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Article

Physicochemical Variability and Biodiesel Potential of Seed Oils of Two *Hibiscus sabdariffa L.* Phenotypes

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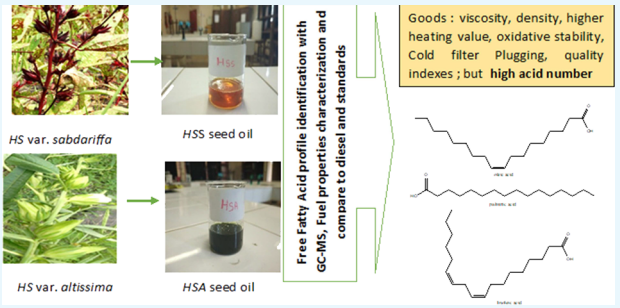
ABSTRACT: Considerable interest is being focused on vegetable oils as fuel. Due to their characteristics being close to diesel and their renewable potential, studies recommend their use for agricultural applications. *Hibiscus sabdariffa* var. *sabdariffa* is widely studied for the nutritional properties of its calyces. Although the seeds of this species are known to be rich in fatty acids, their use is little known in Benin Republic. Similarly, a few studies have attempted to characterize the seeds of the green phenotype of this plant species. By following standard methods, the fatty acid profiles of oils extracted from the seeds of the two varieties (red phenotype, sabdariffa (HSS), and green phenotype, altissima (HSA)) of *H. sabdariffa L.* were established. A comparative study of their physicochemical properties was also performed to highlight their potential use as fuel. It follows that HSS seed oil is yellow while HSA seed oil is dark green. For the two varieties, values obtained for the kinematic viscosity (~4 mm^2/s), cetane number (~55), and density (0.87 g/cm^3) are in accordance with the U.S. and European standards. However, it is observed that HSA oil is significantly more acidic (23.10 ± 0.22 for HSS vs 18.20 ± 0.40 mg KOH/g oil for HSS) with a higher peroxide value (HSA: 0.280 ± 0.002 vs HSS: 0.140 ± 0.001). The major fatty acids are the following: palmitic (HSA: 27.09 vs HSS: 25.48%), oleic (HSA: 31.81 vs HSS: 35.21%), and linoleic (HSA: 31.43 vs HSS: 29.70%) acids. These fatty acid profiles give to the two oils calorific values (~39.45 MJ/kg) lower than that of diesel but good oxidative stability and cold filter plugging. The two oils could be used as fuel oil, after their transesterification to improve their properties.



INTRODUCTION

Hibiscus plant species are distributed worldwide with more than 500 species mostly found in the tropics and subtropics areas. These plants are cultivated by African farmers, and dried bracts are exported to Arabic and European countries.[1] Guinea sorrel (Malvaceae) is an oilseed plant species widely distributed in sub-Saharan Africa. In Benin, calyces of Guinea sorrel are used for a local common drink popularly called bissap. Guinea sorrel has green or red calyces that distinguish two main varieties: the *Hibiscus sabdariffa* variety altissima HSA (green variety) and the *H. sabdariffa* variety sabdariffa HSS (red variety).[2,3]

Different parts of HSS are well known and studied. The cardioprotective, anti-hypertensive, antioxidant, antiseptic, and aphrodisiac properties of extracts obtained from HSS calyces, stems, flowers, and leaves have been reported.[4]On the contrary, HSA is more cultivated for the fibers of its stem and often is confused with *H. cannabinus L.* (kenaf).[5] Unlike kenaf, HSS rods first undergo bacteriological rusting before being used as fibers.

In Benin, the seeds of *H. sabdariffa*, byproducts of the calyces, are not used since they are released into the wild. The fatty acid profile of HSS seed oils have been described to be highly linoleic (39%) and contains saturated fatty acids (~29%).[1] By following Halphen's test and HBR titration, Ahmad et al. (1979) found in the variety *sabdariffa* seed oils the presence of cyclopropene and epoxy acids.[6] Indeed, it was reported in the seed oils of plants belonging to Malvaceae the presence of some unusual fatty acids known as cyclopropenoid fatty acids and epoxy fatty acids.[7] On the basis of the properties[8] and the occurrence or not of these compounds, these oils could be used for food,[9−11] cosmetics, or as fuel. Vegetable oils are used in diesel engines, for agriculture purposes, and for alimentation of biodiesel[12] since they seem to have greater potential.[13] In that capacity, Sahu et al. (2017) explored the performance of HSS oil as a biodiesel blend of diesel engines.[14] To our knowledge, there is no study on the

