PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 23 (12) 2231-2238 December 2019

Characterization of Essential Oils and Fatty Acids Composition of Stored *Ginger (Zingiber officinale* Roscoe*)*

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ABSTRACT: The essential oils and fatty acids composition of stored *Zingiber officinale* Roscoe (Ginger) were characterized using Gas chromatographic-Mass Spectrometric (GC/MS) method. The ginger rhizomes sample was procured from spices store at Bwari main market, Abuja, Nigeria. It was pretreated and the proximate analysis was carried out. The essential oils and fatty acids were extracted using Hydro-distillation unit. It was then analyzed using GC/MS. From the results obtained, the proximate composition showed 8.50% protein content, 3.80% crude fibre, 5.10% fat, 3.60% ash content, 10.20% moisture and 68.80% carbohydrate content. 38 essential oil components were found and the essential oil was dominated by the compound class Sesquiterpenes (64.83%) followed by Esters (13.29%), aldehydes (9.16%), Sesquiterpenols (6.46%) and monoterpenes (5.71%). Monoterpenols constituted 3.35% while ketones and oxides were seen in trace amounts. The specific dominant constituents were zingiberene (28.57%), Arcurcumene (14.22%) and Geranyl Acetate (13.28%). There was a decrease in the number of sesquiterpenes and monoterpenes in the stored ginger compared to the number earlier reported on freshly harvested and dried samples but the reduction did not affect the overall average percentage composition. The fatty acid comprised 50% saturated acids and 50% unsaturated acids and was dominated by Linoleic Acid (23.92%) followed by palmitic acid (22.18%), oleic acid (20.40%) and Lauric Acid (8.34%). The composition of the *Zingiber officinale* essential oil and fatty acid has shown that the plant contains true essential oils, implying that the medicinal and flavouring strength of the plants may be attributed to its essential oil composition.

DOI: https://dx.doi.org/10.4314/jasem.v23i12.22

Dates: Received: 30 November 2019; Revised: 20 December 2019; Accepted: 23 December 2019

Keywords: Essential Oil, Fatty acids, Ginger, *Zingiber officinale*

Zingiber officinale, commonly known as Ginger, is a very useful spicy/herbal plant in African folk medicine. It is known in Hausa language of Nigeria as Cithar, in Yoruba as Atale and in Igbo as Jinja (Dalziel, 1937; Wurochekke *et al*., 2001).The plant species *Zingiber Officinale* belongs to the family of Zingiberaceae. It is grown throughout the tropical areas of the world and commonly found in South - Eastern Asia especially in India (Levine *et al.*, 2008). It is extensively cultivated in Jamaica, Mexico, Hawaii, India, China and Africa (Kamaliroosta *et al.,* 2013). *Zingiber officinale* is an herbaceous perennial plant with broad looking leaves which grows from one to three or four feet in height. The stem is surrounded by the broad leaves. It shoots up a stem with spear-shaped leaves, as well as white or yellow flowers growing directly from the root. Each flower contains a three-lobed calyx and a corolla with one fertile stamen and three seed-filled carpels and its commercial propagated material is the germinated rhizome cuttings (Hassanapouraghdam *et al*., 2011). Fig. 1 shows a typical full grown ginger plant and its rhizome. The fresh or dried ginger rhizomes have common culinary, pharmaceutical and therapeutic applications. For culinary purposes, it is often used as spices or flavouring agent in Nigeria (Wurochekke *et al*., 2001). It is used to spice food in most of the Asian cuisines. For pharmaceutical and therapeutic applications, the plant and its preparations and / or supplements have long been used as digestive, carminative, aphrodisiac, tonic, anti-emetic and stomachic agents (Hassanapouraghdam *et al*., 2011; Abdurahman *et al*., 2013). Ginger extract also has long been used in traditional medical practices to treat inflammation. Many herbalists use ginger to treat inflammatory associated ailments such as arthritis, bronchitis, and ulcerative colitis. Other medicinal applications include the treatment of cold and flu, catarrh, congestion, coughs, sinusitis, sores on the skin, sore throat, diarrhea, colic, cramps, chills and fever (Kanadea and Bhatkhandeb, 2016). Chinese commonly use *Zingiber officinale* in the colic and tonic dyspepsia, treatment of motion sickness and gastrointestinal diseases (Hassanapouraghdam *et al*., 2011). The diverse applications of the plant have been attributed to its chemical compositions. Several studies have shown that *Zingiber officinale* is characterized by the presence of some constituents

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such as essential oils, Oleoresins and fatty acids (Hassanapouraghdam *et al*., 2011; Kizhakkayil and Sasikumar, 2012; Kanadea and Bhatkhandeb, 2016). Interest in essential oils has revived in recent decades with the popularity of aromatherapy.

