



**ESSENTIAL OIL COMPOSITION OF  
SELECTED GHANAIAN SPICES**

**BY**

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## 1.0 INTRODUCTION

Spices are substances of plant origin that are commonly used as condiments or other purposes on account of their fragrance. They may be obtained from the bark, fruits, leaves, flowers, rhizomes, roots, seeds, stigmas and styles of the plant.

The ability of this important group of agricultural commodities to contribute to the aroma, flavor, pungency, color and agreeable taste to foods has rendered them virtually indispensable in the culinary art. Due to their potency, it is easy to use these products in small quantities to impart the desired aroma or flavor to the food product.

Various volatile compounds are responsible for the aromatic and pungent properties of spices. These compounds are found in the spices' essential oils and oleoresins. While the characteristic aroma of spices is the direct consequence of the essential oil component, the oleoresin (non-volatile) component is responsible for the typical flavor and taste associated with spices (Pruthi, 1980; Merory, 1960).

As part of their work on the composition of foods commonly used in Ghana, Eyeson and Ankrah (1975) documented the proximate and some mineral composition of a few local spices. There is however, no documentation on the essential oil composition of spices commonly used in Ghana. The present study was therefore, undertaken to investigate the essential oils that may be associated with the characteristic aroma of four local spices widely used in Ghana, viz: Anise seed, Nutmeg, Garlic and Cloves.

The specific objectives of the study are:

- ◆ To determine the volatile compounds in the selected spices using the GC-MS and
- ◆ To tentatively document the compounds which contribute to the characteristic aroma of spices according to literature.

## 2.0 LITERATURE REVIEW

### 2.1 Spice Categories and their Functional Properties

The use of spices dates back to ancient times. Archaeological excavations have revealed that prehistoric man used leaves of certain plants to enhance the flavor of half-cooked food (Borget, 1993). When the queen of Sheba visited Jerusalem in Bible times to witness the fame and wisdom of King Solomon, she brought among other things spices in such profusion that there was never an occasion in the king's life that such amount of spices were brought as gifts. Spices come in various types and though they have diverse uses, they are more often used because of their functional properties to enhance the flavor, aroma and sometimes color of the food. Table 1 shows the various grouping of spices and their functional properties of.

Table 1: Spice Groups and their Functional Properties

Group Name	Example	Functional Properties
Barks	Cinnamon	Flavor
Buds	Cloves	Flavor and Aroma
Bulbs	Garlic, Onions	Flavor and Aroma
Fruits	Aidon Fruit, Ashanti Pepper, Cayenne Pepper	Flavor, Aroma and Color
Seeds	Anise Seed, Nutmeg	Flavor and Aroma
Rhizomes	Ginger and Tumeric	Flavor and Aroma
Herbs	Rosemary, Mint, Thyme	Flavor and Aroma

### 2.2 Essential Oils of Spices

Spices contain cellulose, proteins, lipids, starch and minerals. However, their most significant composition is their essential oil fraction which is not present in all vegetables and which confer on spices their specific characteristic flavour, aroma and smell (Borget, 1993).

Essential oils were regarded as commodities and became significant when distillation was discovered and then it became possible to separate the flavor chemical mixture from the botanical material of the spices (Wright, 1999). They are typically liquids and possess an aromatic fragrance owing to their volatilization upon contact with air (Schery, 1954). Oils extracted from spices are composed chemically of complex organic substances – a mixture of various aromatic hydrocarbons and their oxygenated derivatives. The aromatic hydrocarbons, belonging to a group of cyclic hydrocarbons with complex structures called terpenes, have the structural formula which are multiples of two, three or four of  $C_5H_8$  (an isoprene group). The monoterpenes have multiples of two, sesquiterpenes have multiples of three and diterpenes have multiples of four (Borget, 1993). In addition to terpenes, they may also contain derivatives of benzene and other straight-chained compounds which sometimes contain sulfur or nitrogen atoms (Schery, 1954). The terpenes have been given names which are generally suggestive of the plant from which they were first found, e.g. Pinene (present in the essential oils of pepper and nutmeg) and limonene (found in the essential oil of cardamom). Due to the presence of a pair of double bonds in its structure, the isoprene group  $[CH_2=CH - C(CH_3)=CH_2]$  can bond with itself and polymerize resulting in the presence of a mixture of terpenes in each spice (Borget, 1993).

