A STUDY ON PHYSICOCHEMICAL PROPERTIES OF OCIMUM GRATISSIMUM AND OCIMUM BASILICUM LEAVES FIXED OIL.

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Abstract

Edible oils from plant sources are of interest in various food applications and industries. They provide characteristic flavours and textures to foods as integral diet components and can also serve as a source of oleo chemicals. This study was conducted to characterize the physicochemical properties of *Ocimum gratissimum* and *Ocimum basilicum* leaves fixed oil. The physicochemical properties were determined, following the method described by the Association of Official Analytical Chemists. The results indicate that *Ocimum basilicum* fixed oil has a higher pH value (7.73±0.000), saponification value (294.44±0.042mg/KOH), and ester value (280.59±0.120mg/KOH) compared to *Ocimum gratissimum* fixed oil (pH 5.86±0.000, saponification value 155.71±0.035mg/KOH, and ester value 139.60±0.042mg/KOH). On the other hand, the specific gravity, peroxide value, acid value, and iodine value of *Ocimum gratissimum* fixed oil are higher (0.95±0.000, 16.25±0.141Meq/kg, 16.105±0.078mgKOH/g, and 21.705±0.177gI_2/100g, respectively) compared to *Ocimum basilicum* fixed oil (0.84±0.000, 2.88±0.000Meq/kg, 13.495±0.431mgKOH/g, and 13.445±0.007gI_2/100g, respectively). The result suggests that both oils are classified as non-drying oils, suitable for soap making and food products.

Keywords: *Ocimum gratissimum*, *Ocimum basilicum*, physicochemical, fixed oil.

Introduction

The term oil is used in generic sense to describe all substances that are greasy or oily fluid at room temperature. Oils are naturally occurring esters of long straight-chain carboxylic acids. They belong to the saponifiable group (contain an ester group) of lipids. Edible oils are constituted of triacylglycerol molecules, mainly formed by unsaturated (oleic, linolenic acids etc.) and saturated fatty acids (myristic, palmitic, stearic acids etc.) esterified to glycerol units (Andersson *et al.*, 2010). They can be formed from a single fatty acid that could be esterified up to three times into glycerol backbone, or at least by three different ones. (Dimberu, and

Behete, 2011). Edible oils from plant sources are of interest in various food applications and industries. They provide characteristic flavours and textures to foods as integral diet components and can also serve as a source of oleo chemicals (Morrison, *et al.,* 2015). A fixed oil is often referred to as a carrier oil and does not evaporate, unlike the essential oil which is volatile oil that evaporate easily. Fixed oil extracted from leaves has gained significant attention in various industries, including chemical products, pharmaceutical, cosmetics, paints and most importantly, food (Chivandi *et al.,* 2018). Leaves of numerous plant species contain valuable bioactive compounds that can be extracted to obtain a versatile fixed oil. These oils are characterized by their unique chemical composition and diverse functional properties, making them a valuable resource for different applications. *Ocimum gratissimum* and *Ocimum basilicum* are the two examples of such plant known for its fixed oil yielding potential.

Ocimum basilicum commonly called curry in Nigeria belongs to the Lamiaceae family and is a popular culinary herb with highly aromatic leaves that has a pleasant and vigorous flavor. This plant is an erect branching herb, 0.6 to 0.9 m high, glabrous, more or less hispidly pubescent. Stems and branches are green or sometimes purplish. Leaves of *Ocimum basilicum* are simple, opposite, 2.5-5 cm or longer, ovate, acute, entire or more or less toothed or lobed with a cuneate and entire base (Akah *et al.,* 2017).

Ocimum gratissimum popularly known as scent leaf, is a widespread and commercially viable perennial herbaceous plant with a very strong aromatic smell. It belongs to the family of Lamiaceae and is found in Africa, Asia, and South America. It is used as a natural flavouring agent, condiment, or vegetable in the preparation of fish, meat, soup, and stew. It is called 'Efirin' in Yoruba, 'Daidoya' in Hausa and 'Nchuanwu' in Igbo (Nweze and Eze, 2009). It is about 1–3cm tall, has an erect stem, and is branched, round-quadrangular, and woody at the base, with opposite, slender, and marginalized leaves (Tanko *et al*., 2008).

The limited comprehensive literature on characterization of *Ocimum basilicum* and *Ocimum gratissimum* fixed oil presents a significant knowledge gap, hindering our understanding of its potential applications in the fields of pharmaceuticals, cosmeceuticals, and food additives. Hence the need to explore the physicochemical properties present in fixed oil of these plants fixed oil using multidisciplinary approach which aligns well with scientific methodology.

Method

Collection and Preparation of Samples

The *Ocimum gratissimum* and *Ocimum basilicum* leaves used in this work were purchased from local markets in Orumba North, Anambra State Nigeria. They were botanically authenticated by a taxonomic from the Department of Science Laboratory Technology, Federal

Polytechnic Oko. The leaves were thoroughly and gently washed with tap water and dried under room temperature.