Fig 1: (a). Grown Ginger Plant at Kachia's Ginger farm, Kaduna, Nigeria (b) Stored Ginger Rhizome

The essential oils are essential to life processes of plant. They are not involved with seed germination and early growth of plants. The oils can pass through tissues, cell walls and cell membranes and are capable of circulating throughout plants and human bodies. Essential oils are not greasy to touch (Stewart, 2005). Some of the common essential oils component of zingiber officinale earlier reported include Zingiberene, ar-curcumene, citral (neral and geranial), sesquiphellandrene, E-farnesene, trans-βocimene, β-bisabolene, nerolidol, 1,8-cineole, β-phellandrene, γ-terpinene and camphene (Onyenekwe and Hashimoto, 1999; Wohlmuth *et al*., 2006; Toure and Xiaoming, 2007; Yang *et al*., 2009; Abdurahman *et al*., 2013). The plant's essential oil is specifically known to be used in the treatment of fractures, rheumatism, arthritis, bruising, carbuncles, and nausea and sea sickness. The essential oil is also used in culinary as a flavouring agent for cookies, biscuits and cake. It is the main flavor in ginger ale, sweet, carbonated and non-alcoholic beverage. Essential oil of ginger has been widely used in beverage and confectionary as well as in fragrance and cosmetic industries (Aziza and Okiy, 2018). Fatty acids (FAs) consist of long hydrophobic, often unbranched chains of hydrocarbons, with hydrophilic carboxylic acid groups at one end. They are an important source of reserve energy and essential components of membrane lipids. The fatty acid composition of plants depends on climatic conditions and the genetics of the plant. Fatty acids are found as both storage oils and membrane lipids (Farmer, 1994; Fafal *et al*., 2016). Unsaturated fatty acids have been found to be important to human nutrition. One of the reasons for their importance is their hypocholesterolemic property related to heart disease. They are also involved in many biological processes in both structural and functional roles as parts of cell membranes, regulators of membrane permeability as well as proteins within the membrane. On the contrary, saturated fatty acids are considered to be unsafe when it becomes the major constituents of the fatty acids. Saturated fats raise low density lipoprotein (LDL) cholesterol and high LDL cholesterol increases the risk of heart disease and stroke (Chowdhury *et al*., 2014). However, well documented reports on the fatty acids compositions of Nigerian *Z. officinale* are scarce. The significance of the chemical composition of ginger with respect to its medicinal and flavouring strength has been a subject of debates. Some argue that the medicinal and flavouring strength depend on the essential oil Composition. The essential oil composition is believed to be a major source of the medicinal or flavouring strength only if the plant contains a true essential oil. According to Stewart (2005), one of the major characteristics of a true essential oil in terms of composition is that it is not dominated by acetic acid and 2-hydroxybutanoic acid. Again reports show that the essential oils composition of *Zingiber officinale* varied based on the geographical origin (Hassanapouraghdam *et al*., 2011; Abdurahman *et al*., 2013). This work therefore studied the essential oils and fatty acids composition of stored *Zingiber officinale* from Nigeria to present a well-documented report on the fatty acids composition of Nigerian ginger as well as the contributions of the essential oil compositions of the plant to its medicinal and flavouring strengths.

MATERIALS AND METHODS

Sample collection and Preparation: The semi-fresh *Zingiber officinale* rhizome samples were procured

from spices store, Bwari Main Market, Abuja, Nigeria. They were cleaned and freed of extraneous debris, chopped into 1-2mm size pieces and air dried for 48hours. The dried rhizomes with less residual moisture of about 4.5% were ground into fine powder using a kitchen blender and stored at 5ºC.

*Proximate Analysis***:** The ash, lipid, protein, moisture, fibre and carbohydrate contents of the ginger rhizome were determined as described by the Association of Official Analytical Chemists (AOAC) (Cunniff, 1995).