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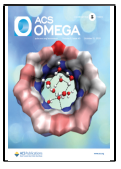

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Table 1. Quality Indices of HSA and HSS Seed Oils[a]

physicochemical parameters	HSA	HSS	diesel[20,17,21]	ASTM D6751[17]	EN 14214[17]
extraction yield (%)	16.01 ± 0.06a	15.34 ± 0.25b			
density 20 ° C (g/cm^3)	0.876 ± 0.001a	0.875 ± 0.001a	0.845	0.88	0.86−0.90
acid number (mg KOH/g oil)	23.10 ± 0.22a	18.20 ± 0.40b	0.17	max 0.5	max 0.5
cetane number	55.376 ± 0.875a	55.515 ± 0.246a	45−55	min 47	min 51
iodine index (g I_2/100 g oil)	90.449 ± 2.960a	90.988 ± 1.665a	6		120
peroxide value (mequiv O_2/kg oil)	0.280 ± 0.001a	0.140 ± 0.001b			
saponification index (mg KOH/g oil)	145.78 ± 2.01a	132.33 ± 2.40b			
refraction index	1.468 ± 0.001a	1.468 ± 0.001a			
kinematic viscosity 20 °C (mm^2/s)	3.820 ± 0,027a	3.833 ± 0,006a	2.91−4	1.9−6.0	3.5−5.0 (40 °C)
higher heating value (MJ/kg)	39.450 ± 0.007a	39.459 ± 0,010a	42−45.9	35	35
oxidation stability (h)	6.286 ± 0.352a	6.418 ± 0.124a			min 6
cold filter plugging point (°C)	−16.265 ± 0.011a	−16.267 ± 0.003a			

[a]Means not sharing a common letter in the same row denote a significant difference at $P < 0.05$

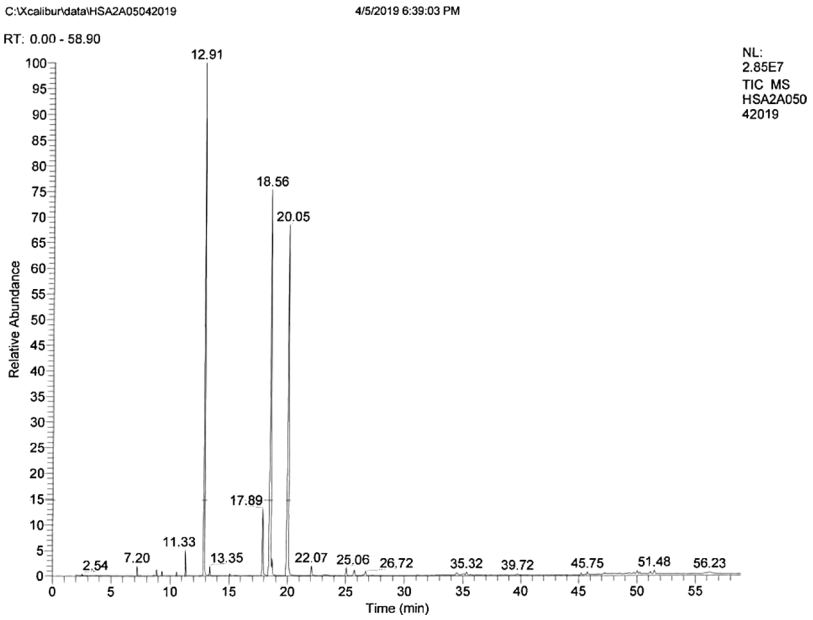

Figure 1. Gas chromatogram of fatty acids in HSA seeds.

biodiesel potential of HSA seed oil. The fuel potential of both seed oils could be linked to the varietal particularities. Therefore, the present study aims to characterize the vegetable oils extracted from the seeds of the two varieties of *H. sabdariffa* in order to evaluate their fuel potential.

RESULTS AND DISCUSSION

Quality Parameters and Fuel Properties of Oils. The quality parameters and fuel properties of the two varieties of *H. sabdariffa L.* seed oil are presented in Table 1.