### 2.2.1 *Nutmeg Oil*

The chemistry of nutmeg, particularly that of its essential oils, has been the subject of many investigations over a considerable number of years. Reports of analyses on nutmeg have been published since the early nineteenth century. (Baldry *et al*, 1976). Its major constituents have been found to be monoterpene hydrocarbons with little amounts of oxygenated hydrocarbons and aromatic ethers. The oil, on extraction, is a pale-yellow to almost water-white liquid with a fresh, warm, spicy and aromatic odor possessing a rich, sweet-spicy body note (Purseglove *et al*, 1981). The monoterpene hydrocarbon components include sabinene (light, peppery, herbaceous note), alpha-pinene (light, peppery note), gamma-terpene (light, citrus, herbaceous note) and limonene (weak, light citrus note). Purseglove *et al* (1981) found the following additional monoterpenes: camphene, delta-3-carene, alpha and beta-phellandrene, alpha-thujen, alpha-terpinene and terpinolene. The only oxygenated monoterpene found in the oil by Wright (1999) was 1,8-cineole which possesses a fresh, eucalyptous flavor note. Additional compounds in this category documented by Purseglove *et al* (1981) are camphor, methone, borneol, citronellol, geraniol, linalool cis-2-menth-2-en-ol, cis-piperitol, alpha and beta-terpineol, terpinen-4-ol, cis and tran-sabinene hydrate, bornyl acetate, geranyl acetate, linalyl acetate, menthyl acetate and terpinene acetate. The essential oil component that falls under the aromatic ether category include: safrole (warm, sweet sassafras note) and myristicin (warm, woody balsamic note) according to Wright, 1999. While the monoterpene hydrocarbon fraction has alpha and beta-pinene as well as sabinene as the predominant constituents, myristicin is the major constituents of the aromatic ether fraction.

### 2.2.2 *Clove Oil*

Clove oil can be derived from three sources, namely, the buds, leaves and stem. (Schery, 1954). A number of studies on the composition of the clove bud oil were

undertaken between the late nineteenth century and early twentieth century in which the identification of 18 components were reported. The major component of the oil is eugenol (Purseglove *et al.*, 1981; Wright, 1999) although its percentage composition differs with higher values being observed for oil distilled from whole spices in contrast to the comminuted spice. Apart from eugenol which has a strong, warm, clove aroma note, other components of the oil that contribute to the characteristic aroma have been identified as caryophyllene (spicy, woody note), alpha-humulene (woody note), and eugenyl acetate (warm, spicy note) Wright (1999).

### **2.2.3 Garlic Oil**

Garlic, a widely consumed spice, is known mainly for its flavor and odor. In recent times however, many medicinal properties have been attributed to it, including anticarcinogenic, antiatherosclerotic, and antithrombotic properties (Bonsu, 2002). The oil from garlic derives its aroma characteristics from sulfide compounds. According to Wright (1999), the major volatile compounds that contribute to the characteristic aroma of garlic are diallyl disulfide (strong, garlic note), diallyl trisulfide (strong, heavy, garlic note) and diallyl sulfide (strong, fresh garlic note). Similar findings were made by Calvo *et al.* (2004). They identified forty seven compounds in garlic oil with the most abundant being diallyl disulfide and diallyl trisulfide.

### **2.2.4 Anise Seed Oil**

Anise Seed oil was found to consist mainly of anethole, trans-anethole (strong, sweet anise note) and methyl chavicol (strong, sweet terragon note) Schery (1954) and Wright (1999).

### **2.3 Isolation of aroma compounds**

Analysis of food aromas using modern techniques such as gas chromatography and mass spectrometry has resulted in the isolation and identification of hundreds of volatile constituents (Meyer, 1960)

In order to be perceived, aroma compounds must leave the food matrix and travel through the air. This characteristic makes them ideal for analysis by gas chromatography, which involves the analysis of compounds that exist in the gaseous phase at typical gas chromatography operating temperatures of between 40 – 300°C (Wampler, 1997). Two most common procedures reported in literature for the isolation of aroma compounds are headspace and distillation/extraction methods (Parliment, 1997). Although it is preferable to take advantage of the volatility of these compounds and rely on techniques of headspace analysis, it is possible, and sometimes most appropriate, to isolate and then concentrate the compounds from the food matrix through solvent extraction and/or distillation.

Schery (1954) emphasized distillation as the most important process for obtaining essential oils from plant products. With the essential oil thus obtained, the GC-MS can be used to investigate their chemical composition and where possible, chemical synthesis of compounds can be affected. According to Parliment (1997) essential oils are best analysed by direct injection of the sample into the GC and in some cases the sample may have to be diluted with a solvent to obtain a response within the limits of the detector.

