

Research Article

Physicochemical Properties of Essential oils of Ginger (*Zingiber officinale*), African Nutmeg (*Monodora myristica*) and Ashanti Black Pepper (*Piper guineense*)

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Abstract: This study was carried out to evaluate the physicochemical characteristics of Ginger (*Zingiber officinale*), African nutmeg (*Monodora myristica*) and Ashanti Black pepper (*Piper guineense*) essential oils. Dried plant materials were obtained from the Oba market in Edo State, Nigeria. All samples were ground into a fine powder and stored in an airtight plastic container at room temperature (28±2°C) until when used. The essential oils were extracted using steam distillation and their physicochemical properties analyzed using standard procedures. The results showed that color varied from yellow for *Z. officinale* to colorless for *M. myristica* and *P. guineense*. The highest yield (3.55%), acid value (6.73 mg KOH/g), saponification value (185.13 mg KOH/g), free fatty acid value (3.37%) and iodine value (69.49 gI₂/100 g) were recorded for *M. myristica*, the highest specific gravity value (0.88) was for *P. guineense* and *M. myristica*, the highest peroxide value (2.67) was for *P. guineense* while the highest refractive index value (1.49) was for both *P. guineense* and *Z. officinale*. There were significant differences (p<0.05) in all physicochemical parameters except for the refractive index and specific gravity of the essential oils. The result indicates that the essential oils of the selected plants can be utilized as edible oils and suitable for nutritional purposes. The oils are stable and would not easily undergo rancidity, thus they can be used as a good component of food additives.

Keywords: Essential oils, *Monodora myristica*, physicochemical properties, *Piper guineense*, *Zingiber officinale*

INTRODUCTION

Spices are aromatic vegetative substances used for seasoning of food and from which no portion of any volatile oil or flavouring principles have been removed and are free from artificial colouring matters, adulterants and impurities (Farrel, 1990). They are Generally Recognized As Safe'' (GRAS) when used at concentrations normally found in foods. In Nigeria commonly used spices include ginger, garlic, onions, nutmeg, clove, Ashanti pepper etc. They can be used whole, pulverized or as extractives such as essentials oils or oleoresins. Most spices contain carbohydrate, protein and small amount of minerals. Only a small fraction of the dry matter which contribute to flavoring for example; tannins, resins, pigments, volatile, essential and fixed oils occur in traces (Cowan, 1999). Essential oils are volatile and aromatic produces or mixtures of produce which are formed in cytoplasm occurring as tiny droplets amongst cells of plants (Toure and Xiaoming, 2007). The plant from which they are gotten gives them a characteristic taste and

odor (British Pharmacopoeia Commission, 2013; Burdock, 2005). This disparity in composition may be as a result of a variety of plants (Sarac and Ugur, 2008), geographical locations (Mechergui *et al.*, 2010; Sarac and Ugur, 2008), harvesting seasons (Hussain *et al.*, 2008; Figueiredo *et al.*, 2008; Taiz and Zeiger, 2010), drying methods (Di Cesare *et al.*, 2003) and extraction methods (Karakaya *et al.*, 2011; Burt, 2004). Quality, freshness and distinctiveness of the oil are main concerns that affect the value of essential oils (Shahidi and Zhong, 2005). Due to the nutritional values of oils and importance for industrial purposes, the use and demand of oils have increased resulting in their exploration from plants (Adolf *et al.*, 2018). Thus, the importance of physicochemical characteristics of these oils is of utmost importance in our daily life's. The quality of essential oils is ultimately affected by the physicochemical properties. These properties are used in regulating their consumption as well as determining their market value (Parthiban *et al.*, 2011; Bamboye and Adejumo, 2010). Despite numerous studies carried out on these spices, there is a dearth of literature on the

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physicochemical characteristics of plant essential oil. This study is intended to uncover the nature and importance associated with the physicochemical characterization of the essential oils to ascertain their suitable application in the food industry.