The oil content of HSA seeds is more important than that of HSS seeds (16.01 ± 0.06 and 15.34 ± 0.25, respectively). However, these oil yields are lower than those (17−24%) reported only for HSS seeds.[15,16] Lipid content of seeds could depend on several parameters such as species, seed maturity, and edaphic conditions.[17] Most of the properties of biodiesel fuel, such as density, kinematic viscosity, iodine number, cetane number, and oxidative stability, are determined by the amounts of each fatty acid in raw materials. In the present study on HSS and HSA seed oils, there are no significant difference between density (∼0.87), kinematic viscosity (∼3.820 mm^2/s), higher heating value (∼39.45 MJ/kg), and cetane number (∼55.37). The density increases with decreasing chain length and increasing number of double bonds and has a direct impact on the injection performance of the fuel.[18] Viscosity plays an important role in the penetration and atomization of the fuel spray. Indeed, a high viscosity creates inadequate atomization of fuel, which causes deposition of impurities and decreases the thermal efficiency.[19] In the current work, kinematic viscosity values obtained are low and are within the range of the U.S. and European standards. The heating value of fuel indicates the quantity of energy that is released when a unit amount of fuel is burned. Both varieties of oil release less energy than diesel but more energy than the minimum value (35 MJ/Kg) recommended by the U.S. and European standards.

The cetane number is a measure of the ignition quality of diesel fuel during combustion. A high cetane number value shows the capacity of a fuel to self-ignite quickly after being delivered into the combustion chamber.[17,19] Values obtained in the current work are close to the maximum value that is obtained with diesel (45−55) and higher than the standard value (min 47 and min 51). The iodine number gives information about the degree of unsaturation of the oil and the

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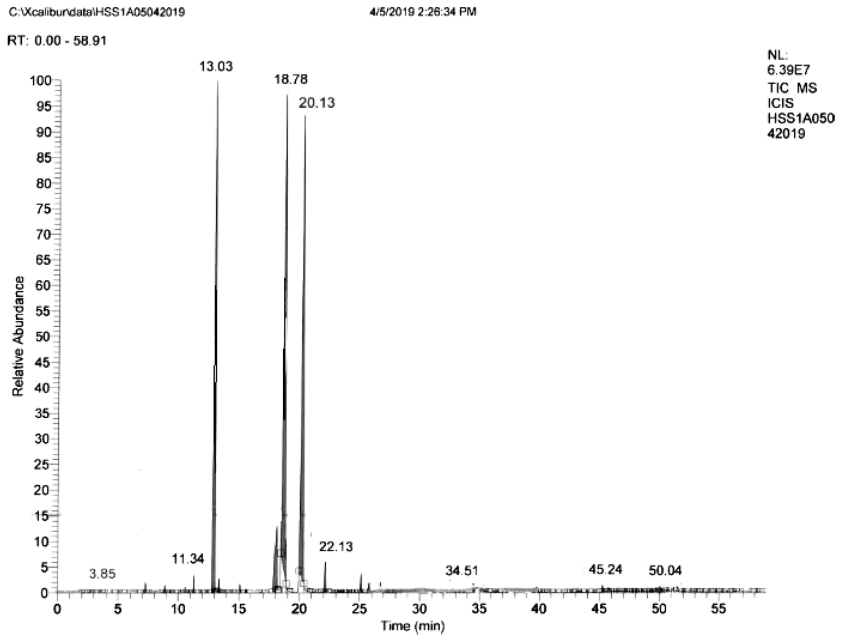

Figure 2. Gas chromatogram of fatty acids in HSS seeds.

oxidation tendency of fuel when it comes into contact with air.[22] The value obtained in the current work complies with the EN14214 biodiesel standard,[17] which sets the maximum value to 120 g I_2/g oil and is indicative of the predominance of monounsaturated fatty acids for both vegetable oils. The acid number depends on the free fatty acids of the vegetable oil. It serves to evaluate the hydrolytic alteration of seed oil. A high acid number creates the issue of corrosion in the fuel delivery channel of the engine.[19] The vegetable oil of the altissima variety has a higher acid number (23.10 ± 0.22 vs 18.20 ± 0.40 mg KOH/g oil for HSS). The high acidity of *Hibiscus* seeds could be linked to the degradation of chlorophyll contained in the seeds because the dyes, by degradation, release acidic substances.[23] Figure 6 shows the difference in color between the two types of seed oils. This difference in oil color could be indicative of a different content of pigments such as anthocyanins and chlorophyll. A study in perspective of this subject is in progress. Likewise, a significant difference between the peroxide index was observed (HSA: 0.280 ± 0.001 vs HSS: 0.140 ± 0.001). These peroxide values meet the normative requirements (<10 mequiv O_2/kg oil). This indicates that seed oils of both *Hibiscus* varieties can be stored without the risk of major alteration. The saponification numbers obtained were 145.78 ± 2.01 mg KOH/g oil for HSA and 132.33 ± 2.40 mg KOH/g oil for HSS. The high value for raw oil shows the existence of a large fatty acid proportion, which promises its use in soap fabrication throughout the transesterification process. The value of saponification varies in the range from 0 to 370 mg KOH/g.[19]

