/w, $AFDEPC_1$ = 40% ethanol extract of *Aframomum danielli* in potato grits carrier at 1:2v/w, $AFDEPC_2$ = 40% ethanol extract of *Aframomum danielli* in potato grits carrier at 1:3v/w.

APPENDIX III
Chromatograms peaks of chemical components in spice samples at 6months storage

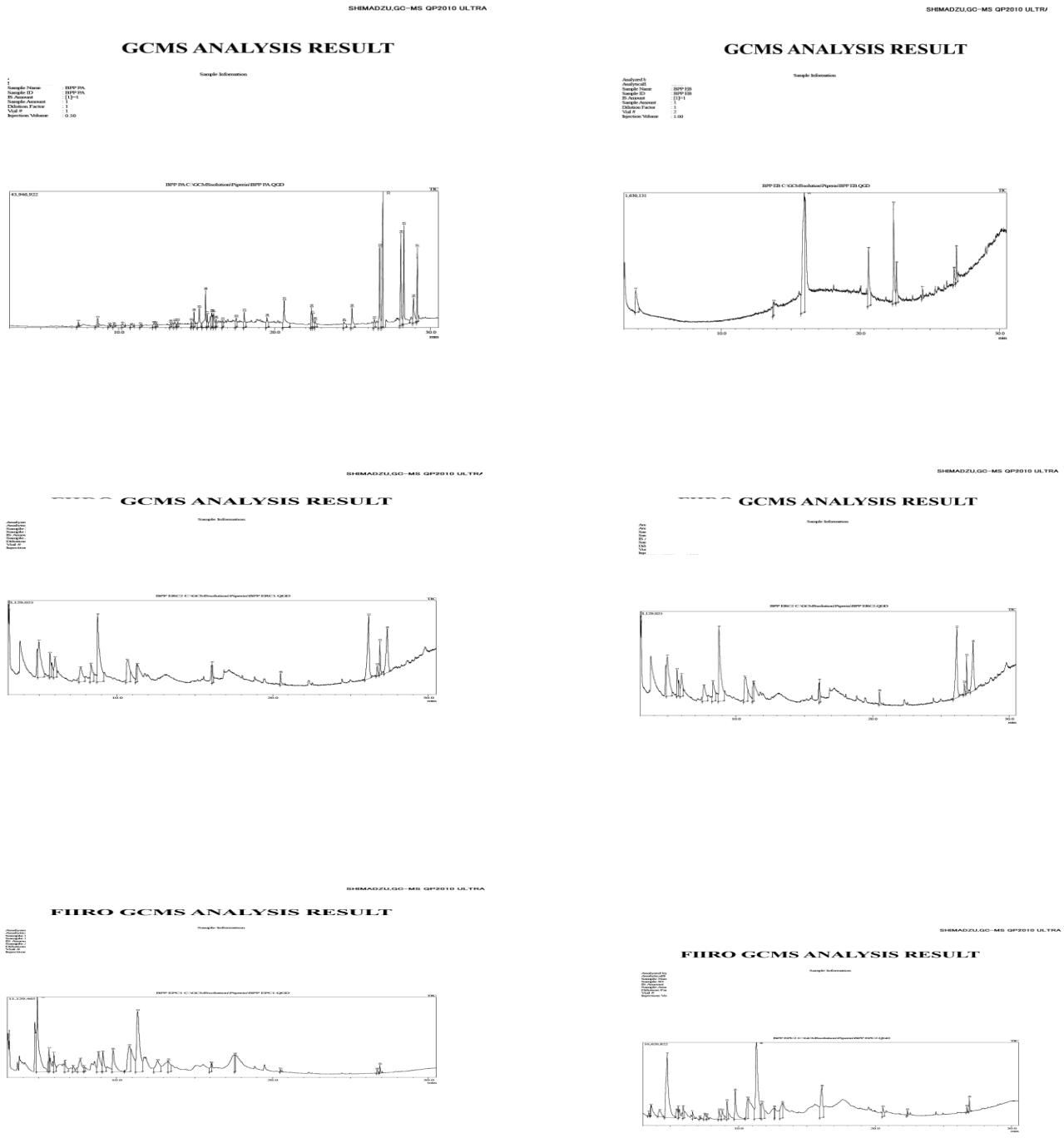

Figure 1: Chromatograms peaks of chemical compounds in *Piper nigrum* products zero hour of storage

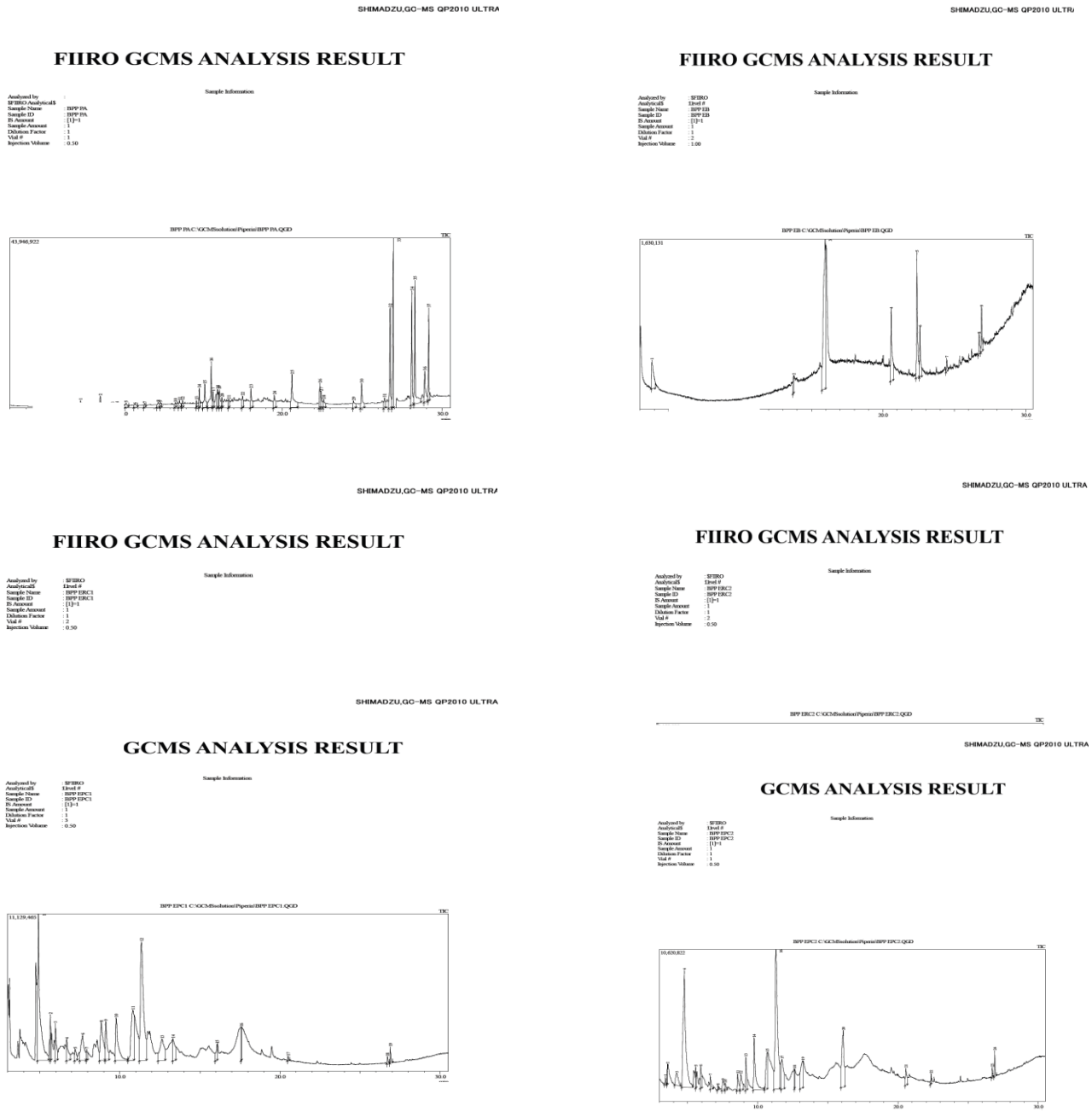

Figure 2: Chromatogram peaks of chemical compounds in *Piper nigrum* products at 2weeks storage

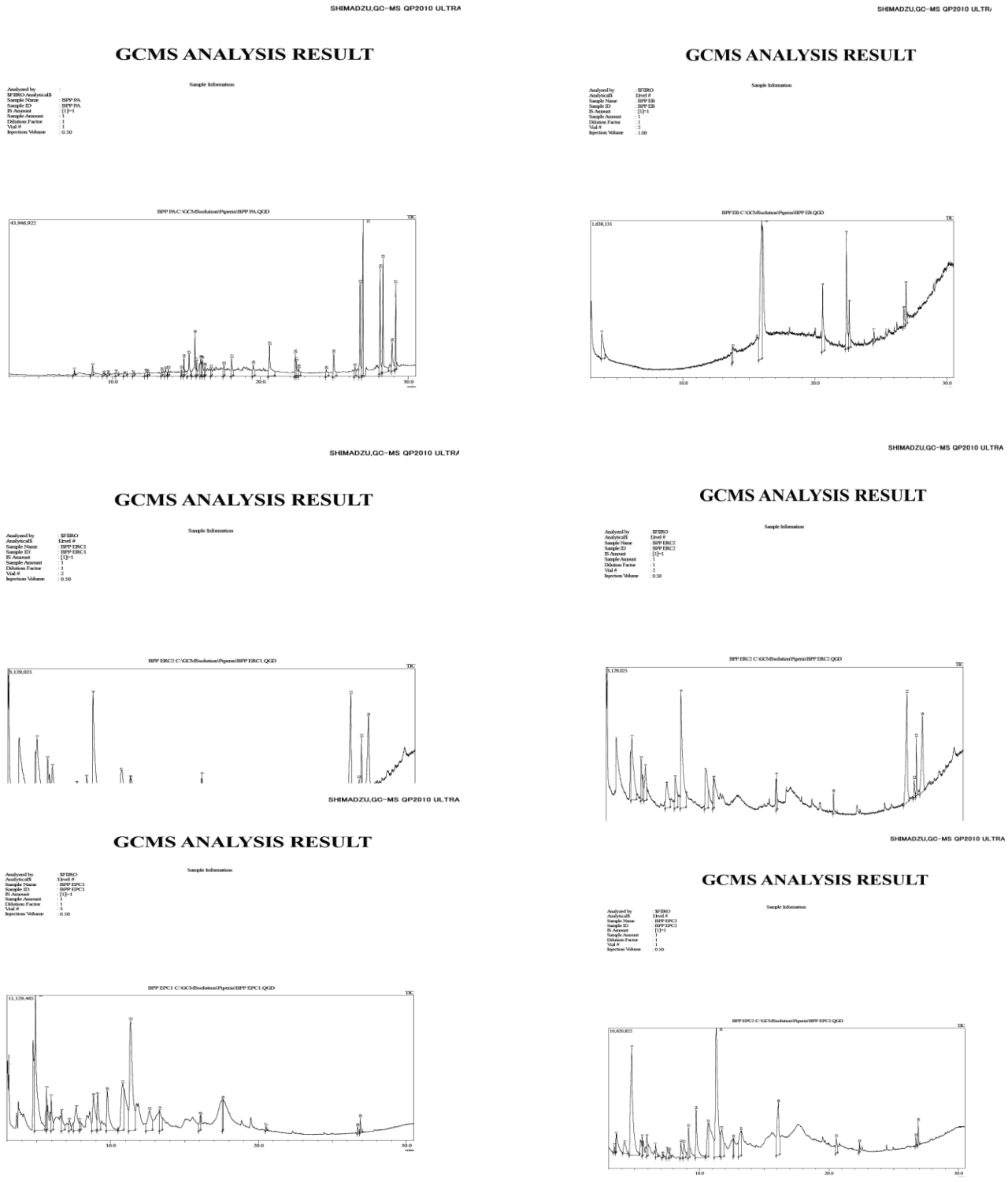

Figure 3: Chromatogram peaks of chemical compounds in *Piper nigrum* products at 4weeks storage.

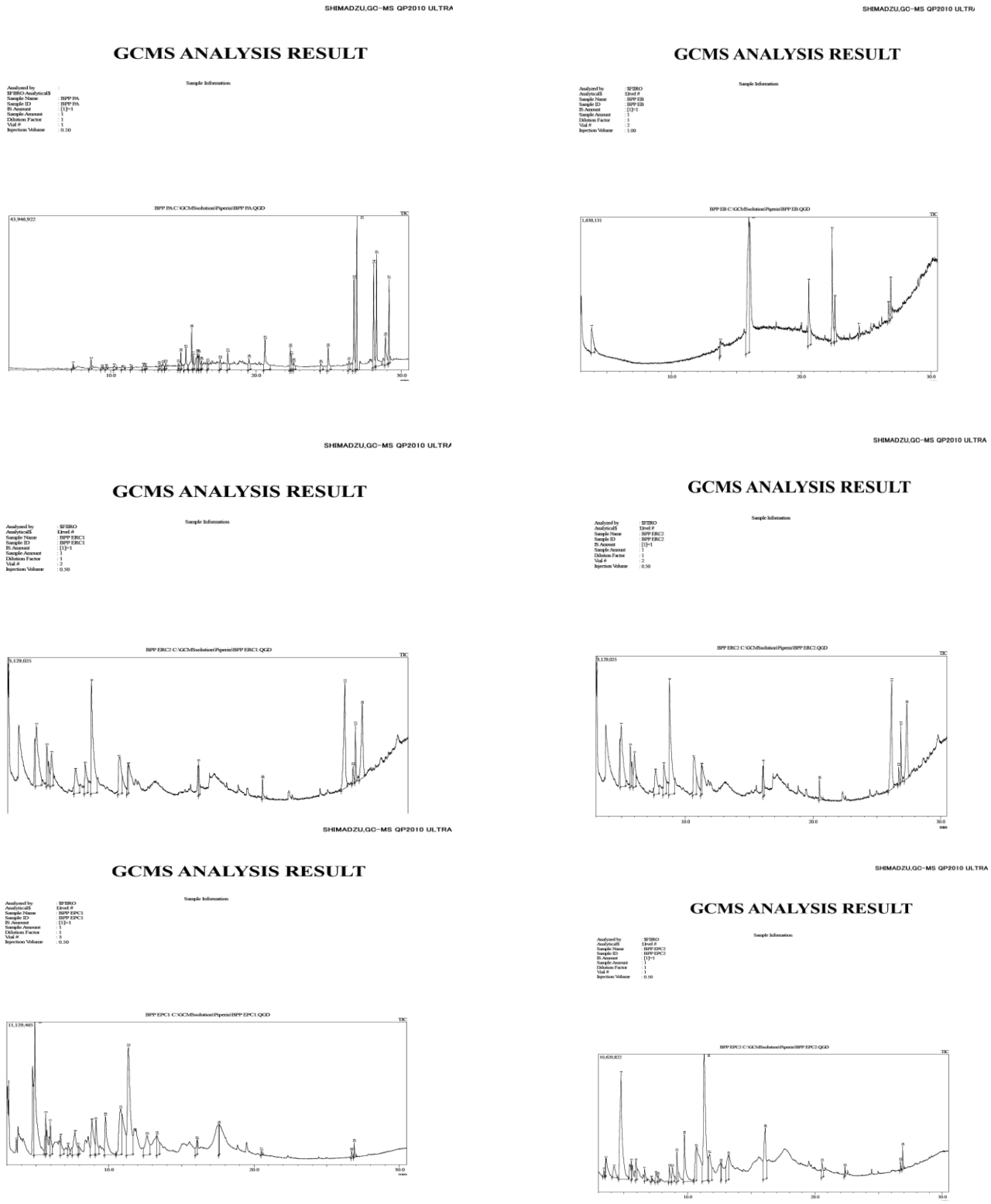

Figure 4: Chromatogram peaks of chemical compounds in *Piper nigrum* products samples 6weeks storage.