### **2.4 Solvent Extraction and Distillation Techniques**

#### **2.4.1 *Direct solvent extraction of aqueous samples***

When relatively large amounts of aqueous samples are available separatory funnels or commercial liquid-liquid extractors may be employed using diethyl ether, diethyl ether/pentane mixtures in order to obtain the extract for analysis. The technique is applied

to carbonated beverages, fruit juices and caffeinated beverages (Parliment, 1997). Extraction is followed by concentration of the solvent on a steam bath and drying it over sodium sulphate or magnesium sulphate.

### **2.4.2 Steam distillation followed by solvent extraction**

Steam distillation followed by solvent extraction has as its primary advantage, the separation of the volatiles through distillation, from the non-volatiles. It works best for compounds that are slightly volatile and water insoluble (Parliment, 1997). Steam distillation may involve direct, indirect or vacuum steam distillations. Indirect distillation is fast and eliminates scorching of samples. It therefore reduces the decomposition of samples since the samples are not directly heated. However, if sample decomposition remains a problem, steam distillation may be done under vacuum (Parliment, 1997). After condensation, the aroma compounds may be extracted by use of semi-micro extractors e.g. Mixer apparatus described by Parliment (1986) or by use of a suitable direct solvent method.

### **2.4.3 Simultaneous Steam Distillation/Extraction**

The simultaneous steam distillation/extraction (SDE) apparatus, first described by Likens and Nickerson (1986) is one valuable technique in flavor analysis. The apparatus comprises mainly a sample flask of 500 ml to 5 liter capacity, a pear shaped flask of 10-50 ml capacity and a central extracting U-tube fitted with a condenser. This ensures a simultaneous condensation of the steam distillate and an immiscible organic solvent, continuous recycling of both liquids and transfer of the steam distillable soluble compounds from the aqueous phase to the solvent (Parliment, 1997).

Variyar, *et al* (1997) used this technique to isolate the essential oils of fresh ginger rhizomes gamma irradiated (60 Gy) for sprout inhibition and non-irradiated rhizomes. Compounds present were identified by GC-MS and GLC.



### 2.5. Solid – Phase Microextraction (SPME)

Solid –phase Microextraction is one of the relatively new techniques used for the rapid, solventless extraction or pre-concentration of volatile and semi – volatile organic compounds (Harmon, 1997). It employs the partitioning of organic components between a bulk aqueous or vapour phase and the thin polymeric films coated onto fused silica fibres in the SPME apparatus. In their studies on the sensitivity of methods for the collection of aroma compounds, Peterson et al. (2000) found SPME to be the least sensitive method compared to dynamic headspace with thermal desorption and the Likens – Nickerson distillation extraction, both of which were found to be most sensitive.

The method is, however, advantageous because it requires no solvent, it is independent of the form of matrix (liquids, solids and gases can easily be sampled) and since heating is optional, the formation of artifacts is reduced and in some cases eliminated entirely (Harmon, 1997). Jirovetz *et al*, (2002) investigated the aroma compounds of the essential oils of dried fruits of black pepper (*Piper nigrum*) and black and white Ashanti pepper (*Piper guineense*) from Cameroon using the Solid State Micro Extraction (SPME) to identify the odorous target components responsible for the characteristic aroma of these valuable spices. Using GC-flame ionization detection (GC-FID) and GC-MS, the main compounds and corresponding peak areas of SPME headspace extracts of the above mentioned spices were found to be germacrene D (11.0%), limonene(10.26%),  $\beta$ -pinene (10.02%),  $\alpha$ -phellandrene(8.59%),  $\beta$ -caryophyllene(7.29%),  $\alpha$ -pinene(6.40%), and cis- $\beta$ -ocimene. Compounds in SPME extracts of *P. guineense* (black) were identified as  $\beta$ -caryophyllene (57.59%),  $\beta$ -elemene(5.10%), bicyclogermacrene (5.05%) and  $\alpha$ -humulene (4.86%) and finally, those constituting the essential oil of *P. guineense* (white) were  $\beta$ -caryophyllene (51.75%), cis- $\beta$ -ocimene (6.61%), Limonene (5.88%),  $\beta$ -pinene (4.56%), linalool (3.97%) and  $\alpha$ -humulene (3.29%).