Extraction of the Oil

The sample (50g) was fed to a Soxhlet extractor fitted with a 2 L round bottomed flask. A clean dry and weighed Soxhlet extraction flask was half filled with petroleum ether, and the flask placed on the heating mantle and heated at 80^{o}C. The fat contents were extracted for three hours. Then the sample holder was disconnected and extraction flask removed and the oil was separated and packaged in a clean container ready for analysis.

Physicochemical Properties Analysis

The Physicochemical Properties was determined, following the method described by the Association of Official Analytical Chemists (2005) as follows:

Specific Gravity

The specific gravity of the essential oil sample was determined using a 50ml capacity density bottle at 30°C. The density bottle was weighed empty and then filled with the oil sample up to the mark on the bottle. The bottle with essential oil samples were weighed again and recorded. The weight of the empty bottle was subtracted from the total weight of the bottle and oil sample. The weight of the essential oil samples was then divided by the weight of an equal volume of water to obtain the specific gravity (SG) of the essential oil samples.

pH

In a dry clean 25 mL beaker, 2g of essential oil sample was placed followed by 13mL of hot distilled water and the mixture was stirred slowly. The mixture was cooled in a cold-water bath to 25˚C. The pH electrode was standardized with buffer solutions (pH 4 and 7) and the electrode immersed into the sample where an average pH of two recordings per sample was recorded.

Acid Value

The fat sample (10g) was weighed and dissolve in about 50ml of fat solvent (chloroform). The solution was titrated with 0.1m KOH using phenolphthalein (1ml) as an indicator until a faint pink colour which persists for 20-30 seconds at the end point was observed. The number of milliters of KOH required was Noted and calculated from the formula below

$$\boldsymbol{Acid\ number = \frac{Vol.of\ 0.1m\ KOH \times 5.61}{weight\ of\ the\ sample\ in\ g}}$$

Note 5.61 = mg of KOH contained in 1ml of a 0.1M solution.

Peroxide Value

Ten gram (10g) of oil sample was dissolved in a mixture of glacial acetic acid and chloroform (3:2). Saturated KI solution (about 0.5ml) was added and allowed to stand for 1min. 30ml of water was added to the solution and it was titrated with solution of 0.1M $Na_2S_2O_3$ (Sodium thiosulphate) using starch as an indicator added towards the end point. A blank titration was also performed and the peroxide value was obtained from the formula below

$$\boldsymbol{iodine\ value} = \frac{\boldsymbol{(b-a)\times 1000\times molarityof\ Na2S2O3}}{\boldsymbol{weight\ of\ sample\ in\ g}}$$

Where b = burette for blank

a= burette reading for sample

Saponification Value

The saponification value was determined by weighing out 5ml of the oil sample into a 250ml conical flask and 50ml of 0.5M alcoholic KOH was added by means of a pipette. A reflux condenser was attached and the mixture was allowed to reflux for exactly 1hour on a water bath with frequently swirling the contents. The water bath was removed from under the flask and 5ml of phenolphthalein was added down the condenser (in order to wash the latter without diluting the content of the flask). The flask was allowed to cool for 5mins under the bath and it was titrated with 0.5M HCL ("a" ml). A blank determination without the oil was perform under the same similar condition and the number of ml of 0.5M HCL required ("b" ml) was noted. A duplicate determination was performed and the saponification value was calculated from the formula below**.**

$$\textbf{Saponification value} = \frac{\boldsymbol{(b-a)\times 56.1\times molarity\ of\ acid}}{\boldsymbol{weight\ of\ sample\ (in\ g)}}$$

Where b = burette for blank

a= burette reading for sample

Iodine Value

Accurately, 0.5g of oil was weighed into a 250ml dry iodine flask and 10ml of carbon tetrachloride was added to dissolve the oil. 20ml of iodinemonochloride (wigs or Hanus reagent) solution was added from a burette and a stopper previously moistened with KI solution was inserted and allowed to stand in the dark for 30mins. The stopper was partly removed and 15ml of 10% (w/v) KI solution was poured over the stopper and into the flask. 100ml of water was added in the same way and the stopper was inserted and shaked vigorously. The content of the flask was titrated with 0.1M sodium thiosulphate solution using starch mucilage, added

towards the end point as indicator. The number of ml required "a" ml was noted and the operation was carried out exactly the same manner, but without the oil and the number of ml of 0.1M $Na_2S_2O_3$ (Sodium thiosulphate) required "b" ml was noted. A duplicate determination was made and the iodine value of the sample was calculated thus.

$$\textbf{\textit{iodine value}} = \frac{(\boldsymbol{b-a}) \times \mathbf{12.7} \times \textbf{\textit{molarityof Na2S2O3}} \times \mathbf{100}}{\textbf{\textit{weight of sample in g}}}$$

Where b = burette for blank

a= burette reading for sample

Ester Value

The ester value was determined by subtracting the value of acid value from the saponification value.