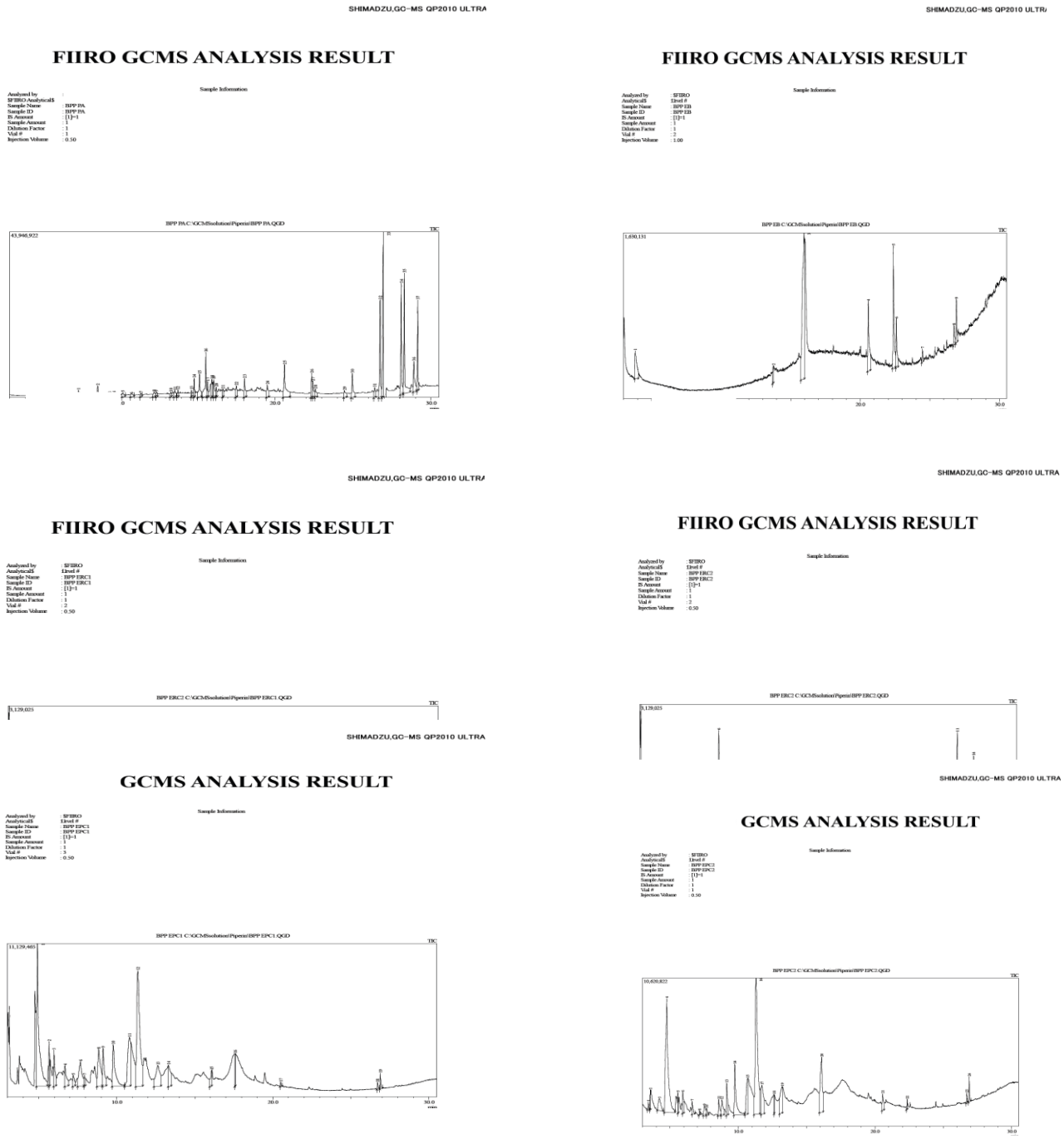

Figure 5: Chromatogram peaks of chemical compounds in *Piper nigrum* products samples at 2months storage.

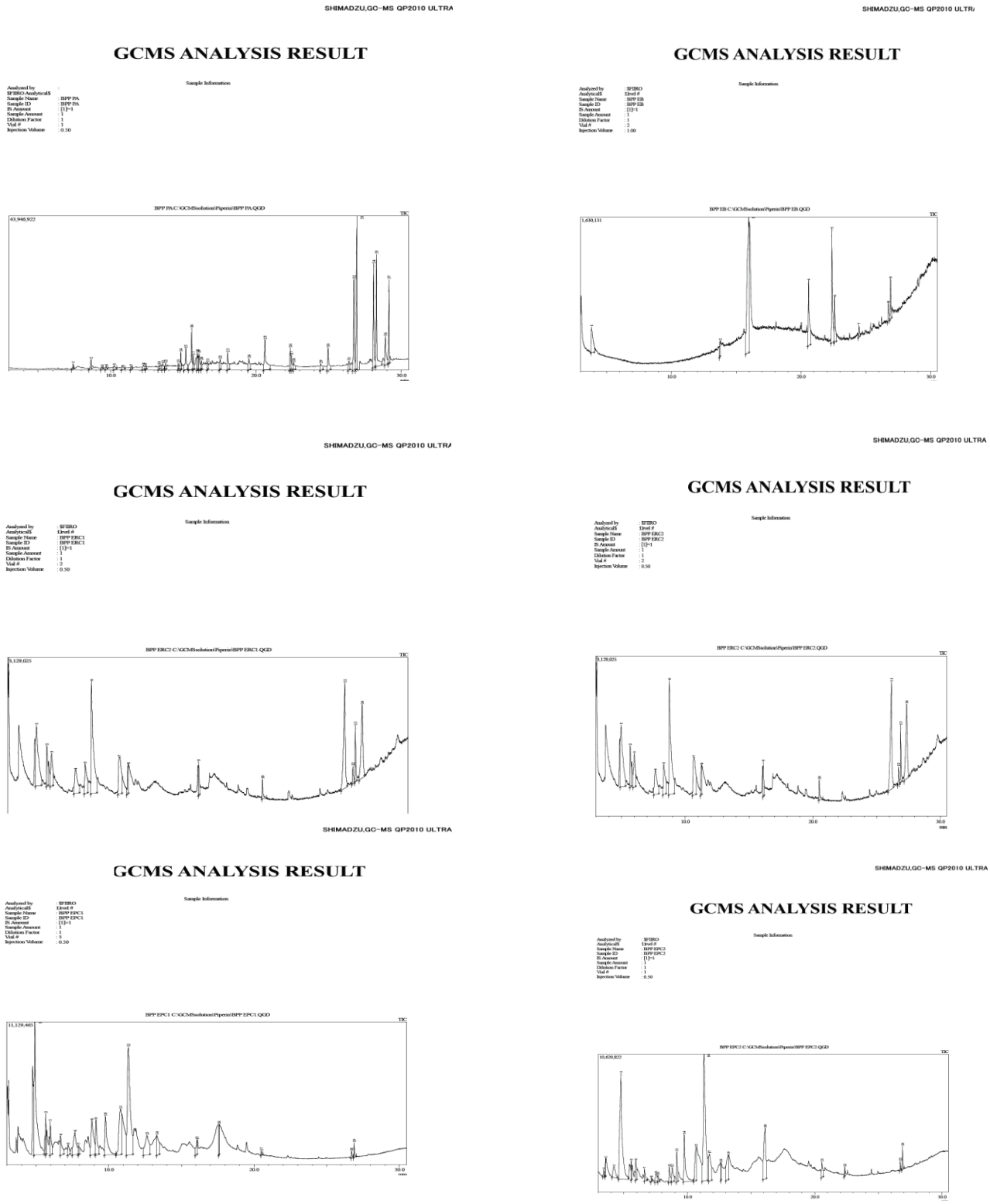

Figure 6: Chromatogram peaks of chemical compounds in *Piper nigrum* products 3months storage.

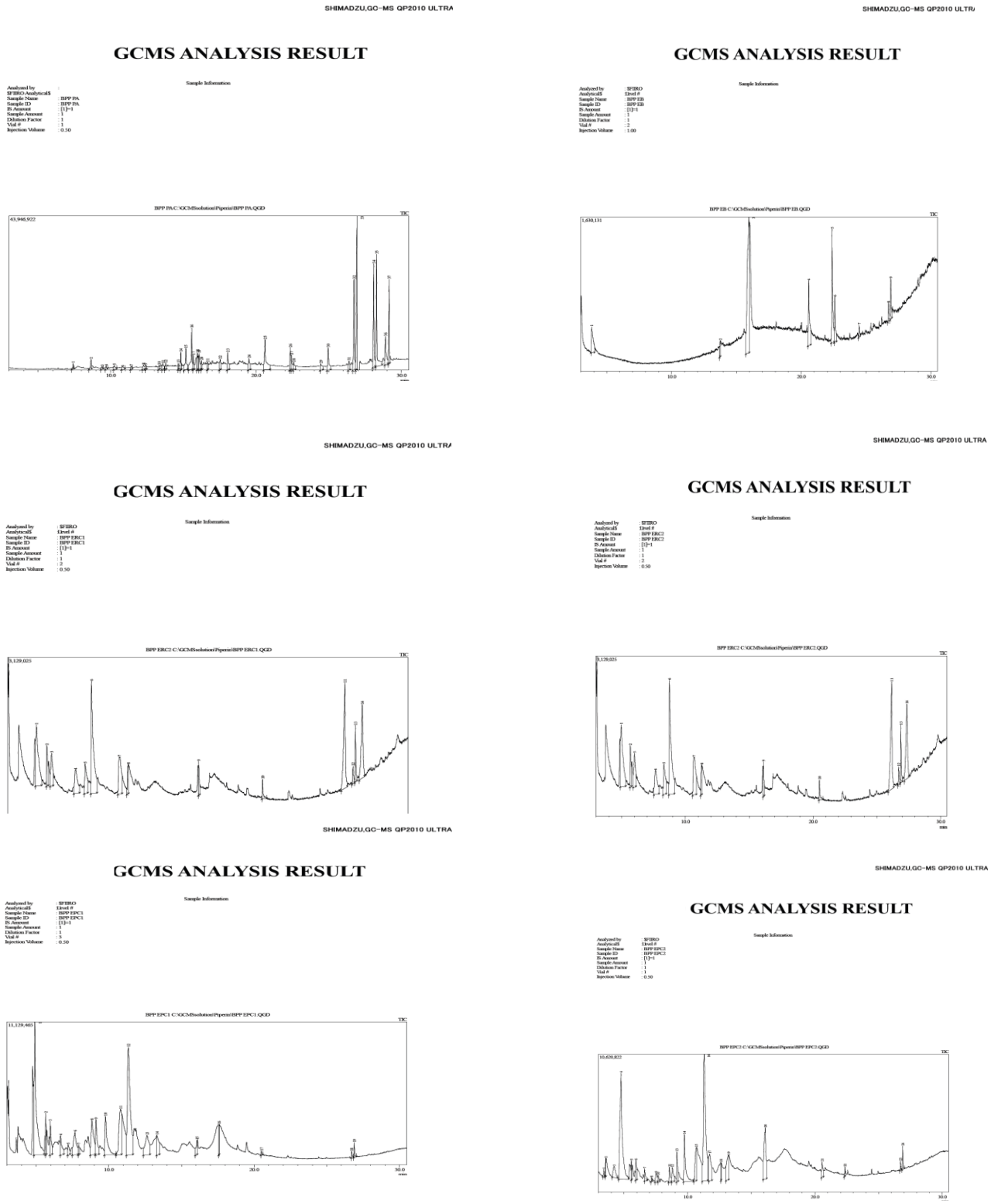

Figure 7: Chromatogram peaks of chemical compounds *Piper nigrum* products samples at 4months storage.

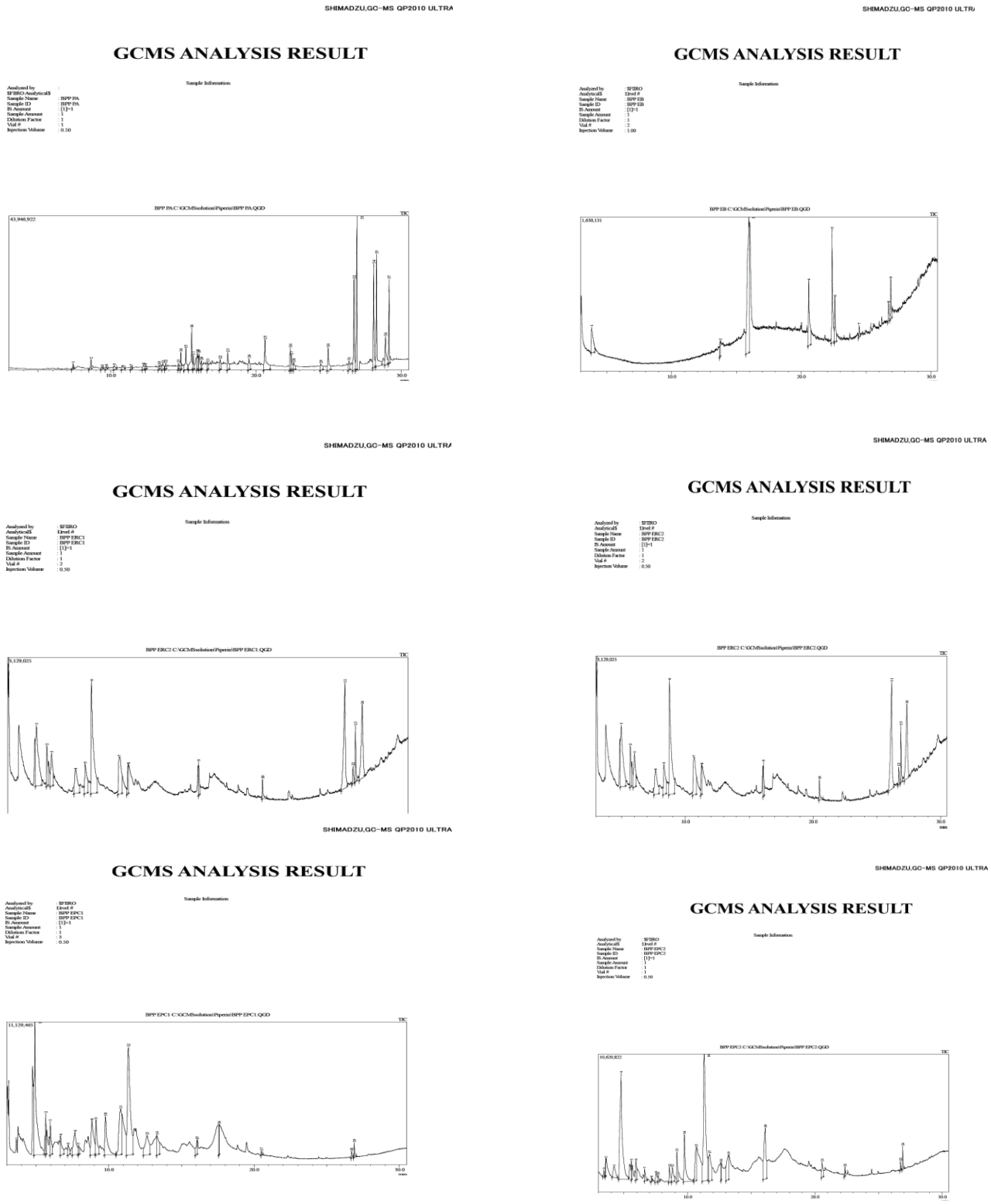

Figure 8: Chromatogram peaks of chemical compounds in *Piper nigrum* products samples at 5months storage.

