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Research Article

Determining the Physicochemical Properties of Lemongrass oil Extract for Suitability in Various Applications

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Abstract:

Essential oil such as lemongrass oil is currently gaining traction globally probably due to the growing concern of the potentially hazardous effect of synthetic spices, gains in consuming organic products, and its medicinal, agricultural, and cosmetics values. For appropriate use of lemongrass oil and its suitability for the intended applications, there is a need to assess its physicochemical properties. In this work, the properties investigated included saponification, acid, free fatty acid, iodine, and peroxide values, respectively, refractive index, viscosity, density, and pH. These were determined mostly according to AOAC (2000). The results obtained were viscosity (211.5 pa.s), saponification value (165.49 mg KOH. g-1), iodine value (84.6 mg/100g of sample), peroxide value (14.2 meq/kg), pH (6.57), acid value (4.488 mg KOH/g), free fatty acid (1.244 % oleic acid), refractive index (1.442), density at 25oC (0.984 g/mL) and specific gravity (0.982). Most of these results are in agreement with prescribed standards, and deviations are attributed predominantly to the difference in the geographical location where the lemongrass was grown. Based on the results, the lemongrass oil is relatively of good quality and preservation status, and is suitable for applications in the food industry, agriculture, cosmetics, beverages and medicine, and bioenergy production.

Key words: lemongrass; physicochemical; properties; essential oil; extraction

1. Introduction

Lemongrass (*Cymbopogon citratus*) is typically a tropical perennial plant that produces aromatic oil that is cultivated in tropical and subtropical areas including Africa, India, Malaysia, Sri Lanka, China, Cambodia, and Guatemala. Its aromatic oil is one of the most popular essential oils. These oils are commonly used traditionally for various applications such as food, agriculture, medicine, and cosmetics. Its applications vary in different countries, for instance in Ethiopia, lemongrass has been used as an additive, killing lice and mosquitoes, treatment of hypertension, abortifacient, bronchitis, cold, fever, malaria, hemorrhoids, and toothache (Letebo, 2018; Maryo, et., 2015). In Nigeria, lemongrass is reported to have been used for the treatment of fever, convulsion in children, throat inflammations, stomach upset, skin diseases, ears/eyes infections, pepper soup ingredient, curries, and as a local drink (Okpo and Edeh, 2022).

Based on the foregoing, there is a need to determine the physicochemical properties of lemongrass oil. Such properties include colour, odour, density, specific gravity, refractive index, optical rotation, acid value, iodine value, and saponification value influence indirectly the quality of both essential and fixed (Ibrar, et al., 2011). These properties determine the storage status and suitability of essential oils for various applications such as human consumption (Bamgboye and Adejumo, 2010; Parthiban, et al., 2011). The iodine value may be used to predict the potential of oil to rancid and to determine the level of deterioration of the oil due to enzymatic or chemical oxidation (Dawodu and Omole, 2000). The acid value helps to determine the quality, age, edibility, and suitability of oil for various industrial applications such as paint (Akubugwo, et al., 2008). This value measures the extent of the hydrolysis of the glycerides in the oil by enzymes such as lipase, and physical factors like light and heat (Demian, 1990). The saponification value indicates the average molecular mass of various fatty acids in the oil, and the lower the molecular weight of fatty acids, the lower the saponification value. The viscosity is a measure of the resistance to fluid motion and determines the rheological properties of the essential oils (Kimbonguila, et. al, 2010). The viscosity of vegetable oils increases with the chain length of the fatty acid constituents (Tovar, 2011). The density is used to determine the quality of essential oil and has a direct relationship with the composition of the essential oil (Florido et al., 2013). The refractive index (RI) is used for the identification of compounds, determination of their purity, and for analyzing the ratio of homogeneous binary mixtures of known components leading to ascertaining the quality of essential oils quickly

