

Characterization and Determination of Nutritional and Anti-Nutritional Values of the Seed Oils of Sorrel (*Hibbiscus Sabdariffa*) and Okro (*Abelmoschus Esculentus*)

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Abstract:-Attempt was made to characterize and determine the nutritional and anti-nutritional composition of Sorrel (*Hibbiscus sabdariffa*) and Okra (*Abelmoschus esculentus*) seed oils using standard analytical methods. The physico-chemical characteristics of the oils were found to be; (Moisture contents = 7.00 %, 6.90 %; Oil yields = 12.80 %, 22.80 %; Specific gravities = 1.00, 0.90; Densities = 0.92 g/cm³, 0.83 g/cm³; Peroxide values = 3.40 ± 0.06 meq/kg, 14.00 ± 0.20 meq/kg; Free fatty acid values = 0.51 ± 0.10 mg KOH/g, 0.28 ± 0.10 mg KOH/g; Iodine values = 0.51 ± 0.10 g/100 g, 1.78 ± 0.10 g/100 g; and Saponification values = 176.72 ± 0.06 meq/kg, 196.35 ± 0.06 meq/kg) for both Sorrel and Okra respectively. The mineral contents were found to be (Mg = 170.80 ± 0.40 mg/100 g, 178.00 ± 0.40 mg/100 g; Ca = 416.00 ± 4.00 mg/100 g, 792.00 ± 0.00 mg/100 g; Na = 200.00 ± 0.00 mg/100 g, 200 .00 ± 0.00 mg/100 g; and K = 160.00 ± 0.00 mg/100 g, 160.00 ± 0.00 mg/100 g) for both Sorrel and Okra respectively. The anti-nutritional compositions were; (Oxalate = 4.95 ± 0.10 mg/100 g, 2.78 ± 0.06 mg/100 g; Phytate = 1.70 ± 0.06 mg/100 g, 2.10 ± 0.10 mg/100 g; and Hydrogen cyanide = 61.32 ± 0.20 mg/100 g, 83.16 ± 0.15 mg/100 g) for both Sorrel and Okra respectively. These results showed that these vegetables contain an appreciable amount of nutrients/mineral elements and low levels of anti-nutrients hence, should be included in diets to supplement the daily allowance needed by the body.

Keywords: *Hibbiscus sabdariffa*, *Abelmoschus esculentus*, Nutritional, Minerals, Antinutritional.

I. INTRODUCTION

Since creation, man has used plants as source of foods and drugs. The use of fats and oils by man dates back to antiquity. Vegetable oils are widely consumed domestically in Nigeria. The interest in vegetable oils with bioactive compounds, such as the ones extracted from fruit seeds, is growing. Almost every part of the tree; roots, trunks, barks, leaves, flowers, fruits and seeds, are known to have some uses. They could also contribute to the supply of nutrients to the soil via nitrogen fixation as leguminous does. Plants were used as a source of medicine since from the centuries ago and today the scientists and the general public recognize the value

of plants as a source of new or complimentary medicinal products [1].

Nuts oils, seed oil and oils of fruit and vegetables are receiving growing interest due to their high concentration of bioactive lipid components, such as polyunsaturated fatty acids and phytosterols, which have shown various health benefits. Fats and oils, and their several lipid components are extensively used in the food and also in cosmetics, pharmaceuticals, oleochemicals and other industries [1-3] their chemical composition and specific properties have allowed them to find uses as foods, fuels and lubricants. Their sources are numerous, encompassing vegetables, animals, and marine sources [1, 4, 5]

Different fats and oils come about due to the fact that there are numerous fatty acids of various kinds and these can be combined in an infinite number of ways on the hydroxyl centers of glycerol. Moreover, the physical properties of fats and oils are dependent on the nature of fatty acids involved in the ester. Hence the traditional distinction of fats as solids and oils as liquids arises from the fact that due to the different chemical structures of the different fatty acids combined in the esters, the bonding forces in existence vary in strength resulting in different melting points. These differences are manifested in different chain lengths, the presence or otherwise of unsaturation as well as geometric conformations [2, 4, 6]

Vegetable oils are obtained from seeds or fruits rich in the oils. They have mostly yellow color or light green color and have a slight smell and a taste of seed or fruit from which they are obtained. They are suitable after extraction as table oils, others are used in preparation of products such as margarine. Vegetable oil can also be used in production of biodiesel [7] and in production of paints and varnishes [6].

