Characterization of Oil Extracted from Agricultural Wastes: Sweet Orange Seeds (*Citrus sinesis*) from Ogbia in the Niger Delta Region of Nigeria.

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Abstract

The physicochemical properties of oil extracted from sweet orange seed fruit discards in Ogbia were investigated. The oil was extracted from the powdered seed samples in a soxhlet apparatus using hexane and petroleum ether as solvents. A higher percentage yield (31.40 %) was recorded in the Hexane extract as compared to a 12.80 % yield for the petroleum ether extract. *Citrus sinesis* seed oil had a moisture/volatile matter content of 31.76 %, a peroxide value of 10.20mg/kg, free fatty acid (4.85 %). The acid value of the oil was 9.65±0.84 mg KOH/g and was in line with the acceptable limits of ≤10 mg KOH/g for edible oils. The iodine value was 102.2±3.66 mg ioding/100g and the oil had a saponification value of 196.65±4.87 mg KOH/g. This finding had shown that the waste seeds of *C. sinesis* could serves as a viable feedstock for the extraction of oil with possible industrial applications.

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INTRODUCTION

The demand for edible oil is on the increase all over the world. Besides its use as food, seed oils have found application in the pharmaceutical, detergent, textile, cosmetics and surfactant industries (Montesano *et al.*, 2018).In order to satisfy this increasing global demand, much interest is presently being focused on exploring the potentials of some newer and underutilized plant resources for the production of edible oils (O'Brien, 2018).

Citrus sinesis seeds which are usually agro-industrial wastes could be put to better use instead of been discarded. Many crop plant seeds are currently being used in the extraction of edible oil for homes and industrial purposes in many developed countries (Wang *et al.*, 2017).Oil was being extracted from fruit seeds many years ago but the olive tree fruit remains the oldest source of oil. Today there are many seeds, kernels and fruits that are potential sources of edible oils (Iwuagwu *et al.*, 2018).

In Nigeria, *Citrus* fruits are largely consumed due to their high ascorbic acid content. The seeds which are fruit discards are presently underutilized and could be explored in edible oil production. Studies on the characterization and possible utilization of citrus seeds in oil production, assessment of sweet orange seed oil for biodiesel production and evaluation of volatile oils extracted from orange peels has been reported (Abdulhamid *et al.*, 2014; El- Demery *et al.*, 2015; Sadiq, 2016). Therefore, the main objective of this study is to analyseand compare the physicochemical and biochemical properties of the seeds oil of *C. Sinesis* grown in Ogbia local government area of Bayelsa State with previous studies.

MATERIALS AND METHODS

Sweet orange seeds were collected at random from Otuoke and its environs in Ogbia Local Government Area in Bayelsa State, Nigeria, between September and October, 2017. The orange seeds were washed and sun dried at intervals for a period of two weeks. The seeds were decorticated (the cellulose seeds coat or testa removed) and subjected to further drying in an oven (50^0C) for four hours and allowed to cool before milling into flakes in a laboratory porcelain mortar. The milled samples were then placed in a desiccator for two hours before extraction.

Extraction of oil from *Citrus sinesis* seeds

Milled seeds (250 g) of *C. sinesis* was extracted using soxhlet apparatus with n - hexane and petroleum ether as solvents respectively, at 40-60^{o}C for six hours. The solvent was separated from the oil at reduced temperature, pressure and refluxing at 70^{o}C. The oil obtained was stored in the freezer until needed for subsequent analysis. All analysis were carried out in triplicate.

Determination of percentage (%) yield of extracted oil

The percentage (%) of oil extracted was determined by the method of Kyari (2008). Solvent was freed from the oil obtained after extraction then placed over a water bath at 70^{o}C for 30mins. The volume of oil was recorded in percentage (%) as calculated below;

$$\text{Oil content (\%)} = \frac{weight\ of\ oil}{weight\ of\ sample}\ x\ 100$$

Physicochemical parameters of oil extract

Moisture/volatile content determination

The moisture and volatile content of the oil extract was estimated by the method of Kyari (2008). A crucible was dried in the oven after washing, then allowed to cool in the desiccator and weighed (W_1). Two grams of the sample was weighed in the crucible and weight was taken, this was named (W_2). The crucible containing the sample was placed in an oven at 105°C for 1 hour. The heated sample was allowed to cool and then reweighed. The crucible was then transferred into the oven again. The process of cooling and weighing at intervals continued until a constant weight was obtained (W_3). Moisture content was calculated using the relation:

$$\text{Percentage (\%) moisture content} = \frac{w_2 - w_3}{w_2 - w_1}\ x\ 100$$

Determination of relative density of oil

The determination of relative density of oil was carried out employing the method of Pearson (1980). A specific density bottle was washed, dried and weighed (W_1) and then filled with distilled water and weighed (W_2).The water was poured off, and then bottle was dried to its previous constant weight. The bottle was filled with the oil sample and weighed (W_3).