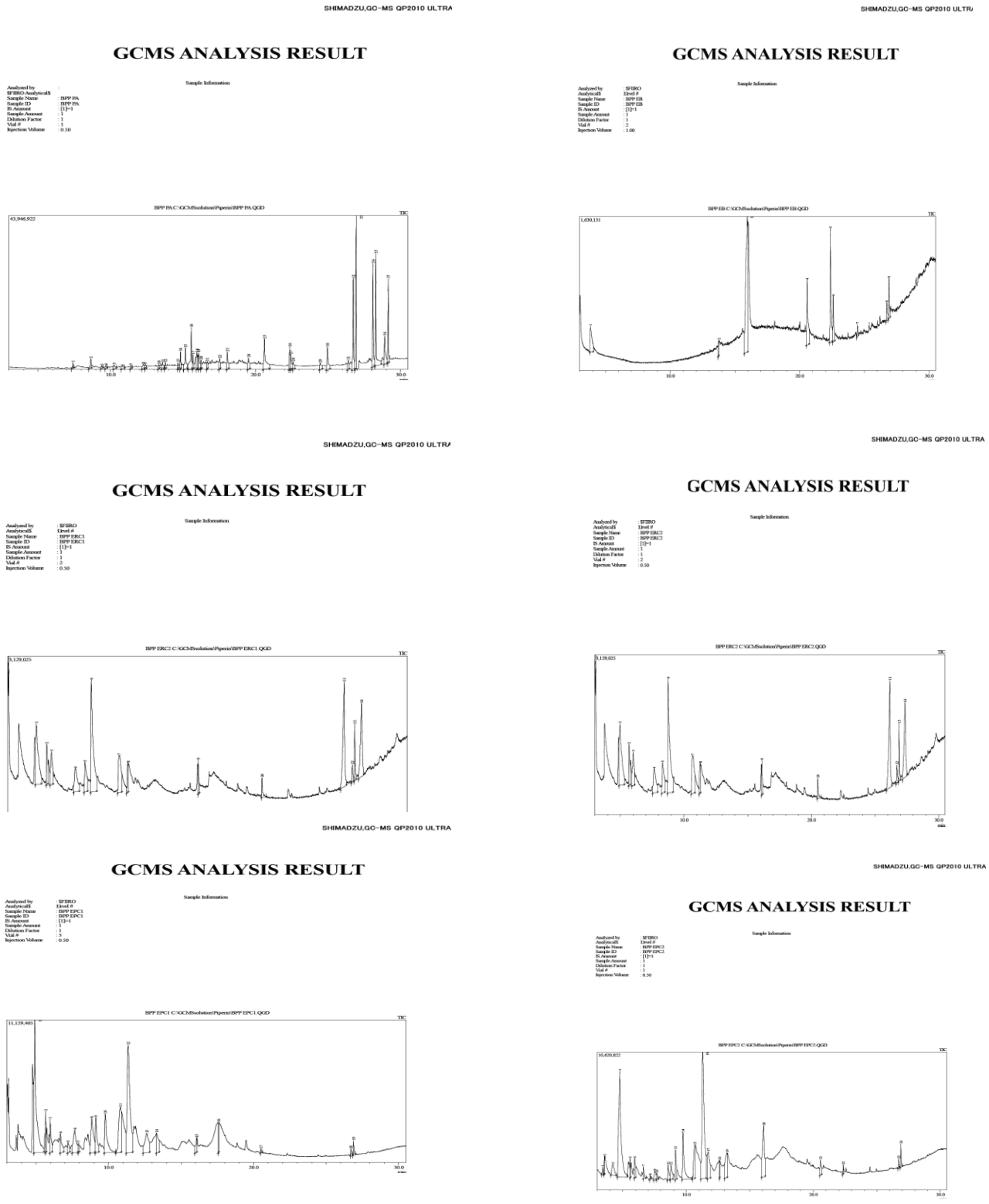

Figure 9: Chromatogram peaks of chemical compounds in *Piper nigrum* products samples at 6months storage.

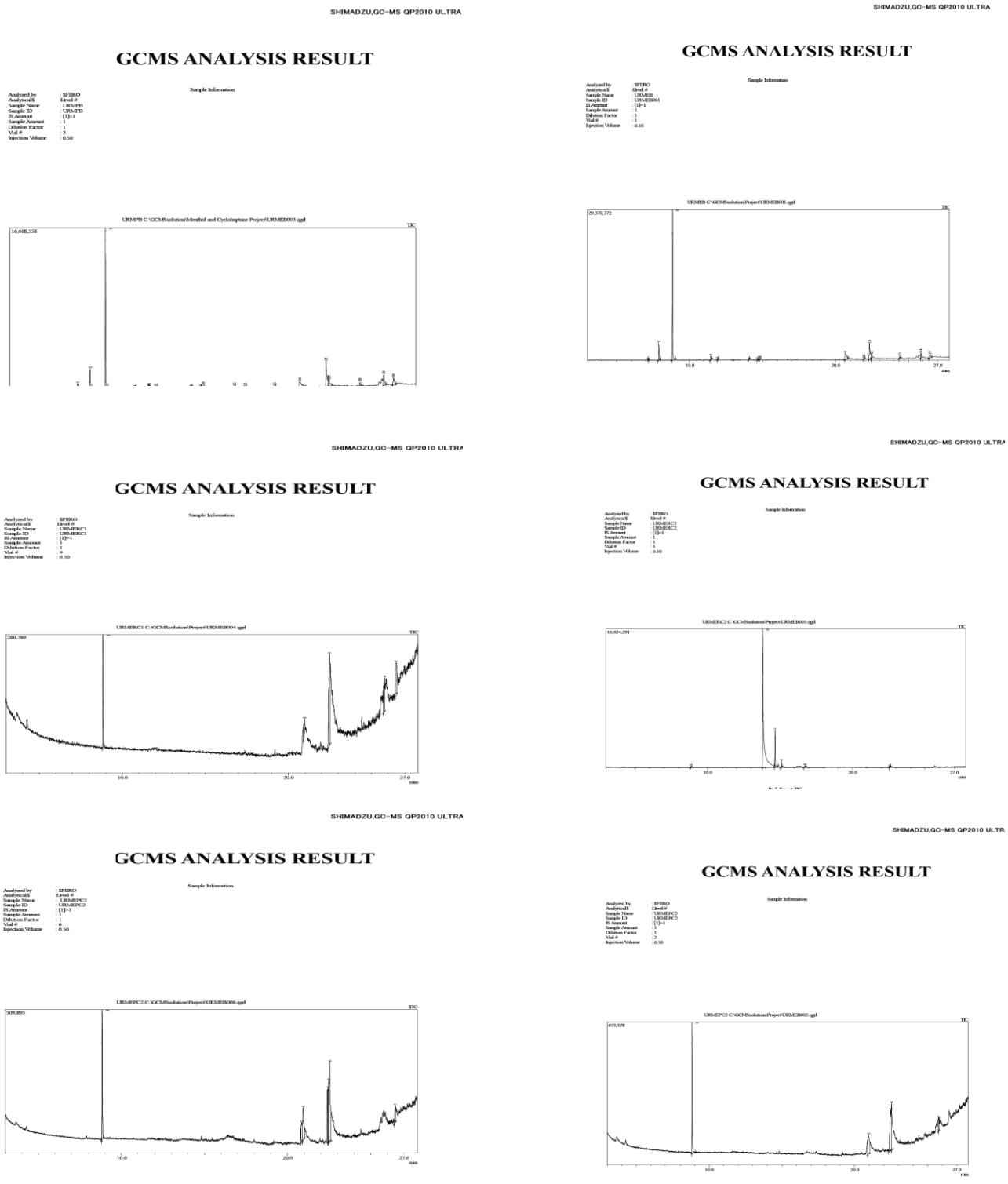

Figure 10: Chromatogram peaks of chemical compounds in Aframomum *danielli* products samples at zero hour storage.

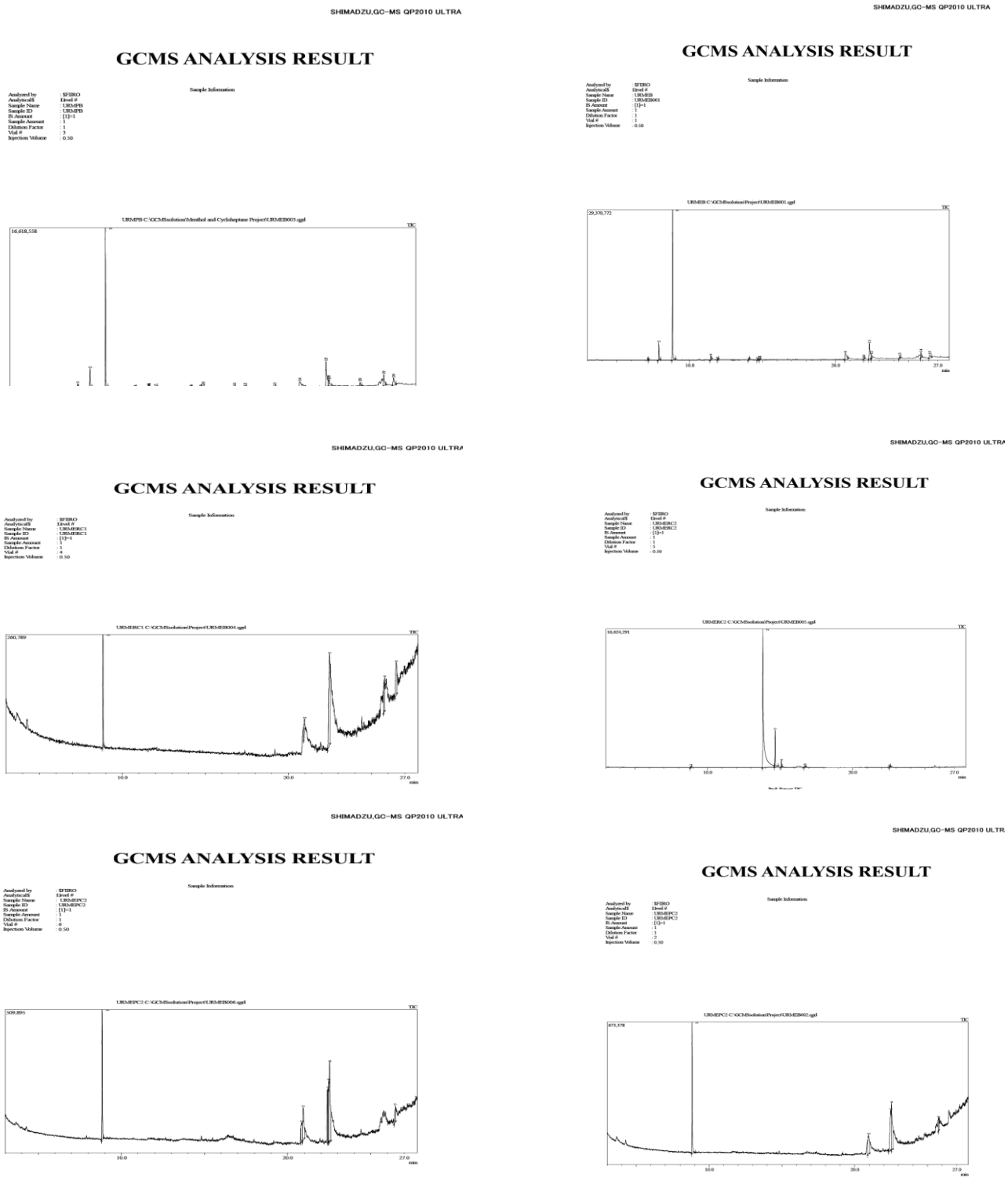

Figure 11 Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 2weeks storage.

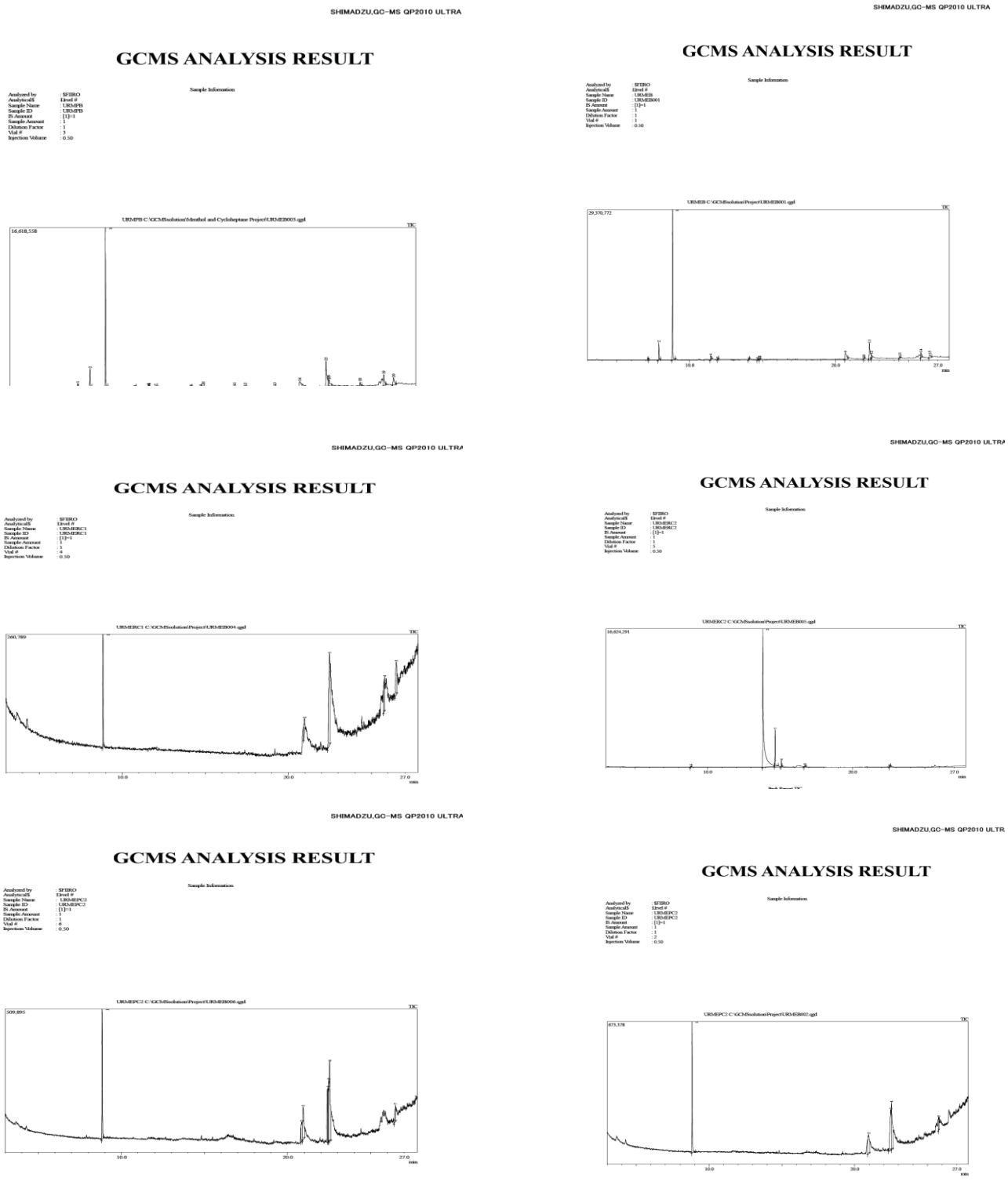

Figure 12: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 4weeks storage.