To measure the level of the biodiesel reaction with air and the level of oxidation, oxidation stability is determinated.[19] It is an important biodiesel fuel quality parameter. The minimum value of 6 h for the induction period at 110 °C, measured with a Rancimat method recommended by the standard, is reached by both oils. Another parameter is the cold filter plugging point, which is the lowest temperature at which a given volume of fuel still passes through a standardized filtration device in a specified time when cooled under certain conditions. It follows that when an oil has a high cold filter clogging point, it will more easily clog the engines of vehicles. The both oils have the same cold filter plugging point (−16.26 °C).

Fatty Acid Profile of the Two Seed Oils. We performed the fatty acid analysis of seed oils extracted from both varieties of *H. sabdariffa*. The chromatograms obtained are shown in Figures 1 and 2. They are almost identical and stackable. The three intense peaks were observed at retention times of 12.91 (13.03), 18.56 (18.78), and 20.05 (20.10) and correspond to palmitic, oleic, and linoleic acids (Figure 3).

Table 2 shows the complete fatty acids profiles of the two varieties of *H. sabdariffa*. Myristic and nervonic acids were not identified in HSA seed oil, while eicosenoic and lignoceric acids were absent from HSS seed oil.

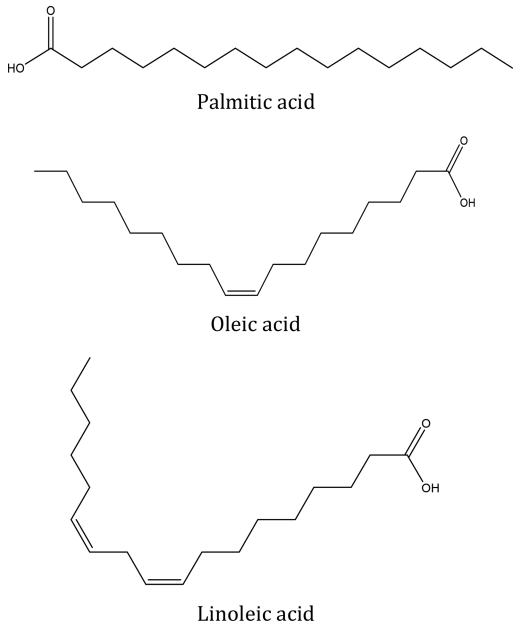

Figure 3. Major fatty acids of the two oils

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Table 2. Fatty Acids Composition (%) of HSA and HSS Seed Oils

free fatty acid		HSA	HSS	works on HSS[1,24,25]
tridecanoic	C13:0	0.30	0.23	
myristic	C14:0		0.15	0.23−0.31
pentadecenoic	C15:1	0.86	0.36	
palmitic	C16:0	27.09	25.48	18.15−21.65
palmitoleic	C16:1	0.38	0.26	0.44
stearic	C18:0	5.01	5.54	4.09−5.47
oleic	C18:1	31.81	35.21	30.90−38.46
linoleic	C18:2	31.43	29.7	38.17−40.12
linolenic	C18:3	0.67	1.13	0.57−2.09
arachidic	C20:0	0.56	0.69	0.72
eicosenoic	C20:1	0.54		0.08
behenic	C22:0	0.33	0.44	0.37
erucic	C22:1	0.27	0.29	
tricosylic	C23:0	0.18	0.25	
lignoceric	C24:0	0.18		
nervonic	C24:1		0.17	
total SFA		33.65	32.78	
total MUFA		33.86	36.29	
total PUFA		32.1	30.83	

In the current work, *H. sabdariffa L.* seed oils have a fairly balanced fatty acid composition: saturated fatty acids (HSA: 33.65% and HSS: 32.78%, m/m), monounsaturated fatty acids (HSA: 33.86 and HSS: 36.29%, m/m), and polyunsaturated fatty acids (HSA: 32.1 and HSS: 30.83%, m/m) (Table 2).