Essential Oil Extraction: The pulverized ginger rhizome sample was hydro-distilled according to Association of Official Analytical Chemists (AOAC) method and the modified method of Jarubol *et al.* (Cunniff, 1995; Jarubol *et al*., 2009). 100g of pulverized sample was weighed into a 1L round bottom flask. The flask, condenser and other gadgets were connected to complete the hydro-distillation arrangement using Clevenger-type apparatus. The crushed sample which was put in the flask was entirely covered with deionized water suspension and placed on the heating mantle. The water was allowed to boil and the essential oil was distilled over with steam through the condenser. The essential oil and the steam were separated through separating funnel and the essential oil was dried over anhydrous sodium sulphate and stored in a sealed Agilent vial protected from light at 4ºC before chromatographic analysis.

GC/MS Analysis of the Essential Oil: The essential oil was analyzed using gas chromatograph model HP6890, powered by HP Chemstation Rev.A09.0 (1206) software. Flame ionization detector (FID) fitted with Cp-Sil5CB column type of length-25mm, internal dimension-0.32µm and 0.12µm film thickness was used. The detector temperature was 300˚C and the carrier gas was hydrogen at flow rate 1.0ml/min, pressure of 22psi with compressed air of 28psi. The oven temperature was programmed from 35˚C -100˚C at 5˚C/minute and run at 100˚C for two minutes. Split injection temperature of 150˚C with split ratio 20:1 was used.

Fatty acid extraction: 8ml of isopropanol was added to 1g of the pulverized sample in a 500ml flask and heated at 80˚C for 5minutes and then cooled to room temperature. 12ml of hexane was then added to the mixture, homogenized and centrifuged to facilitate separation of lipid extract. The upper phase of hexane: isopropanol containing lipids was transferred to another flask. The lower phase of the mixture was re-extracted with 7:3ml hexane: isopropanol and combined with the former upper phase. The combined extract was filtered through anhydrous sodium sulphate (Na_2SO_4) according to Li *et al*. (2006). The fatty acid was recovered by removing the solvent mixture using rotary evaporator.

Methylation of Fatty Acids: Fatty acid profile, saturated, mono-and poly-unsaturated, analysis was carried out following the modified AOAC 965.49 and 996.06 official methods. 50mg of the oil sample was saponified (esterified) for 5mins at 95˚C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. 3ml of 14% boron triflouride in methanol was added. The mixture was heated for 5mins at the temperature of 90˚C to achieve complete methylation. The fatty acid methyl esters (FAME) were extracted three times from the mixture with redistilled n-hexane. The fatty acid content was concentrated to 1ml for gas chromatography analysis.

GC Conditions for the FAME: 1µl was injected into the injection port of GC-model HP6890, powered with HP chemstation Rev.A09.01 (1206) software. Flame ionization detector (FID) with column dimension 30m x 0.25ml x 0.25µm and type HPIN NOWAX was used. The oven temperature was programmed at 60˚C with fist ramping at 12˚C/min for 20mins, maintained for 2mins; second ramping at 15˚C/mins for 3 mins, maintained for 8mins. Split injection temperature of 250˚C with split ratio 20:1 was used. The detector temperature was 320˚C and the carrier gas was nitrogen (N_2). The hydrogen pressure was 22psi and compressed air 35psi. In all, the linear retention indices of the components were determined relative to the retention times of the series of n-alkanes and the percentage compositions were obtained from electronic integration measurements.

RESULTS AND DISCUSSION

Proximate Composition: The result for the proximate composition of the stored pulverized ginger sample is presented in Table 1. Carbohydrate (68.80%) constituted the dominant proximate component of the plant. A comparison of this amount with those obtained from previous studies shows a significant similarity with the 68.15% reported by Otunola et al. (2010) but lower than the 71.46% reported by Ugwoke and Nzekwe (2010) and higher than 58±6.80% reported by Ajayi *et al*. (2013) for Nigerian ginger. However, in all the reports, carbohydrate was the major nutrient composition of ginger. This shows that ginger can serve as a dietary source of carbohydrate but cannot be considered as a major carbohydrate source compared to tubers and cereals which are spread throughout the world. The moisture content of the ginger sample was 10.20%. This amount was different from the reports from Otunola *et al*. (2010), Ugwoke and Nzekwe (2010) and Ajayi *et al*. (2013)