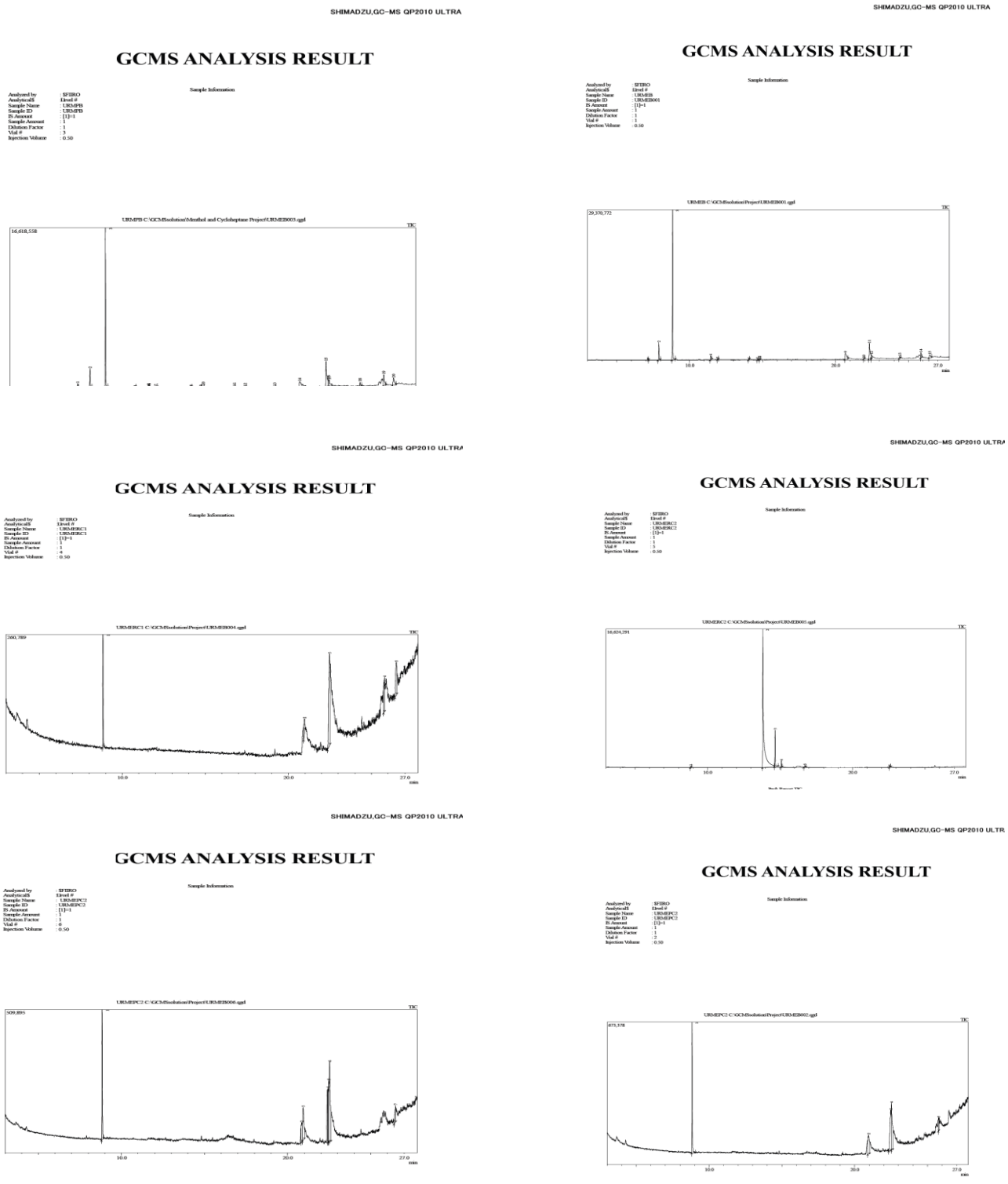

Figure 13: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 6weeks storage.

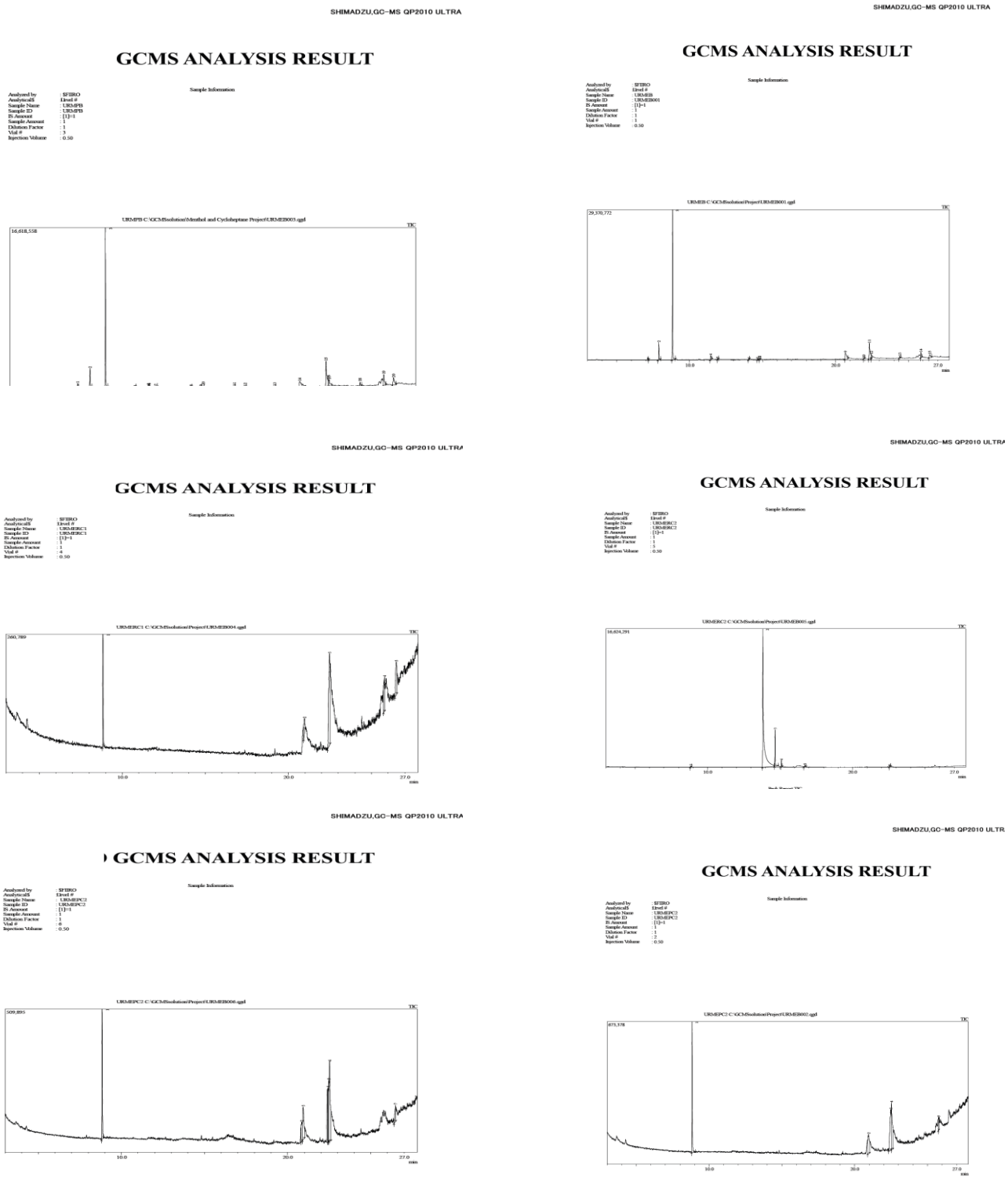

Figure 14: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 2months storage.

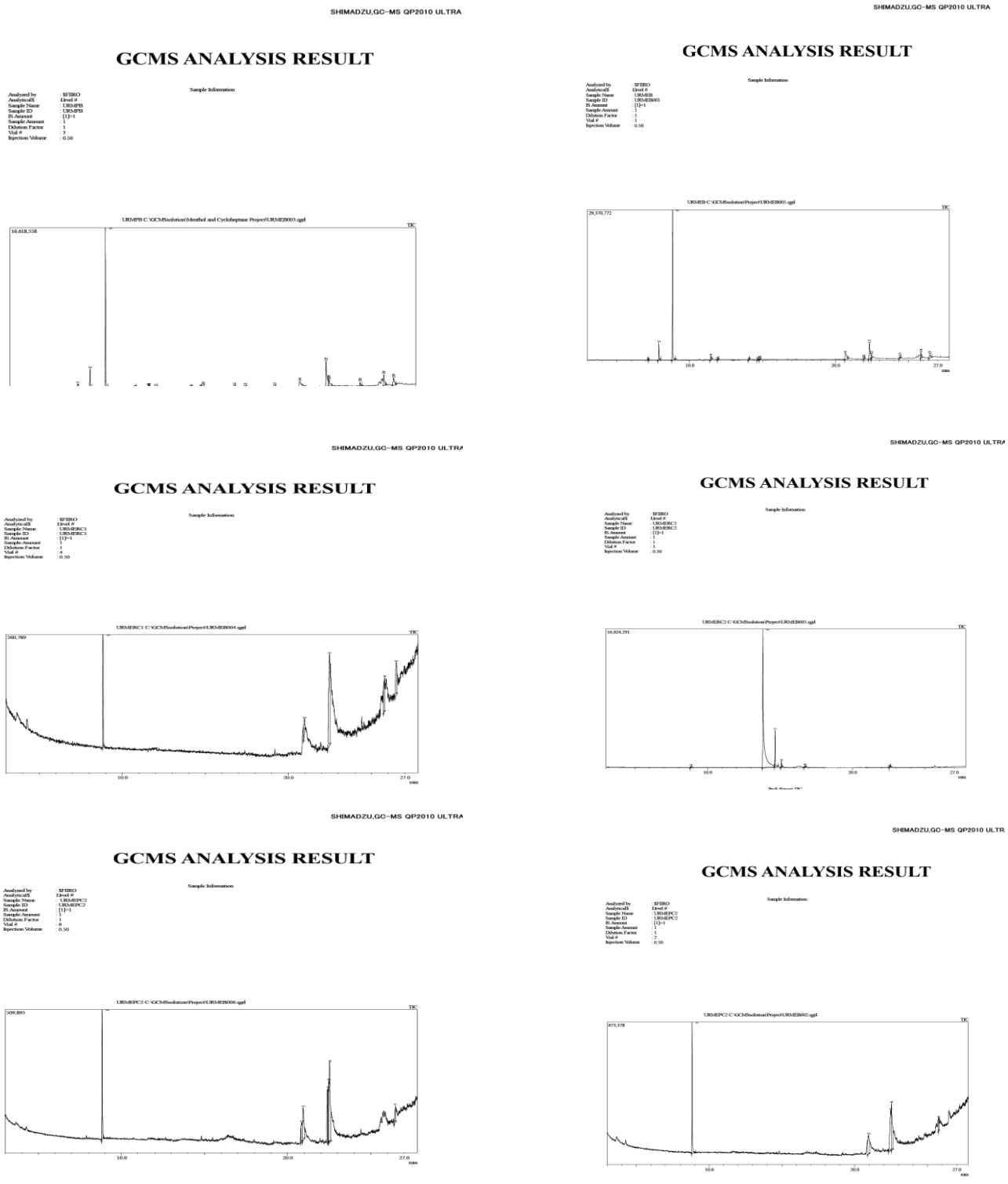

Figure 15: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 3months storage.

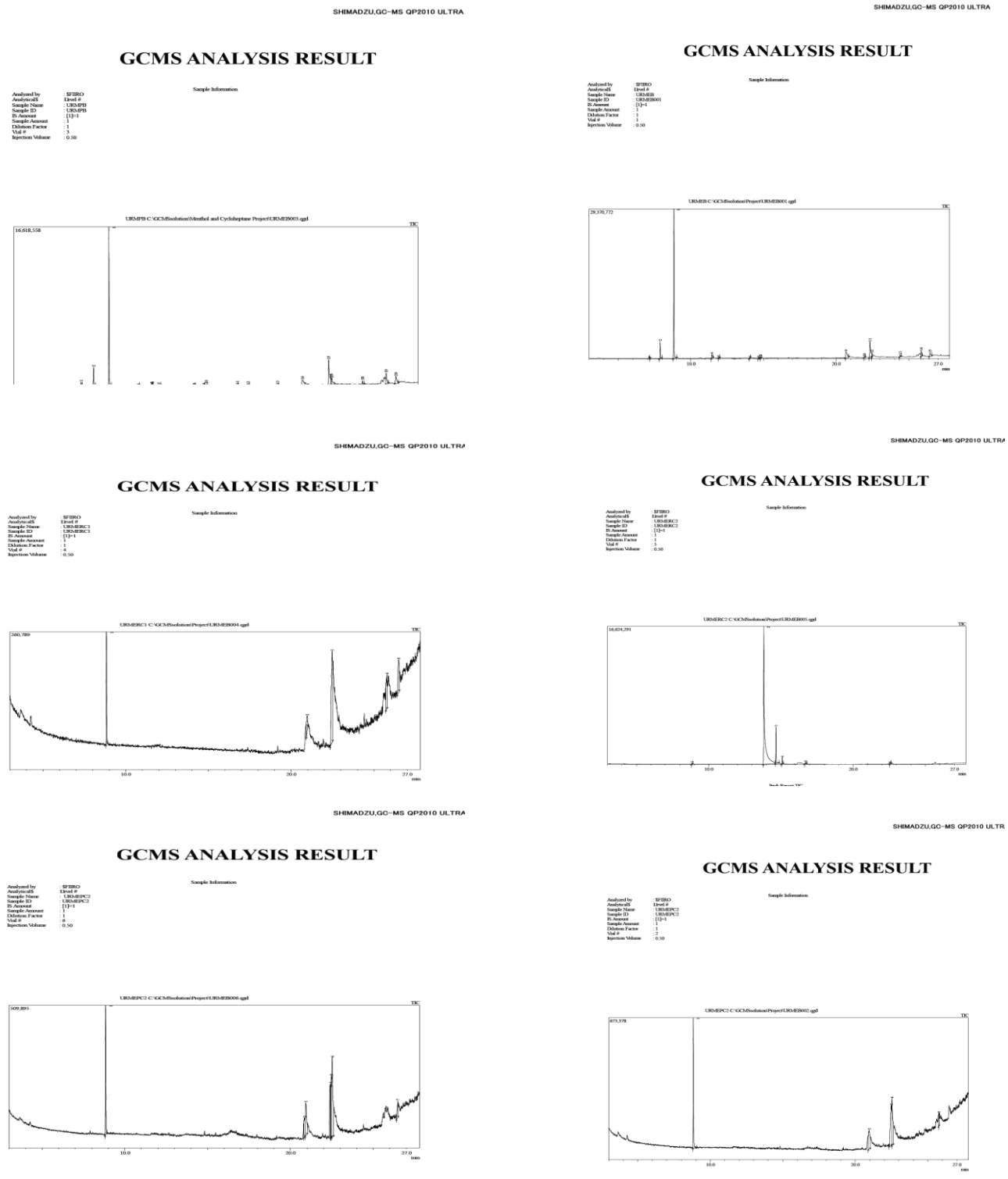

Figure 16: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples 4months storage.