The dominant saturated fatty acids are palmitic acid (HSA: 27.09% and HSS: 25.48%, m/m) and stearic acid (HSA: 5.01 and HSS: 5.54%, m/m). The most abundant unsaturated fatty acids are oleic acid (HSA: 31.81 and HSS: 35.21%, m / m) and linoleic acid (HSA: 31.43 and HSS: 29.7%). The fatty acids profiles of HSS seed oils of our study differ from the results reported by different authors. As an illustration, Elneairy (2014) reported for seed oils extracted from HSS a proportion of unsaturated fatty acids of 75.57 and 66.09% for Egyptian and Libyan seeds, respectively.[26] Meanwhile, for the same variety, Dhar et al. (2015) reported 27 and 73% saturated and unsaturated fatty acids, respectively.[7] That chemical variability in fatty acids may depend on many factors such as location, soil, and climate. However, the ratio (2:1) of unsaturated to saturated fatty acids found for HSS seed oils studied is identical to that found by Elneairy and lower to that of Egyptian HSS seed oils (3:1). In all the cases, oleic, linoleic, palmitic, and stearic acids were the major compounds found in previous studies on HSS.[1,7,24−26]

An organism's phenotype corresponds to the observable characteristics, which are affected both by its genotype and by the environment. Based on plant genotypes, many authors observed high variance for biodiesel properties.[27,28] Thus, the genotype could be a source of variance results observed with the color, yield, acid number, peroxide number, saponification index, and fatty acid content.

CONCLUSIONS

The current work, which focused on two varieties of *H. sabdariffa* seed oils, has demonstrated that only the values of peroxide, saponification, and acid indexes were significantly different. Moreover, density, viscosity, cetane number, refractive index, high heating value, oxidation stability, cold filter plugging point, and fatty acids profiles of these seed oils favor their use as biofuels. However, the two oils have a high acid number and in this case can create corrosion problems in the fuel delivery channel of engines. Using them as fuel will require their transformation. In perspective, studies projected to optimizing their fuel efficiency and to performing engine tests by using them as fuel are planned.

MATERIALS AND METHODS

Materials. HSA (Figure 4) and HSS (Figure 5) seeds were collected in September 2018 from Lobogo in an area of the

Figure 4. *H. sabdariffa* var. altissima seeds.

Figure 5. *H. sabdariffa* var. sabdariffa seeds.

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Bopa region in Mono, Southern department of Benin. Voucher samples were deposited at the National Herbarium of the University of Abomey-Calavi.

METHODS

Seed Oil Extraction. The collected hulls were sun−dried, and the seeds were recovered. After eliminating all the impurities, the seeds were reduced to powder with a grinder. The powders are then extracted with hexane using Soxhlet apparatus for 6 h at 69 °C, and the hexane was released in a rotavapor. The obtained seed oils (Figure 6) were dried in an oven for 20 min at 103 °C, cooled in a desiccator for 30 min, and weighed. Then, they were packaged in dark bottles for later use.[29]

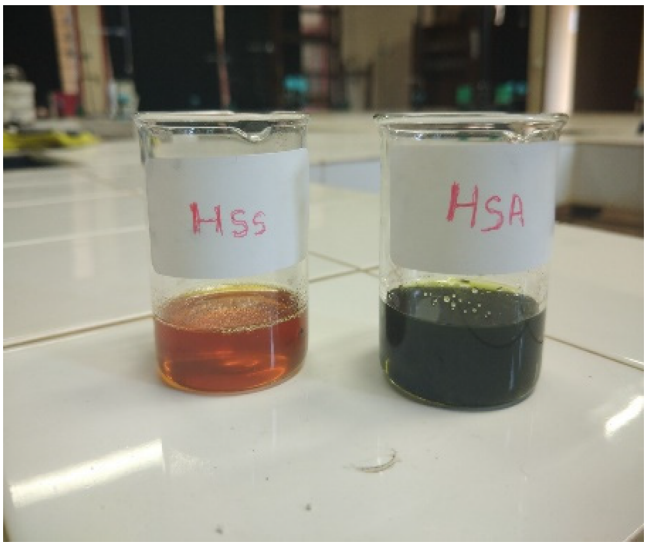

Figure 6. Two varieties of *H. sabdariffa* seed oils.