Oil seed crops are vital sources of oils of nutritional, pharmaceutical, and industrial importance. The characteristics of oils from different sources depends mainly on their

composition and no oil from single source can be suitable for all purposes [8]. Presently, the quest for traditional vegetable oils has increased immensely because of the ever-growing world population and their use for industrial purposes. Several oils such as Moringa oil, Sunflower oil, Soybean oil, and Pumpkin oil have been used for industrial purposes [9-11]. New low-cost oilseed crops are needed to produce inexpensive oils suitable for foods, pharmaceuticals, and industrial applications. One of the possible alternative crops is *Hibiscus sabdariffa* and another is *Abelmoschus esculentus*.

Sorrel, also known as *Hibiscus sabdariffa* is a herb belonging to the malvaceae family, which is grown in Nigeria, India, west Indians, and to some extent in tropical America. The Sorrel seed oil is rich in both linoleic (39.4 - 40.1 %) and oleic (26.2 -28 %) fatty acids [12, 13]. [14] reported that in Sudan, the seeds are used for edible oil manufacture and the by-products of this process are used for poultry feed.

On the other hand, the Okra plant, *Abelmoschus esculentus*, a native plant from Africa, is now widely cultivated in the tropical and subtropical regions of the world [15]. It is one of the most widely known and utilized species of the malvaceae family [16, 17]. Okra, which is currently grown mainly as a vegetable crop, has potential for cultivation as an oilseed crop because its mature pods contain high quantity of seeds containing considerable amount of oil which could be characterized and utilized for commercial purposes. Okra seed oil is rich in palmitic, oleic, and linoleic acids, which shows a high degree of unsaturation with linoleic acid as the major constituent.

Numerous methods exist in oil separation from oilseeds and these include Mechanical pressing, Pressurized solvent extraction, Soxhlet extraction, Ultra-sonic extraction, Aqueous enzymatic oil extraction among others. Mechanical pressing is the most widely used but the oil produced using this method usually have low value. Extraction with solvent has a number of advantages, which include higher yield and less turbidity as well as relatively low operating cost. Previous studies showed that extraction with organic solvents have been one of the major approaches employed.

This study is aim at extraction, characterization and determination of the nutritional and anti-nutritional values of the seed oils of *Hibiscus sabdariffa* and *Abelmoschus esculentus*.

II. MATERIALS AND METHOD

2.1 Seed Collection and Pre-Treatment

The seeds were purchased from Zango market in Bauchi town, Bauchi State, Nigeria. The seeds were oven dried at 50°C, cleaned through winnowing to remove foreign matter. Finally, the cleaned seeds were pulverized into semi powdered form in order to increase the surface area during extraction.

2.2 Solvents and Reagents

All the reagents used are of analytical and BDH grade.

2.3 Oil Extraction

The method adopted by [1, 2, 18, 19] with slight modification. The oil was extracted from the pulverized seeds (530.00 g) in a soxhlet extractor using n-hexane as the solvent for 8 hours until it was certified that at least 90 % of the oil was extracted. The oil was filtered to remove impurities and the solvent was recovered by the use of rotary evaporator. The recovered oil was further evaporated in an oven at a temperature of 105°C, the moisture and traces of solvent were removed. The oil quality parameters such as Acid value, Iodine value, Peroxide value, Specific gravity, Moisture content, Oil yield, Color were determined based on [20] methods.

$$\% \text{ Crude oil yield} = \frac{\text{Weight of the extracted oil}}{\text{Weight of the sample}}$$

2.4 Physical Characteristics of the Seed Oils (*Hibiscus sabdariffa* and *Abelmoschus esculentus* seeds)

2.4.0 Colour, Smell and Texture

The color, smell, and texture were estimated using sensory evaluation.