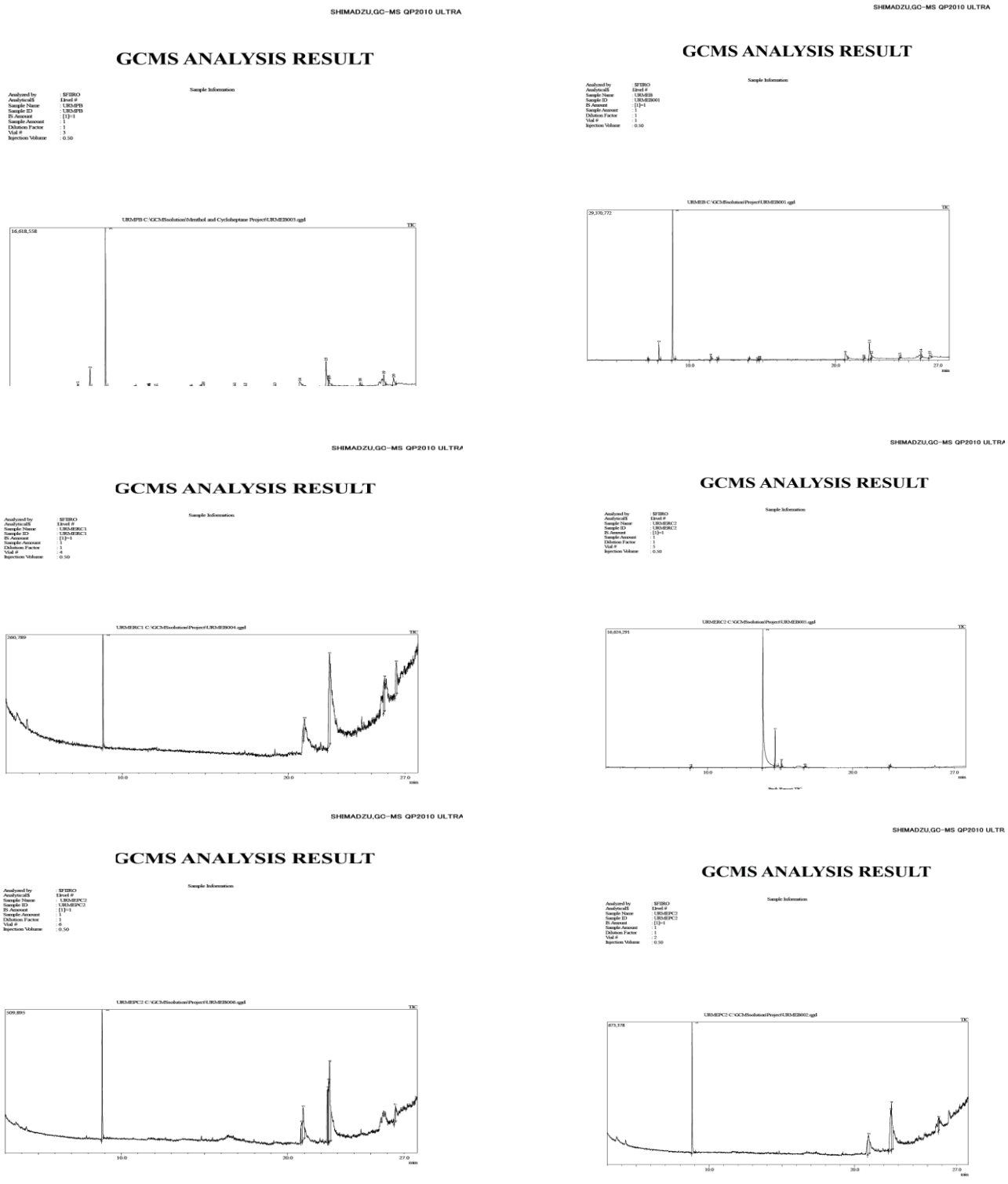

Figure 17: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 5months storage.

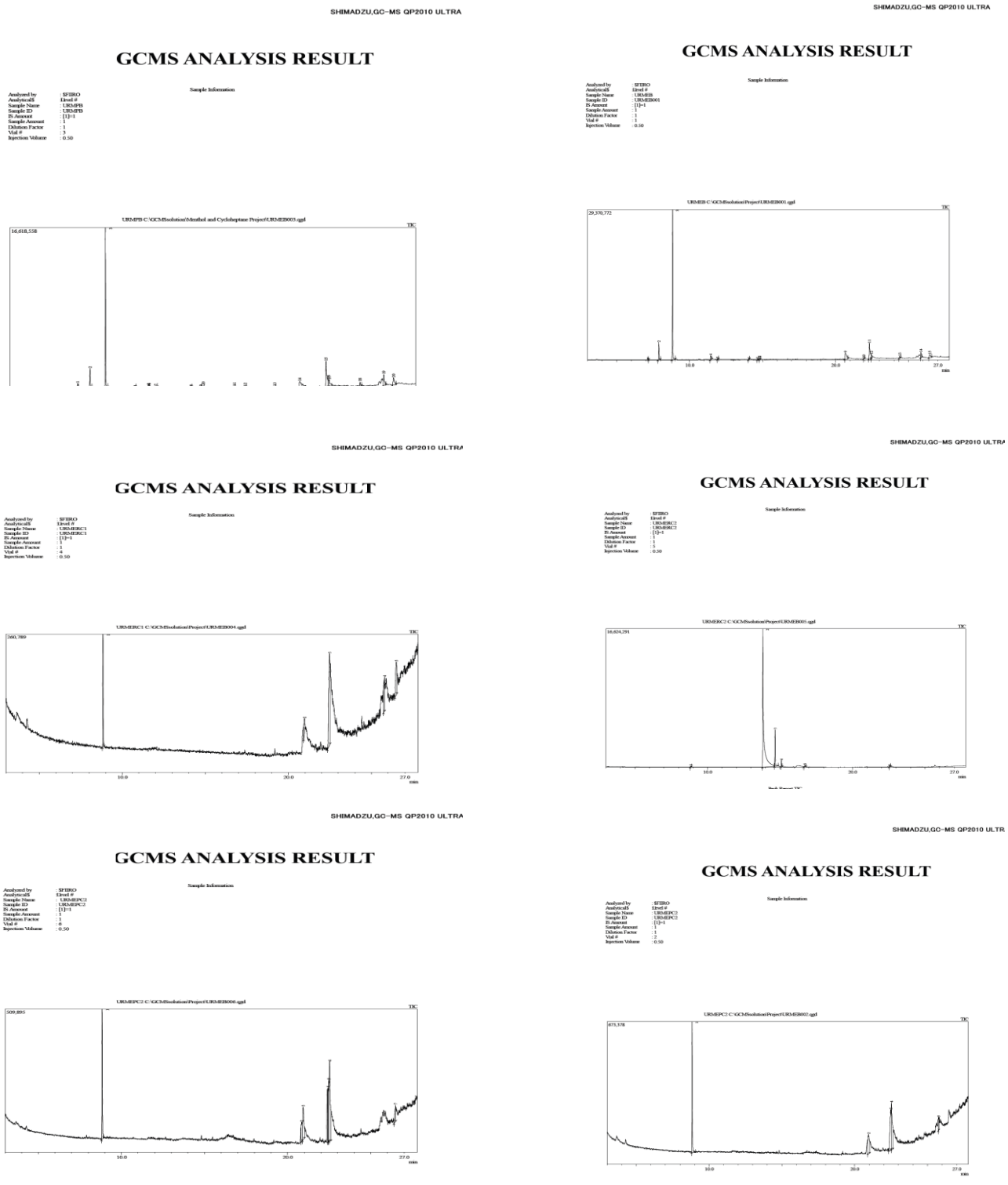

Figure 18: Chromatogram peaks of chemical compounds in *Aframomum danielli* products samples at 6monhs storage.

APPENDIX IV
Results of statistical analysis
Analysis of variance of on moisture content of pulverized and encapsulated spice flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum	Between Groups	5.564	4	1.391	4796.172	.000
	Within Groups	.001	5	.000		
	Total	5.565	9			
Aframomum danielli	Between Groups	1.717	4	.429	1226.757	.000
	Within Groups	.002	5	.000		
	Total	1.719	9			

Homogeneous Subsets pulverized and encapsulated spice flavour products

black pepper

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	RG-C2	2	6.0900				
	PG-C2	2		6.3400			
	RG-C1	2			6.8850		
	PG-C1	2				7.1200	
	powder sample	2					8.2300
	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.

Aframomum danielli

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
Tukey HSD[a]	PG-C2	2	6.0200			
	RG-C2	2		6.1350		
	RG-C1	2			6.4650	
	PG-C1	2				6.9700
	powder sample	2				7.0250
	Sig.		1.000	1.000	1.000	.144

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Analysis of variance on specific gravity of solvent flavour extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
black pepper	Between Groups	.332	5	.066	852.242	.000
	Within Groups	.000	6	.000		
	Total	.332	11			
Aframomum danielli	Between Groups	.276	5	.055	22828.117	.000
	Within Groups	.000	6	.000		
	Total	.276	11			

Homogeneous Subsets on specific gravity of solvent flavour extracts

Piper nigrum

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	hexane extract	2	.3210				
	water extract	2		.4365			
	acetone	2			.5775		
	aqueous 40% ethanol	2				.6275	
	methanol	2					.7650
	absolute ethanol	2					.7850
	Sig.		1.000	1.000	1.000	1.000	.330

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Aframomum danielli

	dependent factor	**N**	**Subset for alpha = 0.05**					
			f	**e**	**d**	**c**	**b**	**a**
Tukey HSD[a]	hexane extract	2	.2290					
	water extract	2		.3210				
	acetone	2			.4930			
	aqueous 40% ethanol	2				.5710		
	absolute ethanol	2					.6130	
	methanol	2						.6335
	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.

Analysis of variance on list of common PFPs in *Piper nigrum* extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cyclohexane1etheny1methyl24bismethyl	Between Groups	41.307	5	8.261	58315.541	.000
	Within Groups	.001	6	.000		
	Total	41.308	11			
cisbetafamescene	Between Groups	91.481	5	18.296	206347.038	.000
	Within Groups	.001	6	.000		
	Total	91.481	11			
humulene	Between Groups	12.501	5	2.500	16667.556	.000
	Within Groups	.001	6	.000		
	Total	12.502	11			
betabisabolene	Between Groups	100.866	5	20.173	60519.540	.000
	Within Groups	.002	6	.000		
	Total	100.868	11			
hexadecanoicacid	Between Groups	21.844	5	4.369	58250.244	.000
	Within Groups	.000	6	.000		
	Total	21.844	11			
piperine	Between Groups	22413.965	5	4482.793	.914	.529
	Within Groups	29420.103	6	4903.350		
	Total	51834.067	11			

Homogeneous Subsets list of common PFPs in *Piper nigrum* extracts

cyclohexane1etheny1methyl24bismethyl

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	methanol	2	.0000				
	water extract	2	.0000				
	acetone	2		2.9450			
Tukey HSD[a]	absolute ethanol	2			4.0100		
	hexane extract	2				4.1000	
	aqueous 40% ethanol	2					4.2700
	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.

Cis-betafamescene

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	acetone	2	.0000				
	water extract	2	.0000				
	methanol	2		1.0850			
Tukey HSD[a]	hexane extract	2			2.9900		
	absolute ethanol	2				6.3940	
	aqueous 40% ethanol	2					6.5650
	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.

humulene

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	methanol	2	.0000				
	water extract	2	.0000				
	acetone	2		1.1900			
Tukey HSD[a]	hexane extract	2			1.9850		
	absolute ethanol	2				2.0350	
	aqueous 40% ethanol	2					2.6600
	Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Beta-bisabolene

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	methanol	2	.0000				
	water extract	2	.0000				
	acetone	2		2.5100			
Tukey HSD[a]	hexane extract	2			3.5500		
	absolute ethanol	2				6.8300	
	aqueous 40% ethanol	2					7.
	Sig.		1.000	1.000	1.000	1.000	1

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Hexadecanoic acid

	Dependent factor	N	Subset for alpha = 0.05	
			b	a
	methanol	2	.0000	
	acetone	2	.0000	
	water extract	2	.0000	
Tukey HSD[a]	aqueous 40% ethanol	2		2.6900
	hexane extract	2		2.6900
	absolute ethanol	2		2.7150
	Sig.		1.000	.166

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

piperine

	Dependent factor	**N**	**Subset for alpha = 0.05**
			a
	water extract	2	.0000
	acetone	2	5.0600
	methanol	2	6.3850
Tukey HSD[a]	aqueous 40% ethanol	2	15.4250
	absolute ethanol	2	17.5100
	hexane extract	2	123.7150
	Sig.		.542

Means for groups in homogeneous subsets are displayed.

Analysis of variance on PFPs in Aframomum danielli flavour extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	5859.514	5	1171.903	3800765.735	.000
eucalyptol	Within Groups	.002	6	.000		
	Total	5859.516	11			
	Between Groups	161.872	5	32.374	228525.518	.000
Alpha terpineol	Within Groups	.001	6	.000		
	Total	161.873	11			
	Between Groups	165.398	5	33.080	305349.369	.000
Hexadeonic acid	Within Groups	.001	6	.000		
	Total	165.398	11			
	Between Groups	603.109	5	120.622	14474625.000	.000
Oleic acid	Within Groups	.000	6	.000		
	Total	603.109	11			
	Between Groups	92.134	5	18.427	442245.000	.000
Beta-pinene	Within Groups	.000	6	.000		
	Total	92.135	11			
	Between Groups	28.989	5	5.798	49695.714	.000
Ethy liso-allocholate	Within Groups	.001	6	.000		
	Total	28.990	11			
	Between Groups	45.248	5	9.050	217191.240	.000
Diethyl phthalate	Within Groups	.000	6	.000		
	Total	45.248	11			

Homogeneous Subsets on PFPs in Aframomum danielli flavour extracts

eucalyptol

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	water extract	2	.0000					
	hexane extract	2		20.5450				
	acetone	2			22.2000			
Tukey HSD[a]	aqueous 40% ethanol	2				50.7200		
	absolute ethanol	2					53.7800	
	methanol	2						62.0600
	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.