Results

Table 1: Shows the results of the characterization of *Ocimum gratissimum* and *Ocimum basilicum* fresh leaves fixed oil

Parameters	***Ocimum gratissimum***	***Ocimum basilicum***
p^H	5.86±0.000	7.73±0.000
Specific Gravity	0.95±0.000	0.84±0.000
Peroxide Value (Meq/kg)	16.25±0.141	2.88±0.000
Acid Value (mgKOH/g)	16.105±0.078	13.495±0.431
Iodine Value ($g|_2$/100g)	21.705±0.177	13.445±0.007
Saponification Value (mg/KOH)	155.71±0.035	294.44±0.042
Ester Value (mg/KOH)	139.60±0.042	280.59±0.120

Results are presented as mean ± standard deviation of the duplicate determinations.

Discussion

The pH of *Ocimum basilicum* (7.73) is notably higher than that of *Ocimum gratissimum* (5.86). pH levels can influence the stability and effectiveness of substances, including in formulations for pharmaceuticals or cosmetics. The higher pH of *O. basilicum* might indicate a more alkaline environment compared to *O. gratissimum*.

The saponification value is an indicator of the average molecular weight of the fatty acids present in the oil, while the ester value is an indicator of the amount of free fatty acids present in the oil. The higher saponification value and ester value of *Ocimum basilicum* fixed oil (294.44±0.042mg/KOH and 280.59±0.120mg/KOH respectively) suggest that it has a higher content of free fatty acids and a lower molecular weight of fatty acids compared to *Ocimum gratissimum* fixed oil (155.71±0.035mg/KOH and 139.60±0.042mg/KOH respectively). This

result on *Ocimum gratissimum* is slightly comparable to saponification value of 164.2mg KOH/g reported by Idris *et al*., (2020). Also, Kadam *et al*., (2012) reported that the saponification value for *O. basilicum* seed oil as 194.94mg KOH/g.

The peroxide value is an indicator of the degree of oxidation of the oil, while the acid value is an indicator of the amount of free fatty acids present in the oil. The higher peroxide value and acid value of *Ocimum gratissimum* fixed oil (16.25±0.141Meq/kg and 16.105±0.078 mgKOH/g respectively) suggest that it is more susceptible to oxidation and has a higher content of free fatty acids compared to *Ocimum basilicum* fixed oil (2.88±0.000Meq/kg and 13.495±0.431 mgKOH/g respectively). A slightly similar peroxide value of 4.6 meq/kg was recorded on physicochemical properties and fatty acid composition of *Ocimum basilicum* L. seed oil and a lower acid value of 4.0mgKOH/g by Idris *et al*., (2020).

The iodine value is an indicator of the degree of unsaturation of the oil, while the specific gravity is an indicator of the density of the oil. The drying quality of the oil can be considered as one of factors of oil classification; it could be non-drying, semi-drying or drying oil through the analysis of the iodine value (Talkit *et al.*, 2012). Iodine value represents true unsaturation of fats only when double bonds are unconjugated and addition of iodine is not interfered by other groups. The higher iodine value, the more unsaturated fatty acid bonds are present in a fat/oil. It is a measure, which indicates the potential of a fat to be oxidized. The higher the iodine value and specific gravity of *Ocimum gratissimum* fixed oil (21.705±0.177gI_2/100g and 0.95±0.000 respectively) suggest that it has a higher degree of unsaturation and a higher density compared to *Ocimum basilicum* fixed oil (13.445±0.007gI_2/100g and 0.84±0.000 respectively). The result suggests that the oils are nondrying oils, and at such they can be used for soap making (hard soaps) and in food products. Non-drying oil is oil which does not harden when it is exposed to air. This is as opposed to a drying oil, which hardens (through polymerization) completely, or semi-drying oil, which partially hardens. Oils with an iodine number of less than 100 gI_2/100g are considered non-drying (Warra *et al.*, 2011). The specific gravity is considered as a good index of purity of oils. The increase in chain length of fatty acid present in oil tends to increase the specific gravity of oils. A study conducted by Idris *et al*., (2020) reported a similar specific gravity (0.9210) and iodine value (108.6g/100g) which was higher compared to this present investigation.

These differences in chemical properties can be attributed to the variation in the growth conditions, such as soil composition, temperature, and humidity, which can affect the synthesis and accumulation of oils in the plant (Idris *et al.*,2020).

Conclusion

The research results show that *Ocimum basilicum* fixed oil has higher pH, saponification value, and ester value compared to *Ocimum gratissimum* fixed oil, while *Ocimum gratissimum* fixed oil has higher specific gravity, peroxide value, acid value, and iodine value compared to *Ocimum basilicum* fixed oil. These differences can be attributed to the variation in the composition of essential oils, which are known to have various biological activities.

Recommendation

Further studies are needed to fully understand the implications of these differences and their potential applications in various industries.

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