2.4.1 Specific Gravity

An improvised specific gravity bottle was washed, rinsed with acetone and dried in an oven. The bottle was weighed empty and further filled with water. The water was discarded and the bottle was dried in an oven. The procedure was repeated using sorrel and okra seed oils and the specific gravity was calculated using the formula below:

$$\text{Specific gravity} = \frac{W_1 - W_2}{W_3 - W_2}$$

Where; W_2 = weight of bottle and oils

W_1 = weight of empty bottle

W_3 = weight of bottle and equal volume of water [18, 21]

2.4.2 Density

An empty cylinder was washed, rinsed, and dried in an oven. The empty cylinder was weighed and 10ml of each oil sample was added separately which was further weighed. The density of each oil was calculated: [18, 25].

$$\text{Density} = \frac{(\text{weight of bottle + oil}) - \text{weight of empty bottle}}{\text{volume of oil}}$$

2.4.3 Moisture Content Determination

Two grams (2 g) of the ground samples were weighed into a pre-weighed porcelain crucible. The crucible was placed in an oven at 100°C for one hour. The dried samples were then cooled in a desiccator for 30 mins and reweighed. The moisture content was calculated using the formula below: [18].

$$\text{Percentage moisture} = \frac{W_1 - W_2}{W_1}$$

Where; W_2 = Final weight of sample

W_1 = Initial weight of sample

2.5 Chemical Properties of the Oil Samples (*Hibiscuss sabdariffa* and *Abelmoschus esculentus* seeds)

The method used for the determination of Free Fatty Acid (FFA), Acid Value (AV), Saponification Value (SV), Hanus [20] while [18] was used for the determination of the Iodine Value (IV)

2.5.0 Free Fatty Acid (FFA) and Acid Value (AV)

Reagents:

The reagents used were prepared using standard method [20].

Procedure:

Oil samples (1 g) each was placed separately in a 250 cm³ conical flask and warmed. 5 cm³ methanol was added with thorough stirring followed by addition of two drops indicator. The solution was titrated against 0.14 N NaOH while shaking vigorously until a permanent pink color which persisted for 15 seconds was observed. The endpoint was recorded and used in calculating FFA and AV as: [18].

$$\text{FFA} = \frac{\text{Titre value} \times M \times 28.2}{\text{Weight of sample}}$$

Where;

M = Molarity of the base

$$\text{Acid Value} = \% \text{ FFA} \times 1.99$$

2.5.1 Saponification Value (SV)

Reagents:

The reagents used were prepared using standard method [20].

Procedure:

Oil samples (1 g) each was placed separately in a 250 cm³ conical flask and 10 cm³ of ethanolic KOH solution was added to each sample. A reflux condenser was attached and the flask content was refluxed in a water bath for 30 minutes. The excess potassium was titrated against 0.5 M HCl using phenolphthalein indicator while still hot. A blank determination was carried out under the same conditions and saponification value was calculated using the formula below [18].

$$\text{S.V} = \frac{(\text{B}-\text{S}) \times 28.05}{\text{W}}$$

Where;

B = titre value of blank,

S = titre value of sample,

W = weight of oil.

2.5.2 Peroxide Value

Reagents:

The reagents used were prepared using standard method [20].

Procedure:

Oil samples (1 g) each was separately placed in a 250 cm³ conical flask and 30 cm³ of glacial acetic acid/Chloroform (3:2 v/v) mixture was added to each sample. Saturated potassium iodide (1 cm³) was added, shaken, and 35 cm³ of distilled water was added. The content was titrated against 0.1 M Na₂S₂O₃ solution until the color turns straw yellow, after which 0.5 ml of 0.5 % starch solution was added. The titration continues until the dark blue color just disappeared. The experiment was again repeated but without oil and this is referred to as blank determination and the peroxide value was calculated using the formula below [18].

$$\text{P.V} = \frac{(\text{S}-\text{B}) \times 1000 \times \text{N}}{\text{W}}$$

Where;

S= titre volume of sample,

B= titre volume of blank,

N= normality of sodium thiosulphate solution,

W= weight of oil sample

2.5.3 Iodine Value

Reagents:

The reagents used were prepared using standard method [20].