which were 6.37%, 6.45% and 4.63% respectively. The differences may depend on the temperature and duration of drying. The moisture content is an indication of the fact that the plant can be more stable when dried during storage and packaging. Other detectable nutritional components were protein (8.50%), Fat or Lipid (5.10%), Crude fibre (3.80%) and Ash (3.60%). The protein content was in agreement with 8.58% and 8.83% earlier reported by Otunola *et al*. (2010) and Ugwoke and Nzekwe (2010) respectively. This shows the relative dietary importance of this spice to the improvement of protein content of food. However, the percentage composition of
protein in the plant does not really portray it being used as a major source of protein. The fat or lipid content of the plant also showed similarities with 5.35% and 5.75% reported by Otunola *et al*. (2010) and Ugwoke and Nzekwe (2010) respectively, while generally low fibre and ash content were recorded. The crude fat or lipid concentration in ginger was not enough to be referred to as oil rich plant. The extent of nutritional composition of the plant may be responsible for why the plant is used as mere spices and not as major sources of food nutrients which of course has no impact on its medicinal values. The essential oil and other chemical composition of the plant may constitute the major source of its medicinal values.

Table 1: Proximate Composition of Stored Zingiber officinale

S/N	Parameter	% Composition
1	Moisture (%)	10.20
2	Protein (%)	8.50
3	Fat (%)	5.10
4	Ash (%)	3.60
5	Carbohydrate (%)	68.80
6	Crude Fibre (%)	3.80

Essential Oil Composition: The identified essential oil constituents of stored Z. officinale and their percentage compositions are presented in Table 2. From the GC/MS analysis, 38 constituents representing about 100% and grouped into eight classes of organic compounds: monoterpenes, monoterpenols, sesquiterpenes, sesquiterpenols, esters, oxides, aldehydes and ketones were identified and confirmed by comparing their retention times, similarity indices and mass spectra with those of reference samples and data. The essential oil was rich in sesquiterpenenes constituting 64.83% of the total oil. The observed total percentage composition of sesquiterpenes in the stored ginger was similar to earlier report by Onyenekwe and Hashimoto (1999) for freshly harvested Nigerian ginger but the differences resided on the number of constituents that made up the total sesquiterpenes percentage. Only 10 sesquiterpenes compounds were identified as against 29 compounds earlier reported for freshly harvested ginger. The following compounds: α-Ylangene, α-Gurjumene, Bergametene, δ-Elemene, α-Calacorene, γ-Calalorene, β-Elemene, γ-Elemene, α-Humulene, α-Himachallene, α-Cubebene, α-Copaene, t-Muurolene , α- Vetivinene, Allaromadendrene, Perillene , Santalene, Calamenene and Eremophyllene earlier reported by Onyenekwe and Hashimoto (1999) were not found in the stored ginger, instead Ar-curcumene (14.22%), β- Bisabolene (5.30%) and γ–Cardinene (2.10%) were identified. The absence of some of these compounds in the stored ginger may be as a result of oxidation, leading to the formation of more monoterpenols and sesquiterpenols which were not identified in freshly harvested ginger. Zingiberene (28.57%) was the dominant constituent in this present study. This was in agreement with earlier reports that Nigerian, Indian and Australian ginger are often dominated by Zingiberene (Onyenekwe and Hashimoto, 1999; Kizhakkayil and Sasikumar, 2012; Wohlmuth *et al.,* 2006). β-Sesquiphellandrene was found in lower quantity (5.97%) compared to 18.42% reported by Onyenekwe and Hashimoto (1999) for freshly harvested ginger. The low percentage composition of β-Sesquiphellandrene and the detection of a new compound ar-curcumene (14.22%) in significant amount confirmed the report that ar-curcumene is an artifact formed when Sesquiphellandrene undergoes rearrangement during storage (Onyenekwe and Hashimoto, 1999; Hassanapouraghdam *et al*., 2011). The antiseptic, anti-inflammatory, antibacterial, analgesic and antispasmodic properties of ginger are mostly attributed to the rich sesquiterpenes contents. The therapeutic effect of ginger essential oil is also attributed to the high sesquiterpenes contents (Sasidharan and Menon, 2010). Esters constituted the second higher percentage composition (13.29%) of the essential oil, followed by aldehydes (9.16%), Sesquiterpenols (6.46%) and monoterpenes (5.71%). Monoterpenols constituted 3.35% while ketones and oxides were seen in trace amounts (i.e. less than 0.1%). Geranyl acetate (13.28%) sometimes referred to as monoterpene was the major ester compound while geranial (9.16%) constituted the major aldehyde compounds of the essential oil (Table 2). The characteristic floral fruity and sweet aroma of ginger is attributed to geranyl acetate, ethyl acetate, and borneol acetate content while the fruity and balsamic odour is attributed to ethyl cinnamate (Yang *et al*., 2009; Sasidharan and Menon, 2010). Out of the six monoterpenes identified, camphene showed the highest concentration (3.53%), followed by trans-