## 2. Literature Review

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The problem with SPME as a method for aroma analysis is the difficulty in obtaining accurate quantitation during extraction. This is because the method is an equilibrium technique and each component behaves differently as the following factors are altered: polarity, volatility, organic/water partition coefficient, volume of the sample or volume of the headspace, the rate of agitation, the pH of the solution and temperature.

Excellent quantitative correlations are, however, usually obtained with the incorporation of an internal standard into the matrix and adhering to specific sampling times (Harmon, 1997).

### 3.0 MATERIALS AND METHODS

#### 3.1 **Materials**

Four local spices were bought from a local retailer in Accra. These were

- Anise seed (*Pimpinella anisum*)
- Nutmeg (*Mondora myristica*)
- Cloves (*Syzygium sp*)
- Garlic (*Allium sativum*)

They were thoroughly cleaned to remove dirt and crushed with a laboratory mortar and pestle. Extraction of volatile compounds was either conducted immediately or samples were sealed in airtight containers and stored in the refrigerator (4°C) for later extraction and analysis.

#### 3.2 **Methods**

##### 3.2.1 **Extraction of volatile components**

The volatile components of the spices were extracted using the Likens-Nickerson simultaneous steam distillation and extraction technique. (Nickerson and Likens, 1966). Using (Micro-SDE, Chrompack, Middelburg, The Netherlands). Two grams of the crushed spice were mixed with 400ml of distilled water to give a slurry of 0.5% (w/v). One millilitre of internal standard solution (50ppm, 4-methyl-1-pentanol in water) was added to the slurry in a 1 litre Erlenmeyer flask and six millilitre of a pentane and dimethyl ether mixture (1:1) transferred into a 9 ml pear-shaped solvent flask. Both flasks were appropriately connected a micro-scale steam distillation low-density solvent extraction device and the solutions brought to the boil. Extraction of volatiles was carried out for 30 minutes, from the beginning of condensation of vapour on the walls of the condenser. The solvent/water collected in the pear-shaped flask was placed in the freezer to freeze-out the water phase. The solvent extract was poured off, dried over approximately 2g of Na<sub>2</sub>SO<sub>4</sub> and concentrated to about

100 mg by gently blowing N<sub>2</sub> gas over the surface. The concentrated extract was analyzed for volatile compounds using the GC-MS.

#### **3.2.2 GC-MS Analysis**

Separation and Identification of volatiles in the spice extract was carried out on a Hewlett-Packard G1800A GCD System (GC-MS, Hewlett-Packard, Palo Alto, CA, USA). The instrument was equipped with a Hewlett-Packard DB-WAX column (30m x 0.25µm i.d; x 0.25mm film thickness). Two microlitre extract were injected (split ratio 1:20) using the temperature program: 10min at 40°C, increased to 240°C at 6°C min<sup>-1</sup>, and held constant at 240°C for 30 minutes. Identification of volatile compounds was determined in the Total Ion mode scanning a mass to charge ration (m/z) of range between 25 and 550. Further identification was obtained by probability- based matching with mass spectra in the G1033A NIST PBM Library (Hewlett-Packard) containing 75000 reference spectra.

## 4. Results and Discussions

### 4.0 RESULTS AND DISCUSSIONS

#### 4.1 Volatile compounds identified in four local Ghanaian spices

Volatile compounds identified in Cloves, Nutmeg, Anise seed and Garlic shown in Tables 1 – 4, respectively.

Table 1: Essential Oils Identified in Cloves.

Monoterpene Hydrocarbons	Oxygenated Monoterpenes	Sesquiterpene Hydrocarbons	Oxygenated Sesquiterpenes	Aromatic Ethers	Miscellaneous Essential oils
.alpha-Pinene .beta-Pinene .alpha-Phellandrene .alpha-Terpinene .gamma-Terpinene Limonene Sabinene Terpinolene	Linalyoxide	.alpha-Cubebene Ylangene Copaene Caryophyllene .alpha-Farnesene Calamene	d-Nerolidol	Eugenol laoeugenol 4-(2-propenyl)-Phenol	Eucalyptol Paracymenyl Durene Germacrene Anethole

Table 2: Essential Oils Identified in Nutmeg

Monoterpene Hydrocarbons	Oxygenated Monoterpenes	Sesquiterpene Hydrocarbons	Aromatic Ethers	Miscellaneous Essential oils
(+)-2-carene 1,3,6-p-menthatriene 3-carene Camphene E.beta-ocimene Sabinene	alpha-cardinol Carvacrol	alpha-Caryophyllene alpha-Cubebene beta-Cubebene Caryophyllene Copaene	Eugenol Myristicin 2,4-bis (1,1-dimethyl)-Phenol	alpha-Phellandrene-epoxide alpha-Santelene Allocimene Anethole cis-Linaloxide Eucalyptol Germacrene D para-Cymenyl Perillene Spathulenol

Table 3: Essential Oils Identified in Anise Seed.