Procedure:

Oil samples (1 g) each was separately weighed in 250 cm³ conical flask which was accompanied by addition of 30 cm³ Hanus solution. The flask was stoppered and the contents were mixed and placed in a drawer for 30 minutes. Potassium iodide (10 cm³) was added to the flask washing down any iodine that may be found on the stopper. This was titrated against 0.1 M Na₂S₂O₃ until the solution becomes light yellow. Starch indicator (2 cm³) was added and the titration continued until the blue color just disappeared. A blank determination was carried out under the same conditions. The titre obtained was used to calculate the iodine value thus:

$$\text{I.V} = \frac{(\text{B}-\text{S}) \times 12.69 \times \text{N}}{\text{W}}$$

Where;

B = blank, S = sample titre,

N = normality of $\text{Na}_2\text{S}_2\text{O}_3$. [18]

2.6 Determination of Nutritional Factors

This was determined using wet acid digestion of each sample using the Buck scientific VGP 210 model of Atomic Absorption Spectrophotometer with appropriate hollow cathode lamp for each element.

2.7 Determination of Anti-Nutritional Factors

2.7.0 Phytate

Reagents:

The reagents used were prepared using standard method [18].

Procedure:

The phytate of each of the samples was determined through phytic acid determination using the procedure described by [1, 23]. Oil samples (2 g) each was weighed into 250 cm^3 conical flask, 100 cm^3 of 2 % HCl was added and the content were allowed to stand for 3 hours and then filtered through a double layer filter paper. The filtrate (25 cm^3) was put into 250 cm^3 beaker and 53 cm^3 of distilled water added to improve proper acidity. Ammonium thiocyanate solution (10 cm^3 of 0.3 %) was added to each sample solution as indicator and titrated with standard iron chloride solution and the end point was signified by brownish-yellow coloration that persisted for 5 min.

2.7.1 Oxalate

Reagents:

The reagents used were prepared using standard method [20].

Procedure

The titration method described by [24] was followed. Oil samples (1 g) each was weighed into 100 cm^3 conical flasks. Followed by addition of 75 cm^3 3 M H_2SO_4 and stirred for 1 hour with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. The filtrate (25 cm^3) was taken and titrated while hot against 0.05 M KMnO_4 solution until a faint pink colour persisted for at least 30 seconds. The oxalate content was then calculated by taking 1 cm^3 of 0.05 M KMnO_4 as equivalent to 2.2 mg oxalate [18, 25, 26].

2.7.2 Hydrogen Cyanide

Reagents:

The reagents used were prepared using standard method [20].

Procedure:

The alkaline titration method of [20] was used for the determination. Oil samples (1 g) each was dissolved in a mixture of 200 cm^3 distilled water and 10 cm^3 orthophosphoric acid. The mixture was left overnight to

release all bonded hydrocyanic acid. The mixture was distilled until 150 cm^3 of the distillate was collected. The distillate (20 cm^3) was taken into a conical flask containing 40 cm^3 of distilled water. Aqueous ammonia (8 cm^3 of 6 M) and 2 cm^3 of 5 % potassium iodide solution were added. The mixture was titrated with 0.02 M silver nitrate to faint but permanent turbidity.

2.8 Statistical Analysis

The statistical analysis was performed using excel 2017. All analyses were performed in triplicate. Data were expressed as mean \pm Standard Deviation and statistical significance was assigned at $P \leq 0.05$ level. An independent sample t-test was conducted to compare the means between the properties of sorrel and okra seed oil samples at 0.05 significance level.

III. RESULTS AND DISCUSSION

The quality of edible oils was analyzed by evaluating physicochemical, nutritional, and anti-nutritional compositions.

3.1 Physicochemical Composition Of Sorrel And Okra Seed Oils

Table 1; Physicochemical properties of Sorrel and Okra seed oils

Parameters	Sorrel	Okra
Moisture contents (%)	7.00	6.90
Oil yield (%)	12.80	22.80
Specific gravity	1.00	0.90
Density (g/cm^3)	0.92	0.83
Peroxide value (meq/kg)	3.40 ± 0.06	14.00 ± 0.20
Free fatty acid value (mg KOH/g)	0.51 ± 0.10	0.28 ± 0.10
Iodine value (g/100g)	0.51 ± 0.10	1.78 ± 0.10
Saponification value (meq/kg)	176.72 ± 0.06	196.35 ± 0.06

Values are mean \pm standard deviation (n=3) Mean difference is significant at $P < 0.05$

3.1.1 Percentage oil yield

The physicochemical properties of the seed oils are shown on Table 1. The percentage oil yield for sorrel and okra seeds are 12.80 % and 22.80 % respectively. The two values obtained were below the standard range of $\geq 32\%$ specified for economically viable seeds [20]. Although okra seed gave an oil yield higher than that obtained from sorrel seed, these amounts may not be considered economical for commercial production of oil in Nigeria.