Determination of Fatty Acid Composition. The split/splitless injector and the ion source temperature were set at 250 °C. Ultrapure helium alpha-gas 2 was the gas carrier set at 1 mL/min constant flow with automatically adjusted pressure. Injections were in split mode. The GC was fitted with a fused silica capillary column (DB-FFAP) with 30 m × 0.25 mm inner diameter (ID) × 0.25 μm film thickness (J&W Scientific, Agilent Technologies). The initial oven temperature was 130 °C, and the program temperature was as follows: equilibration time: 0.5 min; linear increase to 178 °C at 4 °C /min followed by linear increase to 210 °C at 1 °C/min, then an increase to 245 °C at 40 °C/min, and finally, 13 min hold. The duration of the analysis was 60 min. The injected volume was 1 μL, and the injected amount 10 μg/mL. Positive ionization of the FFA was performed by electronic impact (EI), with 70 eV energy and full scan detection mode. The mass spectra range was 50−650 m/z; scan 0.58 s. Precise identification of the analytes was achieved by their relative retention times and mass spectra on the spectral mass database NIST libraries for fatty acid composition.

Physicochemical Property Characterization. Seed oils were analyzed using standard procedures as done previously.[14,30] Acid number (AN), peroxide value (PV), saponification index (SI), and density were determined according to NF T 60-204, NF T 60-220, NF ISO 3657,and NF T 60-214, respectively. The viscosity was measuring at 20 °C with an NDJ-5S digital viscometer.

The cetane number (CN), higher heating value (HHV), and iodine index (II) were calculated using previously developed empirical equations (eqs 1 to 6).[31−33]

$$\mathrm{CN} = \sum_{i=1}^{n} w_i \times \phi_i \tag{1}$$

$$\phi_i = -7.8 + 0.302 \times M_i - 20 \times N \tag{2}$$

where ϕ_i is the cetane number of the ith FFA, M_i is the molecular weight of the ith FFA, and N is the number of double bonds.

$$\mathrm{HHV} = \sum_{i=1}^{n} w_i \times \delta_i \tag{3}$$

$$\delta_i = 46.19 - \frac{1794}{M_i} - 0.21 \times N \tag{4}$$

where δ_i is the higher heating value of the ith FFA in MJ/kg, M_i is the molecular weight of the ith FFA, and N is the number of double bonds.

$$\mathrm{II} = 0.6683 \times \mathrm{DU} + 25.0364 \tag{5}$$

$$\mathrm{DU} = \left(\sum \mathrm{MUFA\ C}_n\text{: 1, wt\%}\right) + 2 \times \left(\sum \mathrm{MUFA\ C}_n\text{: 2, wt\%}\right) + 3 \times \left(\sum \mathrm{MUFA\ C}_n\text{: 3, wt\%}\right) \tag{6}$$

Oxidation stability (Y) and cold filter plugging point (CFPP) were calculated according to Gomas et al. (2017)[33] (eqs 7) and 8

$$Y = \frac{117.9295}{X} + 2.5905 \tag{7}$$

where X is the content of linoleic and linolenic acids ($0 < X < 100$)

$$\mathrm{cFPP} = 3.1417 \times \mathrm{LCSF} - 16.477 \tag{8}$$

$$\mathrm{LCSF} = 0.1 \times \mathrm{C16(wt\%)} + 0.5 \times \mathrm{C18(wt\%)} + 1 \times \mathrm{C20(wt\%)} + 1.5 \times \mathrm{C22(wt\%)} + 2 \times \mathrm{C24(wt\%)} \tag{9}$$

where LCSF is the long chain saturated factor and C16, C18, C20, C22, and C24 are the amount of the corresponding long saturated fatty acids (wt %) present in the oil.

The refractive index (RI) is determined using the Perkins mathematical formula reported by Babatundé and Bello 2016[34] (equation 10).

$$\mathrm{RI} = 1.45765 + 0.0001164\mathrm{II} \tag{10}$$

Statistical Analysis. Assays were run in triplicate, and the results were organized using Microsoft Excel 2007. There were treated with Minitab 16 for the comparison of means and the analysis of variance (ANOVA). The test was considered statistically significant if $P < 0.05$.

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Notes

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■ ABBREVIATIONS

HSA, *Hibiscus sabdariffa* var. *altissima*; HSS, *Hibiscus sabdariffa* var. sabdariffa; FFA, free fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

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