Monoterpene Hydrocarbons	Oxygenated Monoterpenes	Sesquiterpene Hydrocarbons	Aromatic Monoterpenes	Aromatic Ethers	Miscellaneous Essential oils
alpha-Terpinene d-Limonene Limonene	Linalool	beta-Cubebene Caryophyllene Copaene Ylangene	p-Cymene	Eugenol 2,6-bis(1,1-dimethyl ethyl)-Phenol	alpha-Bisabolol alpha-Himachalene alpha-Longipinene beta-Himachalene Anethole Apiol Zingiberene

## 4. Results and Discussions

Table 4: Essential Oils Identified in Garlic.

Aromatic ether	*Sulphur Compounds
2,6-bis (1,1-dimethyl)-Phenol	Allyl Methyl Sulfide Dimethyl –Disulfide Dimethyl –Trisulfide Allyl Sulfide

\* These are the predominant compounds in garlic but they are not essential oils.

Essential oils found in the four spices investigated included monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aromatic ethers and miscellaneous essential oils. The only essential oil found in garlic was 1, 6 – bis (1, 1-dimethyl)-Phenol, an aromatic ether. Garlic was, however, found to possess a sulphur compounds that were significantly absent from the other spices. The characteristic aroma of garlic is reported to be the result of the breakdown of alliinase by alliinase and its conversion to alliinase. The unstable alliinase molecule is rapidly converted into a series of other odorous sulphur-containing molecules (Freeman and Kodera, 1995). Calvino *et al.* (2004) also found a lot of sulphur compounds in garlic oil. Out of the 47 compounds identified in their study, 18 were linear sulphur-containing compounds, 6 were of non-linear, and 23 were cyclic compounds. The most abundant volatile components they found were diallyl disulfide and diallyl trisulfide.

A total of 24 essential oils were found in the clove oil in the present study. Some of the monoterpenes and sequiterpenes found in this investigation were also identified in earlier work by Deyama and Horiguchi (1971) and Walter (1972). They include Ylangene, Caryophyllene and beta-Pinene. Caryophyllene occurs in many essential oils and especially in clove oil (Merck Index, 1989). It has a terpene odour about midway between the odour of cloves and turpentine and is mostly used in perfumery (Merck Index, 1989). Beta-Pinene is found in oils that contain alpha-pinene and has a characteristic odour of turpentine (Merck Index, 1989).

#### 4. Results and Discussions

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In the present study nutmeg was found to contain 26 essential oils. They included sabinene and 3-carene, which have also been previously reported by Sandford and Heinz (1971). Camphene, another terpene found in this study was also reported in nutmeg oil by Dann *et al* (1997) and Baldry *et al* (1976). Camphene volatilizes on exposure to air and has an insipid odour (Merck Index, 1989). Eugenol, the only aromatic ether identified in this investigation was also found in studies by Sandford and Heinz (1971). It has the odour of cloves with a spicy, pungent taste and may be used as an insect attractant as well as a source for the production of vanillin (Merck Index, 1989). The sesquiterpenoids capaene and caryophyllene in nutmeg oil found in this study were also found by Sammy and Nawar (1968) and Baldry *et al* (1976).

The spices showed consistently low numbers of oxygenated monoterpenes and aromatic ethers as compared to the other groups of the essential oils. The only oxygenated sesquiterpen (d-Nerolidol) was found in clove oil while the only aromatic monoterpene (p-Cymene) was found in Anise Seed oil. The miscellaneous essential oils found in the spices may contribute to the aroma and flavour profiles as well as the delicate nuances of these spices.

### **5.0 CONCLUSION**

The essential oils found in the four spices investigated included monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aromatic ethers, sulfur containing compound and several essential oils. The results of this study have tentatively identified and documented for the first time the essential oil composition of selected spices on the Ghanaian retail market. These documented volatile profiles would help to detect the presence of contaminants or adulterations of these spices. It is recommended however, that further studies including Gas Chromatography Olfactometric analysis (GC-sniffing) be conducted to identify the specific aroma characteristic of these essential oils and their aroma impact on the spices. In order to further authenticate the aroma profiles of the spices, measures have to be taken to obtain samples from their original sources before extraction and analysis.



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