3.1.2 Specific gravity

The specific gravities of *Hibiscus sabdariffa* and *Abelmoschus esculentus* was found to be 1.00 and 0.90 respectively. The higher the specific gravity, the higher the energy content. As the specific gravity increases, power output and mileage both increases.

3.1.3 Density

Oils with density of lower values are highly appreciable to consumers. The results tabulated in Table 1 showed that at room temperature, the densities were 0.90 g/cm³ ad 0.83 g/cm³ for Sorrel and Okra respectively. From the results obtained, Okra seed oil is less dense than Sorrel seed oil.

3.1.4 Moisture

Moisture content is one of the key parameters for determining the viability of vegetable oil trans-esterification process. The oil used in trans-esterification should be substantially anhydrous. This is because the presence of water gives rise to the hydrolysis of the esters produced, with consequent soap formation. Soap formation reduces catalyst efficiency, causes an increase in viscosity, leads to gel formation and makes the separation of glycerol difficult. The moisture content of *Hibiscus sabdariffa* and *Abelmoschus esculentus* was found to be 7.00 and 6.90% respectively. These are within the range of 6.5 to 7.5% which gave the best compromise between the various considerations [27].

3.1.5 Peroxide value

Peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage. The quality and stability of fats and oils can be indicated by using peroxide value. From the analysis, peroxide values of sorrel and okra seed oils were 3.40 and 14.0 meqKOH/g respectively which shows significant difference at (P<0.05). Sorrel seed oil fall within the standard range of 2-10 meqKOH/g as reported by [20]. This implies that sorrel seed oil may be more stable to oxidative degradation than okra seed oil which has high peroxide value.

3.1.6 Acid value

Acid value of oils indicates the amount of free fatty acid present in the oil. It determines the purity of oils. The higher the acid value, the lower the suitability of the oil to be used for cooking purpose/consumption. The free fatty acid values obtained from sorrel and okra seed oils are 0.51 and 0.28 mg KOH/g respectively which shows significant difference at (P<0.05). All these values fall below the standard limit value of ≤ 1.3 mgKOH/g as reported [20]. Hence, oils with low free fatty acid value are less susceptible to rancidity. Therefore, okra seed oil which is having lower free fatty acid value (0.28mgKOH/g) possesses better quality for use in cooking/consumption compared with sorrel seed oil.

3.1.7 Iodine value

Iodine value measures the degree of unsaturation in vegetable oils. It determines the stability of oils to oxidation and allows the overall unsaturation of the fat to be determined qualitatively. The iodine values obtained from sorrel and okra seed oils are 0.51 and 1.78 g I₂/100g which are significantly different at (P<0.05) respectively. These oils have low iodine

value which falls below the standard range of (80-100g I₂/100g) as reported by [20]. Therefore, the lower iodine value of sorrel and okra seed oil indicates that about 95 % of fatty acids in the oils are saturated, so they have low C=C which show low iodine number [28]. The lower the values, the greater the oxidative storage stability. Thus, sorrel seed oil will possess greater oxidative storage stability since it has lower iodine value (0.51 g I₂/100g) compared to okra seed oil.

3.1.8 Saponification value

Saponification value provides information of the average chain length and hence the molecular weight of the fatty acid in the oil. The shorter the average chain length of the fatty acid, the higher the saponification value and the lower the average molecular weight [29]. From Table 1, oil fraction with saponification value of ≥ 180 mg KOH/g has been reported to possess low molecular weight fatty acid [20]. The saponification value obtained from sorrel and okra seed oils are 176.72 and 196.35 mg KOH/g respectively. Therefore, saponification value of oil gotten from okra seed which falls within the AOAC range has low molecular weight fatty acid which makes it useful in soap making whereas, the saponification value of oil gotten from sorrel seed falls below the AOAC standard which implies that the oil cannot be used in soap making due to its high molecular weight fatty acid.