and cheaply. The refractive index of vegetable oils has a relationship with the degree of saturation, as cis/trans double bonds, and can be transformed by the incident light used in the assay (Ospina, et. Al., 2016). The iodine value is the number of grams of iodine absorbed per 100 g of fat or oil and it measures the degree of unsaturation with a high value being indicative of a high degree of unsaturation, and the range for semi-drying of oil is 100-300 (Kagwachie and Anozie, 1995). This value helps to predict the drying properties of oils and suggests if the oil is suitable to be used as a drying agent in the manufacture of oil paints, varnishes, cosmetics, and as cocking oil manufacturing index (Akinhanmi et al., 2008; Adelaja, 2006). The iodine value indicates whether essential oils may rancid, and determines the level of oxidative deterioration of the oil due to enzymatic or chemical oxidation (Dawodu and Omole, 2000). Determining the fatty acid helps to ascertain the degree of degradation of the essential oil. The degradation leads to the production of FFA and increases the acidity of the oil (Tovar, 2011). The peroxide value indicates the extent of lipid oxidation with a high value showing high levels of oxidative rancidity of oil which means the absence or low level of antioxidants (Kyari, 2008).

2.0 Materials and Methods

2.1 Materials

Some amount of lemongrass leaves were freshly harvested at Ozoro, Delta State, Nigeria as indicated with the arrow in Figure 1. Shuniu GG-17 Soxhlet apparatus was used to carry out the essential oil extraction, and the reagents used were of analytical grade. Setra analytical weighing balance BL-410s was used to determine the weights of the materials.

2.2 Sample preparation

The freshly harvested lemongrass leaves were washed and dried for 8h in an oven to reduce their moisture content, and preserved in a sealed bag to prevent exposure to direct sunlight. The dried leaves were cut into 0.5cm which was the optimum particle size that gave the highest yield of essential oil in a previous experiment (Okpo and Edeh, 2022).

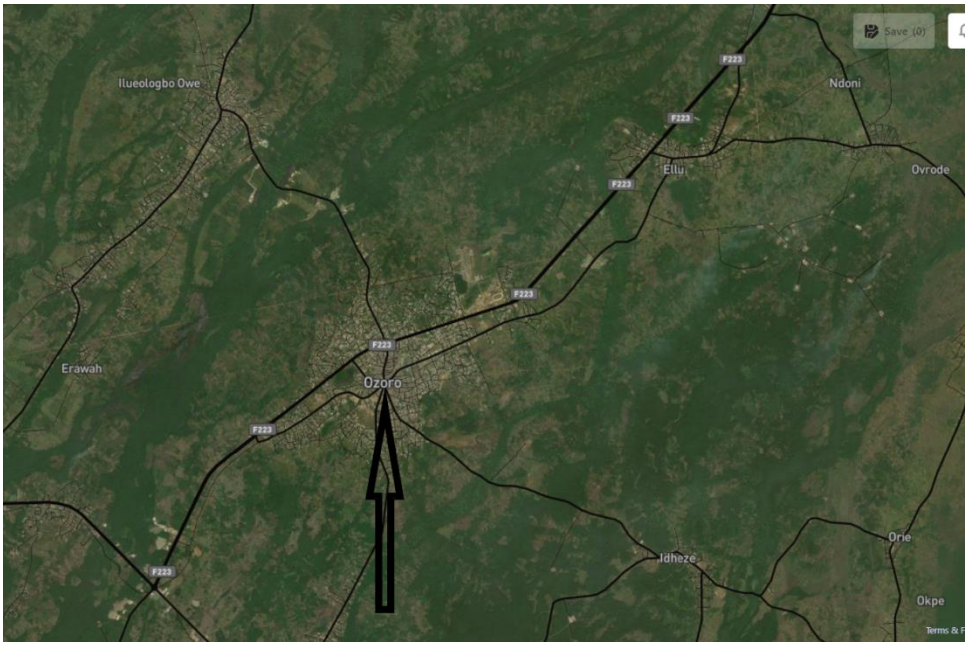

Figure 1: A map showing the location of Ozoro where the sample was collected

2.3 Experimental procedures

Extraction of lemongrass oil

The extraction of the lemongrass oil was conducted according to the method described by Okpo and Edeh (2022) using acetone as the organic solvent. 100g of the dried lemongrass leaves sample and 300 mL of acetone were used. The acetone was recovered in a rotary evaporator operated at a speed of 110 rpm, and the lemongrass oil extract was dried to a constant weight in an oven.

Determination of the physicochemical properties

Determining the physicochemical properties of the lemongrass oil extract makes it possible to ascertain its quality and hence, potential applications. The following properties were analysed saponification values (SV), acid value (number), % FFA, peroxide values (PV), iodine value, viscosity, flash point, melting point, pH value, specific gravity(density), the refractive index of the lemongrass oil were determined.

