Chemical Analysis of (*Lagenaria siceraria*) Calabash Seeds Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Author MH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JI and SYL managed the analyses of the study. Author JS managed the literature searches. All authors read and approved the final manuscript.

Mini-review Article

ABSTRACT

Characterization of fats and oils is important in assessing their potential economic uses. This study was carried out to determine and compare the physicochemical characteristics of a commercial oil and solvent extracted oil from the kernels of calabash seeds (*Lagenaria siceraria*). The range of physicochemical properties are: Solvent extraction recorded a higher yield of (42.3±0.66%) acid value (9.4±1.8 - 8.2±0.5 mgKOH/g of oil), iodine value (4.56±2.6 - 4.46±0.07 mg/100g), saponification value (162.7±4.58 - 159.42±3.68 mgKOH/g), peroxide value (6.0±1.63 - 5.3±2.4 mEq/kg), free fatty acid (10.3±1.76 - 9.89±1.8 % as oleic acid). The low iodine value indicates a high degree of saturation of the fat as well as its non-drying nature.

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1. INTRODUCTION

The legume seeds are sources of human food that provide much-needed essential protein and oils for growth, repairs, etc. They play important roles in the acceptability of monotonous diets and balanced in many parts of the world [1]. Plant sources are the major providers of protein and oil intake in many developing countries [2]. This sample (calabash seeds) are underutilized because it is not widely consumed and their industrial processing is highly limited or non-existence except with some rural farmers using local processing methods. This is because their nutritional potentials have not been analyzed and tapped and only received little attention of food scientists [3].

Calabash is classified to the kingdom of Plantae, division of magnoloiophyta, class of mognliopsida, order of cucurbitacles, family of cucurbitaceous, genus of *Lagenaria* and species of *Lagenaria siceraria*, (synonyms *Lagenaria vulgaris*) commonly known in hausa language as "kwarya" or duma, Igba in Yoruba, Ugba in Igbo and calabash in English. It possesses simple leaves which are 400 mm long and 400 mm broad, oval shape and whitish seeds embedded in a spongy pulp, 7−20mm long [4]. Calabash are dicoteledonous plant, they are an annual leguminous plant that is cultivated under different types of soil and climate condition [5]. They are mainly cultivated in a rare part of the country [5]. Leguminous plants are value for their ability to grow in a symbiotic relationship with nitrogen fixing bacteria and for their drought resistance does to due to the presence of root nodules [6]. They have the ability of converting nitrogen from air in to useful form, thus has a little fertilizer requirement, they also provide nitrogen source to the next crops [7].

Structurally, calabash is a ball like or an orange like structure. it grows best in fertile loamy or clay soil with minimum range of rainfall during growing months. It is traditionally planted after harvested cereal crops, 3-4 seeds are planted per hole, about 2-3 centimeter deep and space of about 2.5-3 meter between the hoes, the seeds are sown as a single plant (crop) between august and September in non Fadama areas while between September and October in Fadama areas, germination occurs 6-7 days after planting, weeding is done at least 2-3 times before covering the soil. Calabash is usually harvested by using a saw (i.e., sawing) which is process of cutting it in to two equal parts, inside the calabash there is a substance which contains the seed, and the seed contain the seed coat that surrounding the embryo [8].

Due high abundance of calabash seeds in our area, but the people are unaware of its nutritional value, in lieu of this, carrying out physiochemical properties of oil would enable us to improve our nutritional, health and economic development of the people in that area. Embarking on a research project on the physiochemical properties and analysis on calabash seed oil will go a long way in guiding us in choosing right amount that would be good for human consumption. The aims of this research work is to characterize the calabash seeds oil based on its chemical composition in order to determine its quality while the objectives of this research are: to extract oil from the kernel seeds of calabash using sox let method, to determine, the chemical composition of calabash seeds oil and to suggest possible recommendation based on experimental outcome [9].