3.2 Nutritional/Mineral Composition Of Sorrel And Okra Seed Oils

Table 2: Mineral composition of Sorrel and Okra seed oils

Mineral Elements	Composition (mg/100 g)	
	Sorrel	Okra
Magnesium, Mg	170.80 ± 0.40	178.00 ± 0.40
Calcium, Ca	416.00 ± 4.00	792.00 ± 0.00
Sodium, Na	200.00 ± 0.00	200.00 ± 0.00
Potassium, P	160.00 ± 0.00	160.00 ± 0.00

Values are mean ± standard deviation(n=3)

Mean difference is significant at P<0.05

Some mineral compositions of the seed oils are shown on Table 2. The results indicate that the oil samples are good sources of Calcium and Sodium with moderate concentrations of Magnesium and Potassium. Okra seed oil contained higher concentrations of Calcium and Magnesium compared to sorrel seed oil. While, Sodium and Potassium have the same concentrations in both samples. Therefore, at (P<0.05), the concentrations of Magnesium and Calcium in both samples are significantly different whereas, Potassium and Sodium shows no significant difference between the two samples at (P>0.05). Minerals are very important in human nutrition. Calcium and Potassium are reported to be responsible for repair of worn out cells, strong bones and teeth, building of red blood cells and for body mechanisms [30]. Epidemiological studies indicate that, diets high in potassium can reduce the risk of hypertension and possibly stroke [31].

Magnesium is beneficial to blood pressure and helps to prevent sudden heart attack. Like calcium, magnesium is an important component of bone and contributes to its development. While calcium stimulates muscles, magnesium relaxes the muscles [32]. Sodium regulates fluid balance in the body and helps in proper functioning of muscles and nerves [33].

3.3 Anti-Nutritional Composition Of Sorrel And Okra Seed Oils

Table 3: Anti-nutritional composition of Sorrel and Okra seed oils

Parameters	Composition (mg/100 g)	
	Sorrel	Okra
Oxalate	4.95 ± 0.10	2.78 ± 0.06
Phytate	1.70 ± 0.06	2.10 ± 0.10
Hydrogen Cyanide	61.32 ± 0.20	83.16 ± 0.15

Values are mean ± standard deviation(n=3)

Mean difference is significant at P<0.05

The anti-nutritional composition such as oxalate, phytate, and hydrogen cyanide of the seed oils are presented in Table 3. The presence of oxalate in the oils has undesirable effects on calcium absorption and utilization. This acid combine with calcium to form calcium oxalate which passes through the intestine unabsorbed. About half of all kidney stones are calcium oxalate either alone or mixed with salts of Calcium phosphate, Magnesium etc. The amount of oxalate formed depends on the amount of oxalic acid in the food [34]. From Table 3, the oxalate content in Sorrel seed oil is higher than that obtained from Okra seed oil however, at (P<0.05), there is no significant difference in the oxalate content between the two oils.

Phytate decreases the bioavailability of minerals especially Calcium, Magnesium, Iron and Zinc [35]. It was also reported to affect the digestion of minerals, protein, and starch solubility and absorption [36]. Nevertheless, phytate has proven to be anti-carcinogenic and potential anti-oxidant [35].

Eating food that is high in the amount of cyanide is associated with a health problem known as spastic paraparesis (Konzo), and tropical Ataxic Neuropathy (TAN) a disease common to cassava based diet consumers [37]. It was reported that only plants with HCN level above 200 mg/100 g are considered dangerous [38]. From Table 3, the hydrogen cyanide present in okra seed oil is higher than that of sorrel seed oil which implies that sorrel seed oil is less dangerous compared to okra seed oil in terms of cyanide content. Therefore, at (P<0.05) the two samples are significantly different in their cyanide content.

IV. CONCLUSION

The anti-nutritional analysis showed that all the samples contained phytate, oxalate and hydrogen cyanide. However, values obtained are lower than the established toxic level. Hence they can be consumed without any restriction.

However, consumption of large amount of fruits with higher levels of anti-nutrients should be avoided.

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