MATERIALS AND METHODS

Collection of materials and preparation of samples:

The dried plant materials of Ginger (*Zingiber officinale*), African nutmeg (*Monodora myristica*), Ashanti Black pepper (*Piper guineense*) were obtained Oba market in Edo State, Nigeria. They were then taxonomically identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin City Edo State. All plant materials were pulverized into fine powder. The powder was stored at room temperature (28±2°C) in an air tight container until it was used.

Extraction of the essential oils: Three thousand gram of the ground dried powder of each plant material was measured into distillation flask fitted with condensers. Steam was provided to the flask through a steam generator at constant flow. The essential oil which vaporizes with the steam was condensed into a collecting separating funnel. The oil was separated by gravity and dried over anhydrous sodium sulphate, filtered and stored at 4°C in a refrigerator until analyzed (Hussain *et al.*, 2008).

Physicochemical analysis of essential oils: The extracted essential oils were analysed for yield, refractive index, relative density (specific gravity), saponification value, iodine value, acid value, peroxide value and free fatty acid value of the essential oils were determined according to the methods below.

Determination of yield: This is the quantity of essential oil extracted from the plant materials. The yield of the oil (hydrophobic fraction) was recorded as volume: weight ratio (Ayoola *et al.*, 2008; Gundidza *et al.*, 2009):

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{weight of dried plant material}} \times 100$$

Color determination: Color of the oils was determined by physical observation in day light and under ultraviolet radiation of 254 and 366 nm by means of an ultra violet chamber (Bamgboye and Adejumo, 2010).

Determination of refractive index: Refractometer was used to determine the refractive index of the extracted oil. One drop of the oil was dropped on the cell compartments of the instruments. The necessary adjustment was made and the result recorded when the lower part became darker (AOAC, 1990).

Determination of specific gravity: The density bottle was cleaned, dried, weighed and filled with distilled

water, then immersed in a bath at 20°C until the water inside reaches 20°C. The outside of the bottle was wiped and weighed. The bottle was emptied, dried and filled with the oil at 20°C. It was later kept in a bath, wiped and weighed again (AOAC, 1990):

$$\text{Specific gravity} = \frac{\text{weight of oil}}{\text{Equivalent weight of water}}$$

Determination of saponification value: The Saponification Value (SV) is a measure of the amount of alkali necessary to saponify all the triglycerides present in the sample. Two grams of the oil was weighed into a 250 cm³ conical flask containing 25 cm³ of alcoholic potassium hydroxide solution. A reflux condenser was attached to the flask and heated on a boiling water bath for 1 h with occasional shaking. One cm³ of phenolphthalein indicator was added and titrated while hot with standard hydrochloric acid (0.5 M) until end point is colourless (AOAC, 1990). Blank determination was carried out:

$$\text{Saponification value} = \frac{(b-a) \times 0.5 \times 56.1}{\text{weight of sample}}$$

where,

- a = Volume of 0.5M HCL used in oil titration
- b = Volume of 0.5M HCL used in Blank titration

Determination of iodine value: The iodine value of oil is a measure of the saturation of fatty acids. Iodine value determination is used to determine the amount of unsaturation in fatty acids. Iodine value is the weight of iodine absorbed by 100 parts by weight of the sample. It was determined using Wij's method. Zero-point five gram of the sample was weighed into a dry glass stoppered flask of about 250 cm³ capacity, 10 cm³ of carbon tetrachloride was added and swirled until it dissolved. Twenty cm³ of Wij's solution was added to it, the solution was mixed and it was allowed to stand in the dark for 30 min with occasional shaking. Fifteen cm³ potassium iodide and 100 cm³ of water were added to the mixture. The whole solution was mixed and titrated with the standard sodium thiosulphate using freshly prepared starch as indicator (AOAC, 1990). Blank determination was carried out.

Calculation:

$$\text{Iodine value} = \frac{(b-a)1.269}{\text{weight of sample}}$$

where,

- b = Titre value of the blank
- a = Titre value of the sample

Determination of acid value: The acid value of an oil or fat is determined by the number of mg of potassium hydroxide required to neutralize the free oil in 1 g of the sample. Twenty-five cm³ of diethyl ether was mixed with 25 cm³ of ethanol and 1 cm³ of phenolphthalein

solution (1%). This was carefully neutralized with 0.1M sodium hydroxide solution. One gram of the oil sample was dissolved in a mixed neutral solvent and titrated with aqueous 0.1M NaOH. This was shaken constantly until a pink colour which persisted for 15 sec was obtained (AOAC, 1990). The process was repeated for three consecutive titre values.