Saponification value

A weight of 2g of the lemongrass oil extract sample was put into a 250 mL Erlenmeyer flask followed by the addition of 25 mL alcoholic KOH solution through a pipette. Similarly, a blank was prepared without the oil extract but contained 25 mL alcoholic KOH. The flask was attached to a condenser for 2 hr to ensure full dissolution of the species, and upon cooling, the unreacted KOH was titrated with 0.5N HCl using 1.0 mL phenolphthalein indicator, until the colour was pink. The saponification value was calculated using Equation 1, according to AOAC (2000)

$$\text{Saponification value} = \frac{56.1(B-S)N}{W} \qquad (1)$$

Where: B = Vol. in milliliters (mL) of standard HCl acid requires for blank titration; S = Vol. in milliliters (mL) of standard HCl acid requires for sample; N = Standard HCl acid Normality; and W = mass of oil sample in (g).

Acid value

The acid value was determined according to AOAC (2000) Official method 920.159 procedure. The oil sample was thoroughly mixed before 2.5g was weighed into a 250 mL conical flask, and 100 mL of freshly neutralized hot ethyl alcohol was added. The mixture was boiled for five minutes and titrated with 10 N KOH using 1 mL of phenolphthalein indicator solution until a pink colour was obtained indicating that the endpoint has been reached. The weight of the oil taken for the estimation and the strength of the alkali used for titration were such that the volume of alkali required for the titration did not exceed 10 mL. The acid value was calculated using Equation 2.

$$\text{Acid value} = \frac{56.1 * V * N}{W} \quad (2)$$

Where: V= Volume in milliliters (mL) of standard KOH or NaOH used; N = KOH solution (or NaOH solution) normality, and W = Weight of sample oil (g)

Free fatty acids-FFA (% oleic acid)

The percentage FFA in the oil was calculated using Equation 3

$$\text{FFA (\% oleic)} = \frac{Acid\ value}{2} \quad (3)$$

Iodine value (IV)

The iodine value was evaluated according to AOAC (2000) Official method 920.159 procedure. Here, 0.3g of the oil was weighed into a 500 mL conical flask, and 25 mL of carbon tetrachloride was added. The mixture was vortex thoroughly. The sample weight was measured such that there was an excess of 50 to 60 % of Wijs solution over what was required. 25 mL of Wijs solution was pipetted into the mixture and stoppered after 15 mL of potassium iodide solution was added, followed by 100 mL distilled water. The mixture was vortexed and the flask was kept in a dark cupboard for 30 minutes. The liberated iodine was titrated with standardized sodium thiosulphate solution, using a few drops of 1% of starch as an indicator until the blue colour disappeared after vortexing vigorously while stoppered. A blank test was carried out without the oil following the above procedure. The iodine value was evaluated applying Equation 4.

$$\text{Iodine value} = \frac{12.69\ (B-S)N}{W} \quad (4)$$

Where: B = volume in mL of standard sodium thiosulphate solution required for the blank; S = volume in mL of standard sodium thiosulphate solution required for the sample; N = normality of the standard sodium thiosulphate solution; and W = weight in grams of the sample.

Peroxide value (PV)

To determine the peroxide value, 1.0g of oil sample was measured into a conical flask, and 25 mL of glacial acetic acid/chloroform solvent (2/1, v/v) was added. Thereafter, 1mL of saturated potassium iodine was added and the mixture was kept in a dark cupboard for 3 min. Thereafter, 30 mL of distilled water was added and the resulting mixture was titrated with 0.02N thiosulphate solution ($Na_2S_2O_3$) using 5 mL starch as an indicator. The procedure was repeated without the use of oil extract to obtain the blank. The peroxide value was obtained using Equation 5.

$$\text{Peroxide value} = \frac{1000 * (V_1 - V_2) * N}{W} \quad (5)$$

Where: W = oil sample weight in grams (g); V1 = volume in (mL) of $Na_2S_2O_3$ used in the test; V_2 = volume in (mL) of $Na_2S_2O_3$ used in the blank; and N = Normality of sodium thiosulphate ($Na_2S_2O_3$)

Refractive index

The Abbe's refractometer was used to find the refractive index of lemongrass oil sample. 2 drops of oil sample were placed on the refractometer prism using 2.5 mL of syringe, thereafter, the prism was secured firmly by using the screw heads. The equipment was allowed to stabilize for 10 min before the reading was obtained from the display screen.