Alpha terpineol

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	water extract	2	.0000					
	methanol	2		4.2400				
	absolute ethanol	2			4.9750			
Tukey HSD[a]	hexane extract	2				6.5400		
	acetone	2					7.1400	
	aqueous 40% ethanol	2						12.2700
	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.

Hexadonic acid

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	methanol	2	.0000			
	hexane extract	2	.0000			
	water extract	2	.0000			
Tukey HSD[a]	acetone	2		4.2700		
	absolute ethanol	2			7.3750	
	aqueous 40% ethanol	2				9.0100
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Oleic acid

	Dependent factor	N	Subset for alpha = 0.05		
			c	**b**	**a**
	methanol	2	.0000		
	acetone	2	.0000		
	hexane extract	2	.0000		
Tukey HSD[a]	water extract	2	.0000		
	aqueous 40% ethanol	2		13.4750	
	absolute ethanol	2			16.3900
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Beta-pinene

	Dependent factor	N	Subset for alpha = 0.05		
			c	**b**	**a**
	methanol	2	.0000		
	acetone	2	.0000		
	hexane extract	2	.0000		
Tukey HSD[a]	water extract	2	.0000		
	aqueous 40% ethanol	2		4.3750	
	absolute ethanol	2			6.9500
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Ethyl iso-allocholate

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	acetone	2	.0000			
	hexane extract	2	.0000			
	water extract	2	.0000			
Tukey HSD[a]	methanol	2		1.6650		
	aqueous 40% ethanol	2			2.6550	
	absolute ethanol	2				4.0400
	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.

Diethyl phthalate

	Dependent factor	N	Subset for alpha = 0.05		
			c	b	a
Tukey HSD[a]	methanol	2	.0000		
	acetone	2	.0000		
	hexane extract	2	.0000		
	water extract	2	.0000		
	aqueous 40% ethanol	2		4.0200	
	absolute ethanol	2			4.2150
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Analysis of variance on Alkaloids of solvents flavour extracts *Piper nigrum* and *Aframomum danielli*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum extract	Between Groups	43.472	5	8.694	86944.533	.000
	Within Groups	.001	6	.000		
	Total	43.473	11			
residue	Between Groups	2.147	5	.429	4683.800	.000
	Within Groups	.001	6	.000		
	Total	2.147	11			
total	Between Groups	39.427	5	7.885	49802.958	.000
	Within Groups	.001	6	.000		
	Total	39.428	11			
Aframomum danielli extract	Between Groups	25.645	5	5.129	51289.133	.000
	Within Groups	.001	6	.000		
	Total	25.645	11			
Aframomum danielli residue	Between Groups	6.944	5	1.389	11111.160	.000
	Within Groups	.001	6	.000		
	Total	6.945	11			
Aframomum danielli total	Between Groups	41.840	5	8.368	66943.480	.000
	Within Groups	.001	6	.000		
	Total	41.840	11			

Homogeneous Subsets on Alkaloids of solvents flavour extracts from *Piper nigrum* and *Aframomum danielli*

Piper nigrum **extract**

	Dependent factor	N	Subset for alpha = 0.05				
			e	**d**	**c**	**b**	**a**
	hexane extract	2	1.0500				
	acetone	2		1.2450			
	methanol	2			2.0850		
Tukey HSD[a]	water extract	2			2.1150		
	aqueous 40% ethanol	2				3.9200	
	absolute ethanol	2					6.5450
	Sig.		1.000	1.000	.147	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
	absolute ethanol	2	.9800					
	acetone	2		1.2400				
	hexane extract	2			1.4250			
Tukey HSD[a]	methanol	2				1.4950		
	aqueous 40% ethanol	2					1.9000	
	water extract	2						2.2650
	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

	Dependent factor	**N**	**Subset for alpha = 0.05**				
			1	**2**	**3**	**4**	**5**
	acetone	2	2.4750				
	hexane extract	2	2.4750				
	methanol	2		3.5800			
Tukey HSD[a]	water extract	2			4.3800		
	aqueous 40% ethanol	2				5.7900	
	absolute ethanol	2					7.5150
	Sig.		1.000	1.000	1.000	1.000	1.000

***Aframomum danielli* extract**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	acetone	2	2.8350					
	hexane extract	2		2.9850				
	water extract	2			3.1050			
Tukey HSD[a]	methanol	2				3.7300		
	aqueous 40% ethanol	2					4.1250	
	absolute ethanol	2						7.0900
	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.

***Aframomum danielli* residue**

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	methanol	2	.3850				
	acetone	2		.6750			
	hexane extract	2			1.2600		
Tukey HSD[a]	water extract	2				1.8000	
	absolute ethanol	2				1.8250	
	aqueous 40% ethanol	2					2.6500
	Sig.		1.000	1.000	1.000	.341	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

***Aframomum danielli* total**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	acetone	2	3.5100					
	methanol	2		4.1000				
	hexane extract	2			4.2250			
Tukey HSD[a]	water extract	2				4.8950		
	aqueous 40% ethanol	2					6.7700	
	absolute ethanol	2						8.8850
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Analysis of variance on Flavonoid content spice flavour extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum extract	Between Groups	18.628	5	3.726	12418.844	.000
	Within Groups	.002	6	.000		
	Total	18.630	11			
residue	Between Groups	10.359	5	2.072	6375.062	.000
	Within Groups	.002	6	.000		
	Total	10.361	11			
total	Between Groups	35.132	5	7.026	44376.684	.000
	Within Groups	.001	6	.000		
	Total	35.132	11			
Aframomum danielli extract	Between Groups	21.784	5	4.357	24895.819	.000
	Within Groups	.001	6	.000		
	Total	21.785	11			
Aframomum danielli residue	Between Groups	9.196	5	1.839	20064.309	.000
	Within Groups	.001	6	.000		
	Total	9.197	11			
Aframomum danielli total	Between Groups	14.915	5	2.983	12784.086	.000
	Within Groups	.001	6	.000		
	Total	14.916	11			

Homogeneous Subsets on Flavonoid content spice flavour extracts

Piper nigrum **extract**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	hexane extract	2	2.0600					
	water extract	2		4.0300				
	methanol	2			4.9100			
Tukey HSD[a]	acetone	2				5.2000		
	aqueous 40% ethanol	2					5.5700	
	absolute ethanol	2						5.6700
	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	2	.0750				
	methanol	2		.4150			
	water extract	2		.4800			
Tukey HSD[a]	hexane extract	2			1.3500		
	aqueous 40% ethanol	2				2.3000	
	absolute ethanol	2					2.4450
	Sig.		1.000	.075	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

total

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	hexane extract	2	3.3800					
	water extract	2		4.4900				
	acetone	2			5.2650			
Tukey HSD[a]	methanol	2				5.3250		
	aqueous 40% ethanol	2					7.8700	
	absolute ethanol	2						8.0850
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

***Aframomum danielli* extract**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	water extract	2	2.4200					
	acetone	2		3.2100				
	methanol	2			3.2800			
	hexane extract	2				3.9200		
	aqueous 40% ethanol	2					5.1050	
	absolute ethanol	2						6.460
	Sig.		1.000	1.000	1.000	1.000	1.000	1.0

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

***Aframomum danielli* residue**

	Dependent factor	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	aqueous 40% ethanol	2	.0900					
	acetone	2		1.5400				
	absolute ethanol	2			1.7150			
	water extract	2				2.1000		
	methanol	2					2.5850	
	hexane extract	2						2.7450
	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.

***Aframomum danielli* total**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	acetone	2	4.7500					
	aqueous 40% ethanol	2		5.1950				
	methanol	2			5.8450			
	water extract	2				6.5200		
	hexane extract	2					6.6650	
	absolute ethanol	2						8.1750
	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.

Analysis of variance on Flavonoid content of spice flavour extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum flavour extract	Between Groups	14.270	5	2.854	21404.250	.000
	Within Groups	.001	6	.000		
	Total	14.270	11			
spice residue from *Piper nigrum*	Between Groups	5.971	5	1.194	4342.430	.000
	Within Groups	.002	6	.000		
	Total	5.972	11			
total *Piper nigrum*	Between Groups	23.452	5	4.690	20101.629	.000
	Within Groups	.001	6	.000		
	Total	23.453	11			
Aframomum danielli flavour extract	Between Groups	4.558	5	.912	9116.133	.000
	Within Groups	.001	6	.000		
	Total	4.559	11			
spice residue *Aframomum danielli*	Between Groups	5.969	5	1.194	7539.632	.000
	Within Groups	.001	6	.000		
	Total	5.970	11			
total *Aframomum danielli*	Between Groups	.937	5	.187	1873.600	.000
	Within Groups	.001	6	.000		
	Total	.937	11			

Homogeneous Subsets on Flavonoid content of spice flavour extracts

Piper nigrum **flavour extract**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	acetone extract	2	4.2100					
	hexane extract	2		4.8500				
	water aqueous flavour extract	2			5.5900			
	methanol extract	2				5.8100		
	aqueous 40% ethanol extract	2					5.9200	
	ethanol extracts	2						7.7300
	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.

spice residue from *Piper nigrum*

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	hexane extract	2	1.1350					
	methanol extract	2		1.2150				
	acetone extract	2			1.3750			
	ethanol extracts	2				1.5700		
	water aqueous flavour extract	2					2.4650	
	aqueous 40% ethanol extract	2						3.0350
	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 *Piper nigrum*

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	acetone extract	2	5.5750					
	hexane extract	2		5.9850				
	methanol extract	2			7.0350			
	water aqueous flavour extract	2				8.0500		
	aqueous 40% ethanol extract	2					8.9100	
	ethanol extracts	2						9.2950
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

***Aframomum danielli* flavour extract**

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	hexane extract	2	2.2550				
	acetone extract	2		2.5050			
	methanol extract	2			2.8400		
Tukey HSD[a]	water aqueous flavour extract	2				3.1050	
	aqueous 40% ethanol extract	2				3.1200	
	ethanol extracts	2					4.1950
	Sig.		1.000	1.000	1.000	.677	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

spice residue *Aframomum danielli*

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	ethanol extracts	2	1.0400					
	aqueous 40% ethanol extract	2		1.4300				
	water aqueous flavour extract	2			1.8450			
Tukey HSD[a]	methanol extract	2				2.0000		
	acetone extract	2					2.8650	
	hexane extract	2						2.9850
	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 *Aframomum danielli*

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	aqueous 40% ethanol extract	2	4.5400				
	methanol extract	2		4.8300			
	water aqueous flavour extract	2			4.9450		
Tukey HSD[a]	ethanol extracts	2				5.2250	
	hexane extract	2				5.2250	
	acetone extract	2					5.3550
	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.

Analysis of variance on Saponin content of spice flavour extracts

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum flavour extract	Between Groups	.759	5	.152	1655.436	.000
	Within Groups	.001	6	.000		
	Total	.759	11			
spice residue from *Piper nigrum*	Between Groups	.178	5	.036	608.714	.000
	Within Groups	.000	6	.000		
	Total	.178	11			
total black pepper	Between Groups	.668	5	.134	5345.133	.000
	Within Groups	.000	6	.000		
	Total	.668	11			
Aframomum danielli flavour extract	Between Groups	1.208	5	.242	9666.200	.000
	Within Groups	.000	6	.000		
	Total	1.208	11			
spice residue *Aframomum danielli*	Between Groups	1.165	5	.233	2329.333	.000
	Within Groups	.001	6	.000		
	Total	1.165	11			
total *Aframomum danielli*	Between Groups	3.522	5	.704	10564.700	.000
	Within Groups	.000	6	.000		
	Total	3.522	11			

Homogeneous Subsets on saponin content of spice flavour extracts

Piper nigrum **flavour extract**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	acetone extract	2	.0600					
	hexane extract	2		.1050				
	methanol extract	2			.4100			
Tukey HSD[a]	water aqueous flavour extract	2				.5250		
	aqueous 40% ethanol extract	2					.6500	
	ethanol extracts	2						.7150
	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.

spice residue from *Piper nigrum*

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	hexane extract	2	.1450			
	methanol extract	2		.1800		
	aqueous 40% ethanol extract	2		.2000		
Tukey HSD[a]	ethanol extracts	2			.2600	
	water aqueous flavour extract	2			.2850	
	acetone extract	2				.5150
	Sig.		1.000	.225	.108	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

total *Piper nigrum*

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	hexane extract	2	.2400				
	acetone extract	2		.5700			
	methanol extract	2		.5850			
Tukey HSD[a]	water aqueous flavour extract	2			.7900		
	aqueous 40% ethanol extract	2				.8450	
	ethanol extracts	2					.9650
	Sig.		1.000	.147	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Aframomum danielli flavour extract

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	hexane extract	2	.0700			
	water aqueous flavour extract	2		.1350		
	acetone extract	2		.1450		
Tukey HSD[a]	methanol extract	2			.3050	
	ethanol extracts	2				.8200
	aqueous 40% ethanol extract	2				.8200
	Sig.		1.000	.434	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

spice residue _Aframomum danielli_

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	acetone extract	2	1.2800					
	hexane extract	2		1.3500				
	ethanol extracts	2			1.4850			
Tukey HSD[a]	water aqueous flavour extract	2				1.7650		
	methanol extract	2					1.9150	
	aqueous 40% ethanol extract	2						2.1450
	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 _Aframomum danielli_

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	hexane extract	2					
	acetone extract	2	1.4050				
	water aqueous flavour extract	2		1.8500			
Tukey HSD[a]	methanol extract	2			2.2150		
	ethanol extracts	2				2.2950	
	aqueous 40% ethanol extract	2					2.9450
	Sig.		.986	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Analysis of variance on tannin content of spice flavour extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum flavour extract	Between Groups	.796	5	.159	1363.886	.000
	Within Groups	.001	6	.000		
	Total	.796	11			
spice residue from *Piper nigrum*	Between Groups	.002	5	.000	36.438	.000
	Within Groups	.000	6	.000		
	Total	.002	11			
total *Piper nigrum*	Between Groups	.796	5	.159	6894.900	.000
	Within Groups	.000	6	.000		
	Total	.796	11			
Aframomum danielli flavour extract	Between Groups	.175	5	.035	13531.000	.000
	Within Groups	.000	6	.000		
	Total	.175	11			
spice residue *Aframomum danielli*	Between Groups	.119	5	.024	1336.269	.000
	Within Groups	.000	6	.000		
	Total	.119	11			
total *Aframomum danielli*	Between Groups	.288	5	.058	19748.349	.000
	Within Groups	.000	6	.000		
	Total	.288	11			

Homogeneous Subsets on tannin content of spice flavour extracts

Piper nigrum **flavour extract**

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	hexane extract	2	.4950				
	acetone extract	2		.6900			
	methanol extract	2			.7700		
Tukey HSD[a]	water aqueous flavour extract	2			.7700		
	aqueous 40% ethanol extract	2				.8250	
	ethanol extracts	2					1.3400
	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.

spice residue from black pepper

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	hexane extract	2	.1100			
	ethanol extracts	2		.1250		
	aqueous 40% ethanol extract	2		.1300	.1300	
Tukey HSD[a]	methanol extract	2			.1420	.1420
	acetone extract	2			.1420	.1420
	water aqueous flavour extract	2				.1520
	Sig.		1.000	.716	.092	.174

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

total black pepper

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	hexane extract	2	.5920					
	acetone extract	2		.8350				
	methanol extract	2			.8900			
Tukey HSD[a]	water aqueous flavour extract	2				.9150		
	aqueous 40% ethanol extract	2					.9430	
	ethanol extracts	2						1.4525
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

***Aframomum danielli* flavour extract**

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	acetone extract	2	.1415				
	methanol extract	2		.2200			
	water aqueous flavour extract	2		.2200			
Tukey HSD[a]	hexane extract	2			.2315		
	aqueous 40% ethanol extract	2				.4410	
	ethanol extracts	2					.4615
	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.

spice residue *Aframomum danielli*

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	water aqueous flavour extract	2	.0710					
	hexane extract	2		.0900				
	methanol extract	2			.1120			
Tukey HSD[a]	ethanol extracts	2				.1420		
	aqueous 40% ethanol extract	2					.2915	
	acetone extract	2						.3265
	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 *Aframomum danielli*

	Dependent factor	N	Subset for alpha = 0.05					
			g	e	d	c	b	a
	hexane extract	2	.3005					
	methanol extract	2		.3315				
	acetone extract	2			.4510			
Tukey HSD[a]	water aqueous flavour extract	2				.5505		
	ethanol extracts	2					.6110	
	aqueous 40% ethanol extract	2						.7420
	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.