Calculation:

$$\text{Acid value} = \frac{V \times M \times 40}{\text{wt. of sample}}$$

where,

v = Volume of NaOH used for titration

M = Molarity of NaOH used

Determination of peroxide value: The amount of peroxides produced in an oil or fat gives an indication of the extent of spoilage. One gram of oil sample was weighed into a 250 cm³ flask and 1 g of ground potassium iodide was added. Then 20 cm³ of the solvent mixture of glacial acetic acid and chloroform (1:2) was added to dissolve it. Twenty cm³ potassium iodide solution and 25 cm³ of distilled water were then added. The mixture was titrated using starch as indicator with 0.002M sodium thiosulphate solution (AOAC, 1990):

$$\text{Peroxide value} = \frac{0.002M \times V \times 1000}{\text{weight of sample}}$$

where,

V = Titre value

0.002M = Molarity of sodium thiosulphate used

Determination of free fatty acid: Twenty-five cm³ diethylether and 25 cm³ ethanol were mixed inside a 250 cm³ capacity of conical flask and warmed on hot plate for 5 min. One g of oil was dissolved in the solvent-mixture and also warmed on hot plate for 5 min and removed. Two drops of phenolphthalein indicator was added and titrated with standardized 0.1M NaOH until a faint colour appeared. The process was repeated for three consecutive titre value (AOAC, 1990):

$$\text{Free fatty acid} = \frac{\text{Acid value}}{2}$$

Statistical analysis: All data obtained in this study were represented as mean±Standard Error (S.E.) of triplicates values. The data were then subjected to a One-way analysis of variance laid in a completely randomized design. Duncan Multiple Range Test at 95% confidence level was used to separate significant.

RESULTS AND DISCUSSION

The physico-chemical parameters of the essential oil's samples are presented in Table 1.

Colour: Physical observation of the essential oils for colour showed that *M. myristica* and *P. guineense* were colourless while *Z. officinale* was pale yellow. These results are in accordance with studies by Udoh *et al.* (2004) for *M. myristica*, Nandi *et al.* (2013) for *Z. officinale* but differed from Uzeh and Oguntosi (2013) who recorded yellow for *P. guineense*.

Yield: The highest yield of 3.55% was recorded for *M. myristica* while the lowest 1.17% was recorded for *Z. officinale*. Significant differences (p<0.05) were recorded in the yield between samples. The essential oil yield for the samples was low. *M. myristica* had the highest yield compared to *Z. officinale* and *P. guineense*. Ifesan *et al.* (2010) recorded a yield that is about 13 times the yield obtained in this study for *Z. officinale*, although the yield was higher than 0.47 and 0.50% reported by Talla *et al.* (2013) and Nguefack *et al.*, (2004). The yield of *M. myristica* and *P. guineense* were also low compared to 2.16 and 37.82% reported by Owokotomo and Ekundayo (2012) and Ogbonna *et al.* (2015) respectively. Generally, the percentage yield of the oils was higher than the essential oils of *Eucalyptus globulus* (0.2%) and *Eucalyptus robusta* (0.13%) as reported by Boukhatem *et al.* (2014). The yield of oil obtained in this study varied with other researchers, this may be due to the application of different methodologies. According to international standards, a product essential oil is deemed commercially acceptable when its concentration is higher than 0.4%, (Hanus *et al.*, 2006; Marquard and Kroth, 2002). This study showed that the essential oils yield from these plants were higher than 0.4% as such can be regarded as commercially acceptable.