$$\text{Relative density} = \frac{w_2 - w_3}{w_2 - w_1}\ x\ 100$$

Determination of iodine value

The iodine value (Wiji's method) was determined by the titration method as described by AOAC (2000). Two grams of oil sample was weighed into a 250 ml flask and then 20 ml of CCl_4 and 25 ml of Wiji's reagent was added to the flask, cocked and vigorously swirled. The flask was placed in the dark for 1 hour 30 minutes. Thereafter, 20 ml of KI solution and 150 ml of water were added. This was titrated with 0.1M $Na_2S_2O_3.5H_2O$solution until a yellow colour was noticed. Afterword, few drops of starch solution was added and, then titration continued until the disappearance of blue colour. The same process was repeated for the blank. The iodine value was calculated using the following mathematical relation;

$$\text{Iodine value} = \frac{\text{(A-B) x (N of } Na_2S_2O_3.5H_2O\text{) x 12.69}}{Q}$$

Where;
A is volume of 0.1M $Na_2S_2O_3.5H_2O$ solution used as blank titration.
B is Volume of 0.l M of $Na_2S_2O_3.5H_2O$ solution used as sample titration.
Q is Weight in grams of the oil sample
12.69 is the Conversion factor.
N is the Normality

Determination of the Saponification value of oil

The method described by AOAC (2000) was used for the determination of saponification value. Two gram of oil sample was weighed into 200ml beaker and 25ml of 0.5M of ethanolic potassium hydroxide solution was than added. The beaker was configured to a condensing set-up then heated on a water-bath for an hour with frequent shaking. The content was then allowed to cool. The solution was titrated with warm 0.5M HCl using 1% Phenolphthalein as an indicator. Same titration procedure was followed for the blank.

$$\text{Saponification value} = \frac{A-B}{Q}\ x\ 28.05$$

Where,
A is volume of 0.5M HCl used in the blank titration.
B is volume of 0.5M HCl used in the sample titration.
Q is weight in grams of oil sample.
28.05 is conversion Factor.

Determination of Acid Value of oil

The acid value of extracted oil was determined by the method of AOAC (2000). Two grams of oil was added into 250 ml beaker; thereafter, 25ml of ethanol and 1 ml of phenolphthalein indicator solution was added. The solution was allowed to boil for 5 minutes and then titrated in the hot state against 0.1 M KOH solution. When pink colour persisted for 30 seconds the endpoint was reached. Acid value was calculated as follows;

$$\text{Acid Value} = \frac{56.1 \; x \; V \; x \; C}{M}$$

Where: V = volume of KOH (ml), C = concentration of KOH, M = mass of the test portion (g), and 56.1 = the molar mass of KOH.

Determination of Free Fatty Acid

The Free fatty acid % is expressed as oleic acid % using the method of AOCS (2009). The acidity is frequently expressed as FFA for which calculation was made using the following equation;

$$\text{FFA (oleic acid)} = \frac{28.2 \; x \; V \; x \; N}{M}$$

Where,
V = volume in ml of standard potassium hydroxide solution used (titrate value)
N = normality of standard potassium hydroxide solution
M = mass in g of the material taken for the test

Determination Peroxide value

The method as described by AOAC (2000) was employed in the determination of the saponification value of oil. Two grams of the oil sample was transferred into 250 ml beaker and one gram of powdered potassium iodide (KI) and a solvent mixture (2:1 of glacial acetic and CCl_4) were then added. The solution was placed on a water bath for 15 minutes for complete dissolution. About 20 ml of 50% KI was added and the sample was titrated with 0.1M $Na_2S_2O_3$. Regular starch solution was used as indicator. Blank experiment was done following same procedure.

$$\text{Peroxide value} = \frac{(R \times B) \times \text{morality of } Na_2S_2O_3}{W}.$$

Where, R = oil and B = blank samples in term of titre values.

RESULTS AND DISCUSSION

The physiochemical analysis result of *C. sinesis* seeds oil in Figure 1, showed that hexane as an extracting solvent had higher percentage yield (31.40 %) when compare with petroleum ether solvent (12.80 %). The values were lower compared to that obtained from *M. peregrine* seed oil (49.80%) reported from Saudi Arabia (Tsaknis, 1998) and that of egusi (53.20 %), pawpaw (40.10 %) and sweet orange seed oils (43.10 %) reported from northern Nigeria (Abdulhamid *et al.*, 2014). The variation in the oil content could be attributable to environmental or geographic conditions which dependent on various region (Manzoor *et al.*, 2007). This relative high percentage oil yield from hexane solvent in the study showed that it could be a viable extracting solvent in production industries. Hence, seed oil extracted with n – hexane was used for further characterization.