Analysis of variance on oxalate content of spice flavour extracts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Piper nigrum flavour extract	Between Groups	6.311	5	1.262	16829.978	.000
	Within Groups	.000	6	.000		
	Total	6.312	11			
spice residue from *Piper nigrum*	Between Groups	.989	5	.198	2967.800	.000
	Within Groups	.000	6	.000		
	Total	.990	11			
total black pepper	Between Groups	7.333	5	1.467	21998.400	.000
	Within Groups	.000	6	.000		
	Total	7.333	11			
Aframomum danielli flavour extract	Between Groups	.946	5	.189	3202.664	.000
	Within Groups	.000	6	.000		
	Total	.946	11			
spice residue *Aframomum danielli*	Between Groups	.171	5	.034	7609.156	.000
	Within Groups	.000	6	.000		
	Total	.171	11			
total *Aframomum danielli*	Between Groups	1.038	5	.208	17060.247	.000
	Within Groups	.000	6	.000		
	Total	1.038	11			

Homogeneous Subsets on oxalate content of spice flavour extracts

Piper nigrum **flavour extract**

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	hexane extract	2	.7150					
	methanol extract	2		.9850				
	acetone extract	2			1.1800			
Tukey HSD[a]	water aqueous flavour extract	2				2.1550		
	aqueous 40% ethanol extract	2					2.4350	
	ethanol extracts	2						2.5150
	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.

spice residue from *Piper nigrum*

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	methanol extract	2	.3600					
	water aqueous flavour extract	2		.4350				
	aqueous 40% ethanol extract	2			.5550			
Tukey HSD[a]	acetone extract	2				.6250		
	hexane extract	2					.9600	
	ethanol extracts	2						1.1650
	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 *Piper nigrum*

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	methanol extract	2	1.3350					
	hexane extract	2		1.6750				
	acetone extract	2			1.7900			
Tukey HSD[a]	water aqueous flavour extract	2				2.5650		
	aqueous 40% ethanol extract	2					2.6350	
	ethanol extracts	2						3.6800
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

***Aframomum danielli* flavour extract**

	Dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	acetone extract	2	.1700			
	hexane extract	2	.1850			
	methanol extract	2		.2650		
Tukey HSD[a]	water aqueous flavour extract	2		.2715		
	aqueous 40% ethanol extract	2			.3700	
	ethanol extracts	2				.9850
	Sig.		.456	.947	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

spice residue *Aframomum danielli*

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	hexane extract	2	.0900				
	ethanol extracts	2		.2615			
	water aqueous flavour extract	2			.3120		
Tukey HSD[a]	methanol extract	2				.3220	
	aqueous 40% ethanol extract	2					.4415
	acetone extract	2					.4420
	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.

total *Aframomum danielli*

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	hexane extract	2	.2715				
	water aqueous flavour extract	2		.5810			
	methanol extract	2		.5850			
Tukey HSD[a]	acetone extract	2			.6105		
	aqueous 40% ethanol extract	2				.7920	
	ethanol extracts	2					1.2420
	Sig.		1.000	.846	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Analysis of variance on piperine content of *Piper nigrum* flavour products

ANOVA

piperine

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	36.926	5	7.385	2014162.855	.000
Within Groups	.000	6	.000		
Total	36.926	11			

Homogeneous Subsets on piperine content of *Piper nigrum* flavour products

piperine

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	BPPEPC2	2	12.1105					
	BPPEPC1	2		13.1100				
	BPPERC2	2			13.6915			
	BPPERC1	2				13.8720		
	BPP-EB	2					15.4215	
	BPP-PB control	2						17.5315
	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.

Analysis of variance on list of common MFPs in Aframomum danielli flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
eucalyptol	Between Groups	3381.262	5	676.252	19321496.192	.000
	Within Groups	.000	6	.000		
	Total	3381.262	11			
alpha-terpineol	Between Groups	27.093	5	5.419	186850.853	.000
	Within Groups	.000	6	.000		
	Total	27.094	11			
oleic acid	Between Groups	345.856	5	69.171	18864873.036	.000
	Within Groups	.000	6	.000		
	Total	345.856	11			
hexadonic acid	Between Groups	90.573	5	18.115	400320.519	.000
	Within Groups	.000	6	.000		
	Total	90.573	11			
beta-pinene	Between Groups	36.170	5	7.234	768215.414	.000
	Within Groups	.000	6	.000		
	Total	36.170	11			
ethy iso-allocholate	Between Groups	4.050	5	.810	18168.856	.000
	Within Groups	.000	6	.000		
	Total	4.050	11			
diethy phthalate	Between Groups	51.822	5	10.364	226957.111	.000
	Within Groups	.000	6	.000		
	Total	51.822	11			

Homogeneous Subsets on list of common MFPs in *Aframomum danielli* flavour extracts

eucalyptol

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFDEPC2	2	20.1510					
	AFDERC2	2		20.3350				
	AFDEPC1	2			27.6550			
	AFDERC1	2				28.7650		
	AFD-EB	2					50.7050	
	AFD-PB control	2						65.2820
	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.

alpha-terpineol

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFDEPC2	2	2.5550					
	AFDERC2	2		2.8250				
	AFDEPC1	2			3.0650			
	AFDERC1	2				3.3920		
	AFD-EB	2					4.7920	
	AFD-PB control	2						6.8720
	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.

oleic acid

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFDERC2	2	2.0615					
	AFDEPC2	2		2.1815				
	AFDEPC1	2			4.0000			
	AFDERC1	2				4.1105		
	AFD-EB	2					10.3315	
	AFD-PB control	2						16.8920
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

hexadonic acid

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	AFDEPC2	2	1.1815					
	AFDERC2	2		1.3315				
	AFDEPC1	2			2.6315			
Tukey HSD[a]	AFDERC1	2				2.8920		
	AFD-EB	2					5.5350	
	AFD-PB control	2						9.0100
	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.

beta-pinene

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	AFDEPC2	2	1.9820					
	AFDERC2	2		2.0610				
	AFDEPC1	2			2.5820			
Tukey HSD[a]	AFDERC1	2				2.6610		
	AFD-EB	2					4.3740	
	AFD-PB control	2						6.8815
	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.

ethy iso-allocholate

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	AFDEPC2	2	1.4050				
	AFDERC2	2	1.4300				
	AFDERC1	2		1.8315			
Tukey HSD[a]	AFDEPC1	2			2.0105		
	AFD-EB	2				2.6515	
	AFD-PB control	2					2.9520
	Sig.		.064	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

diethy phthalate

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFDERC2	2	1.8420					
	AFDEPC2	2		2.0100				
	AFDERC1	2			2.5620			
	AFDEPC1	2				2.6250		
	AFD-EB	2					4.0200	
	AFD-PB control	2						7.8620
	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.

Analysis of variance on piperine content and retention in black pepper products under 6months storage

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PP-EP	Between Groups	7.022	8	.878	24949.901	.000
	Within Groups	.000	9	.000		
	Total	7.022	17			
PP-EB	Between Groups	2.711	8	.339	17581.678	.000
	Within Groups	.000	9	.000		
	Total	2.712	17			
PP-ERC1	Between Groups	18.029	8	2.254	587899.080	.000
	Within Groups	.000	9	.000		
	Total	18.029	17			
PP-ERC2	Between Groups	35.689	8	4.461	872827.723	.000
	Within Groups	.000	9	.000		
	Total	35.689	17			
PP-EPC1	Between Groups	47.766	8	5.971	1919179.330	.000
	Within Groups	.000	9	.000		
	Total	47.766	17			
PP-EPC2	Between Groups	51.127	8	6.391	2212239.192	.000
	Within Groups	.000	9	.000		
	Total	51.127	17			

Homogeneous Subsets on piperine content and retention in *Piper nigrum* products under 6months storage

PP-EP

	Dependent factor	N	Subset for alpha = 0.05						
			g	f	e	d	c	b	a
Tukey HSD[a]	piperine at 6months	2	15.725						
	piperine at 5months	2		16.0106					
	piperine at 4months	2			16.5820				
	piperine at 2months	2				17.0000			
	piperine at 3months	2				17.0000			
	piperine at 6weeks	2					17.0950		
	piperine at 4weeks	2						17.5100	
	piperine at zero hour	2						17.5320	17.5320
	piperine at 2weeks	2							17.5400
	Sig.		1.000	1.000	1.000	1.000	1.000	.070	.892

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PP-EB

	Dependent factor	N	Subset for alpha = 0.05							
			h	g	f	e	d	c	b	a
Tukey HSD[a]	piperine at 6months	2	14.0850							
	piperine at 5months	2		14.8115						
	piperine at 4months	2			14.8615					
	piperine at 2months	2				15.0950				
	piperine at 3months	2				15.0950				
	piperine at 6weeks	2					15.1815			
	piperine at 4weeks	2						15.3100		
	piperine at 2weeks	2							15.3810	
	piperine at zero hour	2								15.4220
	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.

PP-ERC1

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	piperine at 6months	2	10.9615								
	piperine at 5months	2		11.7615							
	piperine at 4months	2			12.0110						
	piperine at 3months	2				12.0800					
	piperine at 2months	2					12.6910				
	piperine at 6weeks	2						13.2815			
	piperine at 4weeks	2							13.7920		
	piperine at 2weeks	2								13.8615	
	piperine at zero hour	2									13.8715
	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.

PP-ERC2

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	piperine at 6months	2	9.1615								
	piperine at 5months	2		10.5820							
	piperine at 4months	2			11.0620						
	piperine at 3months	2				11.1420					
	piperine at 2months	2					11.8215				
	piperine at 6weeks	2						12.7610			
	piperine at 4weeks	2							13.1210		
	piperine at 2weeks	2								13.2815	
	piperine at zero hour	2									13.6915
	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.

PP-EPC1

	Dependent factor	N	Subset for alpha = 0.05								
			i	**h**	**g**	**f**	**e**	**d**	**c**	**b**	**a**
Tukey HSD[a]	piperine at 6months	2	7.5805								
	piperine at 5months	2		9.8400							
	piperine at 4months	2			10.7000						
	piperine at 3months	2				10.771					
	piperine at 2months	2					11.0620				
	piperine at 6weeks	2						11.4215			
	piperine at 4weeks	2							12.562		
	piperine at 2weeks	2								12.9315	
	piperine at zero hour	2									13.1110
	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.

PP-EPC2

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	piperine at 6months	2	7.0620								
	piperine at 5months	2		8.1800							
	piperine at 4months	2			8.4315						
	piperine at 3months	2				8.6610					
	piperine at 2months	2					10.2210				
	piperine at 6weeks	2						10.980			
	piperine at 4weeks	2							11.1815		
	piperine at 2weeks	2								11.6615	
	piperine at zero hour	2									12.1105
	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.

Analysis of variance on eucalyptol content and retention in *Aframomum danielli* products under 6months storage

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AFD-EP	Between Groups	269.965	8	33.746	6902504.824	.000
	Within Groups	.000	9	.000		
	Total	269.965	17			
AFDD-EB	Between Groups	299.691	8	37.461	700212.667	.000
	Within Groups	.000	9	.000		
	Total	299.692	17			
AFD-ERC1	Between Groups	95.441	8	11.930	1350584.143	.000
	Within Groups	.000	9	.000		
	Total	95.441	17			
AFD-ERC2	Between Groups	137.397	8	17.175	7189402.331	.000
	Within Groups	.000	9	.000		
	Total	137.397	17			
AFD-EPC1	Between Groups	212.413	8	26.552	3188536.887	.000
	Within Groups	.000	9	.000		
	Total	212.413	17			
AFD-EPC2	Between Groups	193.487	8	24.186	7034189.002	.000
	Within Groups	.000	9	.000		
	Total	193.487	17			

Homogeneous Subsets on eucalyptol content and retention in *Aframomum danielli* products under 6months storage

AFD-EP

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	eucalyptol at 6months	2	54.4815								
	eucalyptol at 5months	2		55.8200							
	eucalyptol at 4months	2			56.0615						
	eucalyptol at 3months	2				59.1615					
	eucalyptol at 2months	2					60.262				
	eucalyptol at 6weeks	2						62.0815			
	eucalyptol at 4weeks	2							63.4420		
	eucalyptol at 2weeks	2								65.2020	
	eucalyptol at zero hour	2									65.2810
	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.

AFDD-EB

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	eucalyptol at 6months	2	39.1920								
	eucalyptol at 5months	2		40.0000							
	eucalyptol at 4months	2			41.0915						
	eucalyptol at 3months	2				44.5615					
	eucalyptol at 2months	2					45.2210				
	eucalyptol at 6weeks	2						48.015			
	eucalyptol at 4weeks	2							48.0620		
	eucalyptol at 2weeks	2								50.0000	
	eucalyptol at zero hour	2									50.7015
	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.

AFD-ERC1

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
	eucalyptol at 6months	2	22.060								
	eucalyptol at 5months	2		22.9620							
	eucalyptol at 4months	2			24.0150						
	eucalyptol at 3months	2				24.5510					
Tukey	eucalyptol at 2months	2					25.8615				
HSD[a]	eucalyptol at 6weeks	2						27.5200			
	eucalyptol at 4weeks	2							27.8320		
	eucalyptol at 2weeks	2								28.0615	
	eucalyptol at zero hour	2									28.7610
	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.