Table 1: Physicochemical characteristics of *Zingiber officinale*, *Monodora myristica* and *Piper guineense* essential oils

Parameters	Plants		
	<i>Zingiber officinale</i>	<i>Monodora myristica</i>	<i>Piper guineense</i>
Yield	1.17±0.01 ^c	3.55±0.01 ^a	1.43±0.01 ^b
Refractive index	1.49±0.01 ^a	1.48±0.01 ^a	1.49±0.01 ^a
Specific gravity	0.87±0.01 ^a	0.88±0.01 ^a	0.88±0.01 ^a
Acid value (mgKOH/g)	5.01±0.00 ^c	6.73±0.01 ^a	5.61±0.00 ^b
Peroxide value (meq/kg)	2.54±0.02 ^b	2.33±0.01 ^c	2.67±0.00 ^a
Saponification value (mg KOH/g)	173.21±0.52 ^b	185.13±0.50 ^a	171.25±0.65 ^c
Iodine value (g I ₂ 100/g)	39.34±0.37 ^c	69.49±0.42 ^a	65.35±0.44 ^b
Free fatty acid value (% oleic acid)	2.53±0.26 ^c	3.37±0.26 ^a	2.81±0.18 ^b

*: Values are represented as mean±standard error of mean of triplicate values; *Means values in the same column with same superscripts are not significantly different (p>0.05)

Specific gravity: The specific gravity was 0.87 for *Z. officinale*, 0.88 for *M. myristica* and 0.88 for *P. guineense* respectively. There were no significant differences ($p>0.05$) recorded between samples. The ratio of the density of a respective substance to the density of water at 4°C is known as specific gravity (Bamboye and Adejumo, 2010). It is used to assess the purity of the oils (Ogbonna *et al.*, 2015). The specific gravity of all the oils were <1 , this implies water is heavier than the oils. The specific gravity of the oils obtained in this study were lower than 1.05 for *Z. officinale*, 0.96 for *M. myristica* and 0.91 for *P. guineense* reported by Ifesan *et al.* (2010), Adolf *et al.* (2018) and Ogbonna *et al.* (2015) respectively. However, they were within the specific gravity values range of 0.82-0.92 reported by Akubugwo and Ugbogu (2007).

Refractive index: The results of the refractive index recorded for the essential oils samples ranged between 1.48 for *M. myristica* and 1.49 for *Z. officinale* and *P. guineense*. There were no significant differences ($p>0.05$) between samples. The results of the refractive index recorded of the essential oils for the samples were slightly higher than the value of 1.46 obtained for *B. sapida* oil (Akintayo *et al.*, 2002) but comparable with the values for most drying oils whose refractive index are between 1.48 and 1.49 (Oluba *et al.*, 2008). When oils have high number of carbon-atoms in their fatty acids, it indicates the refractive index is high (Adolf *et al.*, 2018). As the double bond increases, the refractive index also increases leading to high degree of unsaturation (Adolf *et al.*, 2018).

Acid value: The highest acid value of 6.73 mg KOH/g was recorded in *M. Myristica* while the lowest 5.01 mg KOH/g was recorded in *Z. officinale*. There were significant differences ($p<0.05$) in acid values recorded among samples. The acid value is a measure of the amount of Free Fatty Acids (FFA) present in the oil (Popoola and Yangomodou, 2006). It is often an ideal measure of the breakdown of triacylglycerols into free fatty acids, which reflects on the essential property or quality of many lipids. Generally, it points to the degree of edibility of the oil (Amira *et al.*, 2014). This has an inverse relationship with the free fatty acid content (Bachheti *et al.*, 2012). These values were found in the permissible limits of 10 mg KOH/g of oil and found suitable for dietary purposes (Essein and Amadi, 2009).

Free fatty acid: The highest free fatty acid values 3.37% was recorded in *M. Myristica* while the lowest 9.56% was recorded in *Z. officinale*. Results showed significant differences ($p<0.05$) recorded between samples. The Free fatty acid is the percentage weight of a specified fatty acid (expressed in oleic acid). According to Oladiji *et al.* (2010) and Oluba *et al.*

(2008), the lower the free fatty acid value, the better the quality of the oil. When there is an increase in the quantity of FFA in a sample of oil or fat, it shows that hydrolysis of tryglycerides has occurred (Akubugwo *et al.*, 2008). The free fatty acids values were greater than one so this indicates the edibility of the oils (Amira *et al.*, 2014). Also, Free Fatty Acids (FFA) values were higher than the minimum limit of 2.0% set for high-grade oils by Codex Alimentarius Commission (1993). The oils of these samples can therefore be used as edible oils and are suitable for nutritional purposes.