Dynamic and kinematic viscosity

A viscometer was used to measure the dynamic viscosity of the lemongrass oil extract. The can containing the oil sample was kept under the viscometer rotor spindle. The spindle was then manipulated to immerse in the oil sample container. Thereafter, the viscometer was switched on and adjusted to 6m/s rotor speed. The rotor spindle was made to rotate in the oil sample for 30 min till stability was attained. At this point, the dynamic viscosity value of the sample oil was noted and recorded at the temperature of 20°C. The kinematic viscosity was calculated using Equation 6.

$$\text{Kinematic viscosity} = \frac{\text{Dynamic viscosity}}{Density\ of\ sample\ oil} \quad (6)$$

Specific gravity

Density bottle also known as pycnometer was used to evaluate oil density (specific density). A clean dry bottle having a capacity of 25 mL was weighed as 'W_1' with digital weighing balance and subsequently, the bottle was filled with oil lemongrass oil sample and the lid was covered and reweighed as 'W_2'. The respective weights were noted and recorded appropriately. Thereafter, oil was replaced with H2O after washing and drying the bottle. The weight of bottle and water was also noted as W3. Equation 7 was used to obtain specific gravity (SP) of the oil sample.

$$\text{Specific gravity} = \frac{W_2 - W_1}{W_3 - W_1} = \frac{Mass\ of\ sample}{Mass\ of\ an\ equal\ volume\ of\ water} \quad (7)$$

pH

2g of lemongrass oil extract was weighed into 25 mL beaker and 10 mL of hot nonionized hot H_2O was added, before, it was stirred gently. Thereafter, the mixture was allowed to cool in a water bath to 25 °C. The pH electrode was standardized with a buffer solution first and then immersed in the mixture and the pH reading was recorded (AOAC, 2000).

3.0 Results and discussion

3.1 Determining the physicochemical properties of lemongrass oil

This was conducted to assess the potential applications of the lemongrass oil extract. The results obtained are presented in Table 1.

Density

The density and specific gravity of the lemongrass oil extract obtained at 25°C were 0.984g/mL and 0.982, respectively. The value of the density is greater than 0.908 and obtained by Tovar et al. (2011) for the distillate and residue streams, respectively at 120°C. The disparity may be attributed to difference in extraction methods, and the geographical location where the lemongrass was grown. The specific gravity of 0.982 is in agreement with the literature value which is less than one (Tovar, et al., 2011).

Viscosity

Properties such as viscosity help to assess the rheological behaviour of essential oil (Santos, 2005). Essentially, the viscosity of essential oil is high because of the presence of a long fatty chain structure. The viscosity of the lemongrass oil extract obtained at 25°C was 211.5 pa.s. Comparing this result with the highest range of 2.430 to 2.491 mPa.s obtained at 60 to 120°C from the residue stream of the short-path-distillation process of lemongrass essential oil recorded by Tovar, et al. (2011), showed that the

viscosity of the lemongrass is low. The disparity may be due to the difference in temperature and geographical location where the lemongrass was grown.

pH

The pH obtained was 6.57 as against 5.6 recorded by Abera (2020) showing that the lemongrass oil extract is slightly acidic.

Refractive index

The refractive index obtained was 1.442 which is lower than 1.48900.0002, 1.4870 0.0002, 1.4883 0.0002 obtained by Mieso and Befa (2020) for Lomisar-I, WG-Lomisar-Java and WG-lomisar-UA (upper awash), respectively. The high refractive index shows that the lemongrass oil extract may have a high long fatty acid chain composition (Ibrar and Inyat-Ur-Rahman, 2012).

Iodine value

The iodine value obtained was 84.6 mg/100g of sample and compared to 100-300 mg/100g of sample prescribed for semi-drying of oil (Kagwachie and Anozie, 1995). The value is out of the prescribed range and this shows that the lemongrass oil extract has a low degree of unsaturation.

Acid value

The acid value obtained was 4.488 mg KOH/g and it is slightly above 4.09 mg KOH/g and 4.35 mg KOH/g reported by Mustapha (2018) and Dutta et al., (2014). Although, this value is within the specification of 30 prescribed by the FAO (2015).