ocimene (1.38%), while α-thurjene and γ-terpinene recorded trace amounts. The total monoterpenene content of the stored ginger essential oil was greater than those for freshly harvested Nigerian ginger earlier reported.

Table 2: Essential Oil Composition of *Zingiber officinale*

S/N	Compound type	Retention Time (Min)	% Composition
Group Name: *Monoterpenes*			
1	*Cymene*	7.283	0.516115
2	*Camphene*	9.670	3.531183
3	*Cis-Ocimene*	12.554	0.291923
4	*Trans- Ocimene*	13.292	1.375295
5	*α-Tinjene*	14.239	0.000069
6	*γ-Terpinene*	14.922	0.000107
			5.71
Group Name: Monoterpenols			
1	*Pinene – 2 – ol*	13.738	0.000094
2	*Endo Borneol*	14.539	0.544089
3	*α- Camphalenol*	17.361	0.000052
4	*Linalool*	17.665	0.000050
5	*Nerol (Geraniol)*	18.492	0.000049
6	*α- Terpineol*	18.664	0.000068
7	*Terpinen – 4 – ol*	18.838	0.000069
			0.54
Group Name: Sesquiterpenes			
1	*Ar-Curcumene*	21.905	14.216349
2	*Germacrene B*	22.063	3.263997
3	*β-Caryophellene*	22.358	0.000082
4	*Zingiberene*	22.599	28.573745
5	*α- Farnesene*	23.469	5.411950
6	*Germacrene D*	24.111	0.000030
7	*β- Bisabolene*	25.001	5.294626
8	*β- Sesquiphellandrene*	25.615	5.973400
9	*γ -Cardinene*	26.014	2.099404
10	*β- Selinene*	26.843	0.000052
			64.83
Group Name: Sesquiterpenols			
1	*Nerolidol*	19.517	2.807983
2	*Spathulenol*	29.165	0.000059
3	*α- Eudesmol*	29.352	3.649689
			6.46
Group Name: Esters			
1	*Ethyl Acetate*	12.821	0.000117
2	*Benzyl Acetate*	20.786	0.000183
3	*Ethyl Cinnamate*	21.434	0.000146
4	*Geranyl Acetate(monoterpene)*	21.819	13.284899
			13.29
Group Name: Oxides			
1	*1,8-Cineole*	16.780	0.000148
2	*Ascaridole*	20.407	0.000094
3	*Caryophyllene oxide*	26.235	0.000030
			Trace
Group Name: Aldehydes			
1	*Geranial*	16.035	9.163571
2	*Citronellal*	18.105	0.000049
3	*Pentadecanal*	19.949	0.000094
			9.16
Group Name: Ketone			
1	*Iso-artemisia*	16.453	0.000061
2	*Camphor (monoterpene)*	17.091	0.000081
			Trace
	GRAND TOTAL		**100**

Ginger essential oil is bound to exhibit mild antiseptic properties and rubefacient effect which provides circulation and pain relief for muscle pain and stiffness due to monoterpenes contents. The presence of significant concentration of monoterpenes in the oil also makes it possess useful decongestant properties for the respiratory and muscular system, effective airborne deodorizers and purifiers, highly lipophilic, hence penetrates the tissues of the body easily. On the other hand, oils rich in monoterpenes can cause skin irritation or sensitivity if significantly oxidized (Peng *et al*., 2012). It is therefore important to store ginger or ginger oil in tightly closed, cool and dark area. Furthermore, three sesquiterpenol compounds: α-eudesmol (3.65%), nerolidol (2.81%) and spathulenol (trace) were detected. Literature has shown that essential oil with eudesmol content is not safe for patients with peptic ulcer or bleeding disorders. It is not also safe for pregnant women, especially, during the first trimester. Sesquiterpenols such as eudesmol have anti-angiogenic effects, that is, ability to suppress new blood vessels from forming (Plengsurinyakarn *et*

al., 2012). The major monoterpenol compounds detected was endo-borneol (0.54%), while others were seen in trace amount. Although the oxide 1,8-cineole was detected in trace amount, studies have shown that essential oil with 1,8-cineole content can set off an attack on asthmatic patients (Peng *et al*., 2012). Hence caution should be taken when using ginger on asthmatic patients.