Fig. 1. Most popular calabash

Fig. 2. Calabash seed

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The seeds of *Lagenaria siceraria* (calabash) were obtained from calabash scrappers in new market Gummi town, Zamfara State, Nigeria. It was taxonomically classified at Biological science department, Botany unit of Usmanu Danfodiyo University Sokoto, as *Lagenaria siceraria*. The calabash seeds were soaked in to water overnight it was later remove out from water and sundried on mat for about two hours. The seed were gathered together and collected for decortications. The seeds were then placed on the floor and small object were used manually to crush the seed and this breaking it, to expose the inner content of seeds. The mixture was later winnowed in order to separate the inner content of seeds and broken shell. The shells were thrown away and the seed were retained for further processing. It then shed dried for about two days; the kernel seeds were later grinded using pastel and mortar which make it easier for oil extraction as described by [10]

2.2 Preparation of Reagents

2.2.1 1% Starch solution (W/V)

1g starch powder was dissolved in warm distilled water and made up to 100 mls with distilled water.

2.2.2 1% Phenolphthalein (W/V)

1g phenolphthalein powder dissolved with little quantity of ethanol; it was later transferred to 100mls with the same solvent.

2.2.3 10% Potassium iodide (W/V)

10g of potassium iodide was weighed in a beaker and dissolved with sufficient amount of distilled water and transferred in to 100mls conical flask, it was made up to 100mls.

2.2.4 0.1 M NaOH (W/V)

1 g of NaOH was weighed in a beaker and dissolved with sufficient amount of distilled water and finally transferred in to 250 mls where it was made up to 250 mls mark.

2.2.5 0.1N Sodium thiosulphate (Na2S2O3) (w/v)

6.2 gram of sodium thiosulphate was weighed in a beaker and dissolved in sufficient amount of distilled water and finally made up to 250 mls using stand flask.

2.2.6 Ethanolic potassium hydroxide (w/v)

20 g of potassium hydroxide was weighed in a beaker and dissolved bit by bit with ethanol and transferred in to 250 mls stand flask where it was made up to 200 mls.

2.3 Solvent (Mixture of Glacial Acetic Acid and Chloroform, (2:1)

80 mls of glacial acetic acid was poured into a conical flask and made up to 120mls with chloroform.

2.3.1 0.5 N HCL

About 10.6 mls of concentrated HCL was poured in a beaker containing distilled water and made up to 250 mls with distilled water.

2.4 Deracination of Crude Lipid by [11]

Crude lipids were extracted from the respective sample by the AOAC (198O) sox let method the principle is based on the solubility of lipid in organic solvent when heated. The procedure is as follows: 50 g 0f the grinded sample was weighed and transferred into empty a clean thimble and the mouth covered with cotton wool, the thimble was placed into the main chamber of sox let extractor fitted to a pre- weighed round button flask containing 250 mls n-hexane.

2.5 Iodine Value Determination by [11]

2.5.1 Principle

The method is based on the treatment of known weight of oil with a known volume of standard solution of iodine monochlorid (ICl) estimation of ICl by titrating iodine, liberated by adding an excess of KI. The titration was done against Na2S2O3 with starch as an indicator.

$$ICl + KI \; ---------------- \; KCl + I_2$$

$$2Na_2S_2O_3 + I_2 \; --------- \; Na_2S_4O_6 + 2NaI$$
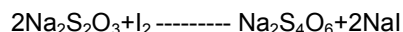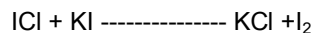
2.5.2 Procedure

0.5 g of oil was weighed in to a conical flask and then dissolved by adding 10 mls of carbon tetrachloride (CCl_4), 25 mls of wij's iodine was added to the mixture, it was shaken and allowed

to stand in a dark for one hours,15 mls of 10% potassium iodide and 100 mls of distilled water was also added, the resulting solution was then titrated against 0.1 N $Na_2S_2O_3$ until the initial yellow color almost disappear, few drops of starch solution was added and titration was continued until the blue color disappear which indicate the end point. The blank was performed at the same time, the test was done in triplicate, and the iodine value was then calculated using the formula below:

$$\text{Iodine value (mg/100 g)} = \frac{(b-s) \times N \times 1.269}{W}$$

b = volume (ml) of thiosulphate solution used in blank,
s = volume (ml) of thiosulphate solution used in test,
w = weight of sample,
N = normality of sodium thiosulphate.