Physicochemical properties/Units	value
Viscosity at 25°C (pa.s)	211.5
Saponification value (mg KOH.g^{-1})	165.49
Iodine value (mg/100g of sample)	84.6
Peroxide value (meq/kg)	14.2
pH	6.57
Acid value (mg KOH/g)	4.488
Free fatty acid (% oleic acid)	2.244
Refractive index	1.442
Density at 25°C (g/mL)	0.984
Specific gravity	0.982

Table 1: Physicochemical properties of the lemongrass oil

The difference may be attributed to the geographical location where the lemongrass was grown and the method of extraction (Dao, et al., 2020). Acid value impacts the storage quality of oil, with a higher value indicating lower storage quality (Abera, 2020). Thus, an acid value of 4.888 mg KOH/g obtained in this work, shows that the quality of the lemongrass oil extract will not deteriorate significantly upon storage. Since the acid value is lower than the 10 acceptable limits for edible oil, the lemongrass oil extract can be used in food manufacturing industries as an additive (Sampson, 2005).

Saponification value

The saponification value obtained was 165.49 KOH/g as shown in Table 1. This result is higher than 140.25 mg KOH/g and 144.16 reported by Abera (2020) and Dutta et al. (2014), respectively. The disparity could be due to environmental factors or the method of drying Abera (2020). Although the saponification values are out of the range 189-199 prescribed by the FAO (2015), lemongrass has a potential for application in the cosmetic industry for soap and bioenergy production, as the higher saponification values are an indication of high oil content (Dutta et al., 2014; Haque, 2018).

Free fatty acid

Free fatty acid helps to determine the extent of degradation of essential oil. The free fatty acid obtained as shown in Table 1 was 2.244 % of oleic acid. The value is less than 2.36 - 3.50 wt % of oleic acid obtained by Tovar, et al. (2011) in the residue stream, but greater than 0.5 prescribed by the FAO (2015). The result shows that the lemongrass oil extract is of better quality compared to that obtained by Tovar, et al. (2011), but may not be suitable for consumption, as the value is greater than 2.0% required for human consumption (ANVISA, 1999). The high FFA is an indication that the essential oil extract is degrading increasingly and there is losses in its composition. The lemongrass oil extract, although has the potential of being used for bioenergy production may face the challenge of saponification reaction resulting in soaps formation, especially if used for biodiesel production (see Figure 2). The soap may cause more catalysts to be consumed, lower catalyst efficiency, and increase the viscosity of the biodiesel mixture. This gives rise to a low yield of biodiesel, especially if an alkali catalyst is used and difficulty in purification (Saraf, 2007).

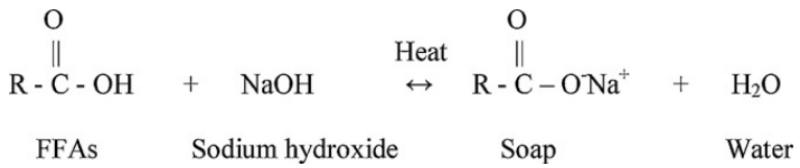

Figure 2: Saponification from FF

Peroxide value

The peroxide value of lemongrass oil obtained was 14.2 meq/kg and this result is higher than less than 10 meq/kg required for fresh oil. The high value shows that the oil extract has high oxidative rancidity and low level of antioxidants (Kyari, 2008).

Conclusion

The physicochemical properties of lemongrass oil extract have been assessed. The acid and saponification values of 4.488 mg KOH/g and 165.49 mg KOH.g-1, respectively demonstrate that the oil can be used in the manufacture of cosmetics. The good acid value suggests that the lemongrass oil extract may be used as a food additive. Although the value of the free fatty acid is greater than 0.5, the lemongrass oil can be used for the production of bioenergy such as biodiesel and renewable diesel, preferably using acidic catalysts. These bioenergies can also be effectively produced due to the high free fatty acid with two-stage processes involving esterification before transesterification using acidic and alkaline catalysts, respectively. The pH of 6.57 shows that lemongrass is slightly acidic and can be used for human consumption as it is projected to have some health benefits. The peroxide value of slightly greater than 10 meq/kg showed that the lemongrass oil extract is relatively of good quality and storage status.

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