AFD-ERC2

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
	eucalyptol at 6months	2	12.0610								
	eucalyptol at 5months	2		14.2800							
	eucalyptol at 4months	2			15.0000						
	eucalyptol at 3months	2				16.9805					
Tukey	eucalyptol at 2months	2					18.0810				
HSD[a]	eucalyptol at 6weeks	2						18.661			
	eucalyptol at 4weeks	2							20.0000		
	eucalyptol at 2weeks	2								20.0815	
	eucalyptol at zero hour	2									20.3420
	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.

AFD-EPC1

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	eucalyptol at 6months	2	18.0817								
	eucalyptol at 5months	2		19.3315							
	eucalyptol at 4months	2			20.9715						
	eucalyptol at 3months	2				22.8200					
	eucalyptol at 2months	2					23.6210				
	eucalyptol at 6weeks	2						26.1210			
	eucalyptol at 4weeks	2							26.9815		
	eucalyptol at 2weeks	2								27.5810	
	eucalyptol at zero hour	2									27.6550
	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.

AFD-EPC2

	Dependent factor	N	Subset for alpha = 0.05								
			i	h	g	f	e	d	c	b	a
Tukey HSD[a]	eucalyptol at 6months	2	11.442								
	eucalyptol at 5months	2		12.0000							
	eucalyptol at 4months	2			13.6015						
	eucalyptol at 3months	2				16.4315					
	eucalyptol at 2months	2					18.2815				
	eucalyptol at 6weeks	2						19.0000			
	eucalyptol at 4weeks	2							19.3910		
	eucalyptol at 2weeks	2								19.9217	
	eucalyptol at zero hour	2									20.1530
	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.

Analysis on variance on descriptive test on sensory attributes of *Piper nigrum* products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
color	Between Groups	12.846	5	2.569	716977.716	.000
	Within Groups	.000	6	.000		
	Total	12.846	11			
flavour pungency	Between Groups	9.742	5	1.948	51501.387	.000
	Within Groups	.000	6	.000		
	Total	9.743	11			
sweet-flavour	Between Groups	2.100	5	.420	180036.171	.000
	Within Groups	.000	6	.000		
	Total	2.100	11			
minty-flavour	Between Groups	2.437	5	.487	129970.689	.000
	Within Groups	.000	6	.000		
	Total	2.437	11			
sweet-taste	Between Groups	1.307	5	.261	89623.366	.000
	Within Groups	.000	6	.000		
	Total	1.307	11			
bitter-taste	Between Groups	20.424	5	4.085	4392213.294	.000
	Within Groups	.000	6	.000		
	Total	20.424	11			
harsh-in-taste	Between Groups	4.710	5	.942	513791.891	.000
	Within Groups	.000	6	.000		
	Total	4.710	11			
hot-in-taste	Between Groups	29.102	5	5.820	1317847.506	.000
	Within Groups	.000	6	.000		
	Total	29.102	11			
after-taste	Between Groups	.805	5	.161	64395.440	.000
	Within Groups	.000	6	.000		
	Total	.805	11			
overall acceptability	Between Groups	2.566	5	.513	6523.298	.000
	Within Groups	.000	6	.000		
	Total	2.566	11			

Homogeneous Subsets on descriptive test on sensory attributes of *Piper nigrum* products

colour

	dependent factor	N	Subset for alpha = 0.05				
			e	**d**	**c**	**b**	**a**
	BPP-EPC2	2	3.7520				
	BPP-EPC1	2		4.0000			
	BPP-ERC2	2			4.3315		
Tukey HSD[a]	BPP-ERC1	2				4.5715	
	BPP-EB	2					6.2900
	BPP-PB	2					6.2915
	Sig.		1.000	1.000	1.000	1.000	.959

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

flavour pungency

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	BPP-ERC2	2	3.8620					
	BPP-EPC2	2		4.6310				
	BPP-EPC1	2			4.8615			
Tukey HSD[a]	BPP-ERC1	2				5.5100		
	BPP-EB	2					5.7115	
	BPP-PB	2						6.7120
	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.

sweet-flavour

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	BPP-ERC2	2	2.5010				
	BPP-EPC2	2		2.6015			
	BPP-ERC1	2			2.7105		
Tukey HSD[a]	BPP-EPC1	2			2.7105		
	BPP-EB	2				3.4315	
	BPP-PB	2					3.5710
	Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

minty-flavour

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	BPP-EPC2	2	1.4315					
	BPP-ERC2	2		1.8315				
	BPP-ERC1	2			2.0000			
Tukey HSD[a]	BPP-EPC1	2				2.1315		
	BPP-EB	2					2.4315	
	BPP-PB	2						2.8615
	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.

sweet-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	BPP-EPC1	2	1.5015					
	BPP-EPC2	2		1.5700				
	BPP-ERC1	2			1.7105			
Tukey HSD[a]	BPP-PB	2				2.0000		
	BPP-EB	2					2.2915	
	BPP-ERC2	2						2.3320
	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.

bitter-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	BPP-EPC1	2	1.6332					
	BPP-EPC2	2		1.7105				
	BPP-ERC2	2			2.0000			
Tukey HSD[a]	BPP-ERC1	2				2.7015		
	BPP-PB	2					4.2905	
	BPP-EB	2						5.0000
	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.

harsh-in-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	BPP-EPC1	2	.5000					
	BPP-EPC2	2		1.1400				
	BPP-ERC2	2			1.1710			
Tukey HSD[a]	BPP-ERC1	2				1.5715		
	BPP-EB	2					2.0000	
	BPP-PB	2						2.4315
	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.

hot-in-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	BPP-EPC2	2	1.4315					
	BPP-EPC1	2		1.6315				
	BPP-ERC2	2			1.8315			
Tukey HSD[a]	BPP-ERC1	2				3.1420		
	BPP-EB	2					4.7105	
	BPP-PB	2						5.4315
	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.

after-taste

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	BPP-EPC2	2	1.0000				
	BPP-EPC1	2		1.3810			
	BPP-ERC2	2			1.5015		
Tukey HSD[a]	BPP-ERC1	2				1.5710	
	BPP-EB	2				1.5715	
	BPP-PB	2					1.8610
	Sig.		1.000	1.000	1.000	.999	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

overall acceptability

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	BPP-PB	2	4.2915					
	BPP-EB	2		4.5715				
	BPP-ERC2	2			5.1705			
	BPP-EPC1	2				5.2650		
	BPP-ERC1	2					5.4320	
	BPP-EPC2	2						5.5715
	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.

Analysis of variance on consumers' preference test on sensory attributes of *Piper nigrum* flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	2.404	5	.481	134171.577	.000
	Within Groups	.000	6	.000		
	Total	2.404	11			
Flavour pungency	Between Groups	10.305	5	2.061	26034.477	.000
	Within Groups	.000	6	.000		
	Total	10.306	11			
aroma	Between Groups	8.390	5	1.678	529920.442	.000
	Within Groups	.000	6	.000		
	Total	8.390	11			
Overall acceptability	Between Groups	7.376	5	1.475	276611.650	.000
	Within Groups	.000	6	.000		
	Total	7.376	11			

Homogeneous Subsets on consumers' preference test on sensory attributes of *Piper nigrum* flavour products

colour

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	BPP-ERC2	2	5.0000				
	BPP-EPC1	2	5.0000				
	BPP-EPC2	2		5.4515			
	BPP-ERC1	2			5.6015		
	BPP-EB	2				5.9520	
	BPP-PB	2					6.2015
	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.

Flavour pungency

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	BPP-ERC2	2	4.3015				
	BPP-EPC2	2		4.6500			
	BPP-EPC1	2		4.6515			
	BPP-ERC1	2			5.5520		
	BPP-EB	2				6.3150	
	BPP-PB	2					6.8020
	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.

aroma

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	BPP-ERC2	2	4.4500				
	BPP-EPC1	2	4.4500				
	BPP-ERC1	2		5.3515			
	BPP-EPC2	2			5.4015		
	BPP-EB	2				6.1510	
	BPP-PB	2					6.7520
	Sig.		1.000	1.000	1.000	1.000	1.000

Overall acceptability

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	BPP-EPC2	2	4.4000					
	BPP-EPC1	2		4.9020				
	BPP-ERC2	2			5.1000			
	BPP-ERC1	2				5.8020		
	BPP-EB	2					6.1520	
	BPP-PB	2						6.7020
	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.

Analysis of variance on descriptive test on sensory attributes of *Aframomum danielli* flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	7.118	5	1.424	355888.133	.000
	Within Groups	.000	6	.000		
	Total	7.118	11			
flavour pungency	Between Groups	2.144	5	.429	14.195	.003
	Within Groups	.181	6	.030		
	Total	2.325	11			
sweet-flavour	Between Groups	.876	5	.175	8512.299	.000
	Within Groups	.000	6	.000		
	Total	.876	11			
minty-flavour	Between Groups	1.276	5	.255	135.863	.000
	Within Groups	.011	6	.002		
	Total	1.287	11			
sweet-taste	Between Groups	4.292	5	.858	396174.338	.000
	Within Groups	.000	6	.000		
	Total	4.292	11			
bitter-taste	Between Groups	5.022	5	1.004	1205346.160	.000
	Within Groups	.000	6	.000		
	Total	5.022	11			
harsh-in-taste	Between Groups	19.031	5	3.806	1038047.164	.000
	Within Groups	.000	6	.000		
	Total	19.031	11			
hotness-in-taste	Between Groups	6.912	5	1.382	691171.700	.000
	Within Groups	.000	6	.000		
	Total	6.912	11			
after-taste	Between Groups	9.026	5	1.805	1274324.671	.000
	Within Groups	.000	6	.000		
	Total	9.026	11			
overall acceptability	Between Groups	10.021	5	2.004	601283.380	.000
	Within Groups	.000	6	.000		
	Total	10.021	11			

Homogeneous Subsets on descriptive test on sensory attributes of *Aframomum danielli* flavour products

colour

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFD-ERC2	2	4.1420					
	AFD-ERC1	2		4.2905				
	AFD-EPC2	2			4.4210			
	AFD-EPC1	2				4.8615		
	AFD-PB	2					5.5715	
	AFD-EB	2						6.2915
	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.

flavour pungency

	dependent factor	N	Subset for alpha = 0.05		
			c	b	a
Tukey HSD[a]	AFD-EPC2	2	4.1420		
	AFD-EPC1	2	4.4210		
	AFD-ERC2	2	4.7100	4.7100	
	AFD-ERC1	2	4.7105	4.7105	
	AFD-EB	2		5.1425	5.1425
	AFD-PB	2			5.4110
	Sig.		.108	.259	.654

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

sweet-flavour

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
Tukey HSD[a]	AFD-EPC2	2	4.7115			
	AFD-ERC2	2		5.0000		
	AFD-PB	2		5.0050		
	AFD-EB	2		5.0055		
	AFD-EPC1	2			5.2920	
	AFD-ERC1	2				5.5715
	Sig.		1.000	.818	1.000	1.000

Means for groups in homogeneous subsets are displayed.

minty-flavour

	dependent factor	N	Subset for alpha = 0.05		
			c	b	a
	AFD-EPC2	2	4.7100		
	AFD-EPC1	2	4.7115		
	AFD-ERC2	2	4.8615		
Tukey HSD[a]	AFD-EB	2		5.1420	
	AFD-ERC1	2		5.1420	
	AFD-PB	2			5.6470
	Sig.		.084	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

sweet-taste

	dependent factor	N	Subset for alpha = 0.05			
			d	c	b	a
	AFD-EPC2	2	4.0000			
	AFD-PB	2		4.4315		
	AFD-EPC1	2		4.4315		
Tukey HSD[a]	AFD-EB	2		4.4320		
	AFD-ERC2	2			5.0000	
	AFD-ERC1	2				5.8620
	Sig.		1.000	.999	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

bitter-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	AFD-EPC2	2	1.4315					
	AFD-EPC1	2		1.7110				
	AFD-ERC2	2			1.8630			
Tukey HSD[a]	AFD-ERC1	2				2.0000		
	AFD-EB	2					3.0000	
	AFD-PB	2						3.1425
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

harsh-in-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	AFD-EPC2	2	1.4300					
	AFD-ERC2	2		1.5715				
	AFD-EPC1	2			1.8615			
Tukey HSD[a]	AFD-ERC1	2				2.4315		
	AFD-PB	2					2.8615	
	AFD-EB	2						5.1420
	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.

hot-in-taste

	dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
	AFD-EPC2	2	3.5705				
	AFD-ERC2	2	3.5710				
	AFD-EPC1	2		4.0000			
Tukey HSD[a]	AFD-ERC1	2			4.4315		
	AFD-EB	2				4.8615	
	AFD-PB	2					5.7105
	Sig.		.999	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

after-taste

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
	AFD-EPC2	2	3.5710					
	AFD-EPC1	2		4.0000				
	AFD-ERC2	2			5.0000			
Tukey HSD[a]	AFD-ERC1	2				5.1410		
	AFD-PB	2					5.8620	
	AFD-EB	2						5.8715
	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.

overall acceptability

	dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFD-ERC2	2	4.5715					
	AFD-EPC2	2		4.7105				
	AFD-EPC1	2			4.8620			
	AFD-ERC1	2				5.1415		
	AFD-EB	2					6.5720	
	AFD-PB	2						6.8615
	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.

Analysis of variance on consumers' preference test on sensory attributes of *Aframomum danielli* flavour products

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	4.393	5	.879	234306.227	.000
	Within Groups	.000	6	.000		
	Total	4.393	11			
flavour pungency	Between Groups	4.324	5	.865	225586.748	.000
	Within Groups	.000	6	.000		
	Total	4.324	11			
aroma	Between Groups	.834	5	.167	33908.695	.000
	Within Groups	.000	6	.000		
	Total	.834	11			
overall acceptability	Between Groups	6.030	5	1.206	237263.859	.000
	Within Groups	.000	6	.000		
	Total	6.030	11			

Homogeneous Subsets on consumers' preference test on sensory attributes of *Aframomum danielli* flavour products

colour

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFD-ERC2	2	4.3000					
	AFD-ERC1	2		4.9520				
	AFD-EPC2	2			5.0000			
	AFD-EPC1	2				5.3010		
	AFD-PB	2					5.4520	
	AFD-EB	2						6.3015
	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.

flavour pungency

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	AFD-EPC2	2	4.4020				
	AFD-ERC2	2		4.9035			
	AFD-ERC1	2			5.0000		
	AFD-EB	2				5.4015	
	AFD-EPC1	2				5.4020	
	AFD-PB	2					6.3520
	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.

aroma

	Dependent factor	N	Subset for alpha = 0.05					
			f	e	d	c	b	a
Tukey HSD[a]	AFD-EPC2	2	4.7515					
	AFD-ERC2	2		4.8020				
	AFD-ERC1	2			4.9020			
	AFD-EPC1	2				5.0000		
	AFD-EB	2					5.0515	
	AFD-PB	2						5.5515
	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.

overall acceptability

	Dependent factor	N	Subset for alpha = 0.05				
			e	d	c	b	a
Tukey HSD[a]	AFD-EPC2	2	4.4500				
	AFD-ERC2	2	4.4520				
	AFD-ERC1	2		4.8515			
	AFD-EB	2			5.4510		
	AFD-EPC1	2				6.1020	
	AFD-PB	2					6.1520
	Sig.		.937	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.