2.6 Determination of Saponification Value by (12)

2.6.1 Principle

Saponification value is based on the number of milligram of KOH require to saponify or hydrolyze one gram of fat or oil, in it is estimation the KOH react with oil to produce potassium salt of component fatty acid (soap).

$$C_3H_5 (OCOR) + 3 KOH \text{ ----------------} C_3H_5 (OH)_3 + 3 RCOOK$$

Potassium hydroxide is added in excess and the amount remaining after saponification is determined through titration with standard acid.

2.6.2 Procedure

2g of oil was weighed in to conical flask, 20mls alcoholic potassium hydroxide was added, a reflux condenser was attached and the flask was heated for a 30 minute with thoroughly shaking to ensure that the sample was fully dissolve. After that the sample was left to cool and 1mls of 1% phenolphthalein indicator was added and later titrated against 0.5N HCl. the blank was perform at same time, the test was done in triplicate, the saponification was then calculated using the formula below:

$$\text{S.V (mgKOH/g of oil)} = 56.1 \frac{\times N \times (b-s)}{W}$$

b = volume (ml) of HCl used in blank,
s = volume (ml) of HCl used in test,
w= weight of oil
N= normality of HCl
56.1= molecular weight of KOH

2.7 Determination of Acid Value by [12]

2.7.1 Principle

The acid value of oil is determined by titration known weight of oil against 0.1N NaOH is using phenolphthalein as an indicator.

$$C_{17}H_{33}COOH + NaOH \text{ -----------} C_{17}H_{33}COONa + H_2O$$

2.7.2 Procedure

1g of oil was weighed in a conical flask, 25mls of denatured alcohol was added and shaken, 2 drops of phenolphthalein indicator was added, it was then titrated against 0.1N NaOH vigorous shaking, until pink color was obtained. The test was done in triplicate. The acid value was then calculated using the formula below:

$$\text{Acid value (mgKOH/g of oil)} = 56.1 \frac{\times N \times V}{W}$$

W = weight of oil
N = normality of NaOH used
V = volume (ml) of NaOH solution 56.1= molecular weight of KOH

2.8 Determination of Free Fatty Acid by [11]

This was determined by method as described by Devine and Williams (1995).

2.8.1 Principle

This is based on the amount of free acid present per gram of sample, which was determined by titration with a standard solution of NaOH.

2.8.2 Procedure

2 g of oil was weighed in a conical flask, 25 mls of denature alcohol was added and shaken, 2 drops of phenolphthalein indicator was also added, it was then titrated against 0.1N NaOH until pink color was obtained, The test was done in triplicate, the %FFA was calculated (as oleic acid) using the formula below:

%FFA = $\frac{2.82 \times N \times V \times 100}{W}$

%FFA = percentage free fatty acid
V = volume (ml) of NaOH solution
N = normality of NaOH used
W = weight of sample 282 = molecular weight of oleic acid

2.9 Determination of Peroxide Value by [11]

This was determined by the method as described by Devine and Williams (1995).

2.9.1 Principle

This is based on the oxidation of double bonds of unsaturated fatty acids by absorbing oxygen to peroxide. The peroxide liberates oxygen from potassium iodine. The liberated is titrated against the sodium thiosulphate solution.