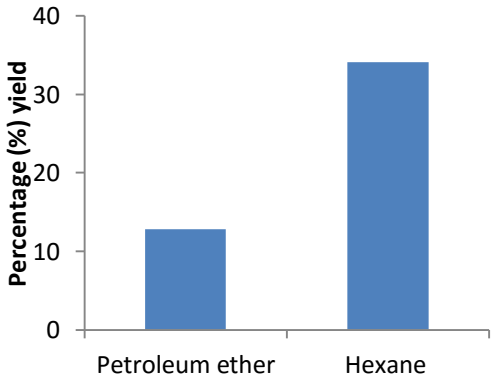

Figure 1: Percentage yield of *C.sinensis* seed oil using petroleum ether and hexane as extracting solvents.

Table 1: Physicochemical properties of sweet orange oil extracted with n-hexane

Parameters	Value
Moisture/volatile matter (%)	31.76±2.03
Relative density (gcm^{-3})	0.92±0.06
Peroxide value (mEq/kg)	10.20±1.10
Free fatty acid (%)	4.85±0.21
Acid value (mg KOH/g)	9.65±0.84
Iodine value (mg iodine/100g)	102.20±3.66
Saponification value (mg KOH/g)	196.65±4.87

Values are expressed as Mean±SD of triplicate determinations

The moisture/volatile matter value of the orange seed oil (31.76 %) was higher than 4.91% that was obtained from water melon seed oil by Taiwo *et al.*

(2008). This might have been as a result of the difference in chemical composition of the fruits, age and time of harvest. However, this is advantageous and very important in terms of shelf life of the seed, with lower moisture, the can be preserved for a longer time. The relative density of the *C. sinesis* seed oil was found to be 0.915 g/cm^3 at 25°C (figure 2), which falls within the range of recommended values of 0.914-0.917 for edible vegetable oil (Iwuagwu *et al.*, 2018).

Deterioration of oils is related to its peroxide value. Akubugwu and Ugbogu (2007) reported that fresh oils have peroxide values less than 10mEq/Kg^{-1} and oils with values between 20 – 40 results to rancid taste. The peroxide value for *C. sinesis* seed oil was found to be 10.20 mEq/kg. The peroxide value in this study was above the maximum limit of 2.0 mEq/kg reported by Codex Alimentairus Commission (1993). The peroxide value was also higher than that of egusi 5.80 mEq/kg and pawpaw 3.12mEq/kg (Abdulhamid *et al.*, 2014). High rancidity rate is associated with high peroxide value.

Free fatty acid values of less than 3 in seed oils could be used as edible oils (Akubugwu and Ugbogu, 2007). In this study, oleic acid was determined in form of free fatty acid in the orange oil seeds with 4.85 %. Values for free fatty acids in this study is relatively lower than that reported by Waheed *et al.*, (2009) for *C. aurantium* (4.66%), and *C. paradise* (3.58%). Oil of sweet orange from Otuoke and environs (study site) may not be used as edible oil since its free fatty acid value is more than 3.

Acid number is usually used to measures the presence of oxidative products and corrosive FFA. It is also an indicator for edibility of oil and suitability for use in the paint industry (Akubugwu and Ugbogu, 2007). The acid value for *C. sinesis* seed oil was 9.65±0.84% and was within the acceptable limits for edible oils ≤10 (Balley, 1982). However, the oil still need to be refined before they it can be utilized.

The iodine value of the *C. sinesis* seed oil was 102.2±3.66 mg iodine/100g. The iodine value according to Amoo *et al.* (2004) is an index employed to evaluate the ability of oil to go rancid. Hence, the greater the degree of unsaturation, the higher the iodine value and thus, the risk of the oil or fat to become rancid by oxidation is high (Egan *et al.*, 1981).However, Mahmud *et al.*(2009) reported that high content of unsaturated acid is expected in quality edible oil The iodine value (102.2±3.66 mg iodine/100g) obtained in this study is higher than that (56.40 mg iodine/100g) reported by Sadiq (2016) for oil from sweet orange seeds collected from Bauchi town, Nigeria. However, is relatively low when compared to the iodine value reported by Nzikou *et al.* (2007). Oils with iodine value of 112 could be utilized for cooking and many industries for the manufacture of ice-cream (vegetable oil-based ice-cream) (Nzikou *et al.*, 2007). Saponification value is usually use for checking adulteration. Saponification value of *C. sinesis* seed oil was196.65 mg KOH/g. The high saponification value of the orange seed oil in this study indicates the presence of high percentage of fatty acids in the oil. The saponification value is in line with the value 189.20 – 190.50mg KOH/g and 190.34 mg KOH/g obtained for *Psophocarpus tetragonolobus* seed oil by Rahman *et al*. (1997) and Amoo *et al.* (2006).According to Akubugwo and Ugbogu (2007), the relatively high value obtained indicates its potential for use in industries.

CONCLUSION

The orange seeds had higher yield of oil with hexane as an extracting solvent than petroleum ether solvent, making the solvent selective suitable for use. The seeds of *C. sinesis* contain high level of oil, which is of great potential for industrial and domestic purpose. Orange seed utilization economically will help in controlling the indiscriminate disposal of the seeds in the environment.

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