Peroxide value: Peroxide values ranged between 2.33 meq/kg for *M. myristica* and 2.67 meq/kg for *P. guineense*. There were significant differences ($p<0.05$) recorded between samples. Peroxide Value (PV) is used to measure the extent to which rancidity reactions have occurred during storage. It could be used to show the quality and stability of fats and oils (Stoilova *et al.*, 2007; Gulfranz *et al.*, 2006). The oils in this study recorded low peroxide values compared to the maximum acceptable value of 10 meq/kg specified by Standard Organization of Nigeria (SON) (2000) and the Codex Alimentarius Commission (2001). Consequently, the oils are thus stable and would not undergo rancidity easily.

Saponification value: Saponification values obtained were 173.21 mg KOH/g for *Z. officinale*, 185.13 mg KOH/g for *M. myristica* and 171.25 mg KOH/g for *P. guineense* respectively. Significant differences ($p<0.05$) were recorded between samples. The saponification value is a measure of the proportion of low molecular weight triacylglycerols in oil (Oladiji *et al.*, 2010). The high values of saponification in this study indicates high mean molecular weight of fatty acids or high number of ester bonds (Zahir *et al.*, 2014). Furthermore, saponification value indicates the average molecular mass of fatty acid which is inversely proportional to the chain length of fatty acid in fats and oils (Oladiji *et al.*, 2010). This signifies that; a longer average fatty acid chain length, results in a smaller saponification value (Mansor *et al.*, 2012). Therefore, *P. guineense* has fatty acid with the longest chain length followed by *Z. officinale* and *M. myristica* being the shortest. As such, the high saponification values recorded for these oil samples in this study, are indications of their possible use in the industry (Aolf *et al.*, 2018; Ardabili *et al.*, 2011; Amoo *et al.*, 2004).

Iodine value: The highest iodine value 69.49 gI₂/100 g was recorded in *M. myristica* while the lowest 39.35 gI₂/100 g was recorded in *Z. officinale*. There were significant differences ($p<0.05$) recorded between samples. The iodine value is a marker for the extent of unsaturation of the oil (Shad *et al.*, 2012). It influences the stability of oils to oxidation and also determines the

quality of the overall unsaturation of the fat (Asuquo *et al.*, 2012; Bello *et al.*, 2011; Bello and Makanju, 2011). The iodine value of *M. myristica* indicated the greatest degree of unsaturation therefore has the highest ability to remain in liquid form at room temperature than other oils. The values in this study are comparable to (64.9 g I₂/100 g) reported by Adolf *et al.* (2018) for *M. myristica*, but higher than (17.1 and 13.66 g I₂/100 g) reported by Aletor (2014) and Ogbonna *et al.* (2015) for *Z. officinale* and *P. guineense* respectively. However, the iodine values for the oils were lower than those recorded for vegetable oils like peanut (86-107 gI₂/100 g) cottonseed (100-123 gI₂/100 g), sesame (104-120 gI₂/100 g), soyabean oil (124-139 gI₂/100 g) as well as sunflower oil (118-141 gI₂/100 g) (Aremu *et al.*, 2006). The fact that iodine values less than 115 gI₂/100 g are regarded as non-drying oils and those above 130 gI₂/100 g are considered as drying oils (Ogbonna *et al.*, 2015). These oils can be categorized as non-drying oils. Higher iodine values in oils are desirable nutritionally. Nevertheless, higher iodine value indicates a lesser stability of the oils and greater vulnerability to undergo oxidation and production of free radicals. Oils with low iodine values have been reportedly used as a component of food additives because they are less liable to oxidative rancidity and it is effective in controlling microbial growth (Stoilova *et al.*, 2007).

CONCLUSION

The result of this study suggests that the essential oil of the selected plants can be utilized as edible oils and suitable for nutritional purposes, stable and would not easily undergo rancidity as such can be used as a good component of food additives.

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