2.9.2 Procedure

5 g of oil was weighed in a conical flask and 25 mls of solvent (glacial acetic acid and chloroform) was added, 1 mls of potassium iodide was also added and allowed to stand in a dark for 1minute so that the solution become straw yellow, after that 35 mls of distilled was added and then follow with 0.5 mls of 1% of starch indicator this was titrate against 0.1N sodium thiosulphate until blue color disappear. A blank was also performed at the same time. The test was done in triplicate. The peroxide value was calculated using the formula below:

Peroxide value (mEq/kg)= $\frac{(V_1 - V_2) \times N \times 1000}{W}$

V_1 = volume (ml) of Na2S2O3 solution used in test
V_2 = volume (ml) of Na2S2O3 solution used in blank
W = weight of oil sample.
N = normality of Na2S2O3 solution

3. RESULTS AND DISCUSSION

The chemical analysis of crude extract and commercial oil of *Lagenaria siceraria* seed oil were presented in Table 1 where the acid value of crude extract and commercial oil are 6.4 ± 0.43 and 5.7 ± 0.43 (mgKOH/g), these values however accounted for the presence of free fatty

acids in the oils as an indicator of presence and extent of hydrolysis by lipolytic enzymes and oxidation [12] also it is higher than that of reported value of ground nut oil 2.52 mgKOH/g [11].

As shown in Table 1 the saponification values for crude extract and commercial oil were 160.42± 2.96 and 159.42 ± 4.51 (mgKOH/g). It has been reported by [11,13] that oils with saponification values contain a high proportion of lower fatty acids. Therefore, the high saponification value obtained from this research indicated that they contain a low proportion of lower fatty acid and can be regarded as edible oils.

The iodine value is a measure of the degree of unsaturation and it is an identity characteristic of seed oils, making it an excellent raw material for soaps cosmetics industries [14]. The iodine value obtained from crude extract and commercial oil represented in Table 2 were 6 ± 0.26 and 4.49 ± 0.08 (mg/100 g) these values are higher than the standard range stipulated by [15] of 45-53 (mg/100 g). Oils with an iodine value less than 100 gI2/100 g of oil are non-drying oils; correspondingly, [8,16] reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity. Therefore, the value obtained from this research showed that, the oil is non- drying oils and is not suitable for ink and paint production due to their non-drying characteristics, but may be useful in manufacturing of soaps [17] and can be regarded as liquid oils.

Peroxide values are the most common indicator of lipid oxidation. As shown in Table 1 the peroxide value of crude extract and commercial oil were 7.47 ± 2.8 and 5.3 ± 3.1(mEq/kg). These values were less than the standard peroxide values (10 mEq/kg) as stipulated by [1,12]. Therefore is suitable for human consumptions, high peroxide values are indicative of high levels of oxidative rancidity of the oils and also suggest absence or low levels of antioxidants [11,18].

Free fatty acid is the percentage by weight of a specific fatty acid (e.g. percent oleic acid) [19]. From the Table 1 the results obtained for the crude extract and commercial oil were 4.4 ± 0.77 and 4.37 ± 1.3 (%). These values are higher than the reported values of ground nut oil 2.15% [11,20].

Table 1. show the results of chemical analysis of crude extract and commercial oil of
***Lagenaria siceraria* (calabash) seed**

Parameters	Crude extract	Commercial oil
Percentage yield (%)	42.3 ± 0.8	------------
Iodine value (mg/100g)	6 ± 0.26	4.49 ± 0.08
Peroxide value (mEq/kg)	7.47 ± 2.8	5.3 ± 3.1
Free fatty acid (% oleic)	4.4 ± 0.77	4.37±1.3
Acid value (mgKOH/g oil)	6.4 ± 0.43	5.7 ± 0.43
Saponification value (mgKOH/g oil)	160.42±2.96	159.42±2.96

The values are presented as mean ± standard deviation of triplicate determination

4. CONCLUSION

Due to low acid value, higher saponification value of *Lagenaria siceraria* seed oil could be a useful source of oil for both domestic and industrial usage, it is also non- drying oil and hence unsuitable for production of paint, varnishes, ink, and coating since its iodine value is less than 100 mg/100 g.

5. RECOMMENDATION

It is recommended that further research can be done on anti- nutritional factor of the oils to improve our health knowledge of the oil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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