Fatty Acids Composition: Table 3 shows the fatty acids composition of the stored ginger sample recorded according to their retention time.

Table 3: Fatty Acids Composition of *Zingiber officinale*

	Group Name	Retention Time (Min)	% Composition
1	Caprylic Acid Methyl Ester (C8:0)	8.922	1.80
2	Capric Acid Methyl Ester (C10:0)	11.065	3.81
3	Lauric Acid Methyl Ester (C12:0)	12.830	8.34
4	Myrystic Acid Methyl Ester (C14:0)	14.440	5.85
5	Palmitic Acid Methyl Ester (C16:0)	16.011	22.18
6	Palmitoleic Acid Methyl Ester(C16:1)	16.677	0.58
7	Margaric Acid Methyl Ester (C17:0)	17.369	0.04
8	Stearic Acid Methyl Ester (C18:0)	18.060	3.45
9	Oleic Acid Methyl Ester (C18:1)	18.941	20.41
10	Linoleic Acid Methyl Ester(C18:2)	19.526	23.92
11	Linolenic Acid Methyl Ester(C18:3)	20.658	5.66
12	Arachidic Acid Methyl Ester (C20:0)	21.911	0.43
13	Arachidonic Acid Methyl Ester(C20:4)	22.998	0.06
14	Behenic Acid Methyl Ester (C22:0)	23.972	0.32
15	Erucic Acid Methyl Ester(C22:1)	24.885	0.23
16	Lingoceric Acid Methyl Ester (C24:0)	25.623	2.94
	TOTAL		100

The fatty acids contents of *Z. officinale* were dominated by Linoleic acid (23.92%) followed by Palmitic acid (22.18%) and Oleic acid (20.41%). Other acid compositions with significant concentration include lauric acid (8.34%), myrystic acid (5.85%), linolenic acid (5.66%), capric acid (3.81%), stearic acid (3.45%), lingoceric acid (2.94%) and caprylic acid (1.80%). None of the 16 fatty acids identified was seen in trace (les that 0.1%) amount. The comparison between the fatty acids content of *Z. officinale* with results reported for *Tetrapleura tetraptera* shows *Z. officinale* has well distributed acid contents than *T. tetraptera* which was chiefly dominated by four (4) types of fatty acids (Udourioh and Etokudoh, 2014). The ginger rhizome is made up 50% saturated fatty acids and 50% unsaturated fatty acids. Omega-6 fatty acid (linoleic acid) constituted the highest fatty acid composition of the plant with about 24%; Omega-9 fatty acid (oleic acid) constituted about 20% while omega-3 (linolenic acid) also constituted a significant percentage (about 6%). Although the saturated and unsaturated fatty acids contents of the plant is 50:50 percent, it is still not advisable to consume the plant frequently or in large quantity since the saturated acid, especially palmitic acid, is capable of suppressing the unsaturated acids and thus raise the blood cholesterol leading to hypertension. On the other hand, the presence of some essential fatty acids such as omega-6 makes the plant useful in retention of a healthy lipid level in the blood and control of inflammation in cases of infection or injury.

Conclusion: The essential oil composition of the stored Nigerian ginger was dominated by sesquiterpenes in which Zingiberene was the major sesquiterpene constituent with about 28%. The fatty acids content consists of 50% saturated acids and 50% unsaturated acids with omega-6 as the dominant constituent. There was a decrease in the number of sesquiterpenes and monoterpenes in the stored ginger compared to the number earlier reported on freshly harvested and dried samples, however, the reduction did not affect the overall average percentage composition. *Zingiber Officinale* contains true essential oil based on the fact that no trace of carboxylic acids was found in the oil. The study has also revealed that the essential oil composition of ginger is a major contributor to its medicinal and flavouring strengths.

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