

Refining, Toxicology Study and Biodiesel Potentials of Used Vegetable Oils

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Abstract This study examined the effect of refining process on physicochemical properties of used vegetable oils from two restaurants in Ile-Ife Nigeria, and their biodiesel potential using biological and chemical based heterogeneous catalyst. The refining stages are degumming, alkaline treatment and bleaching. Physicochemical properties of both refined and the used oils were carried out using the AOAC methods. Transesterification of biodiesel was carried out using KOH and plantain peel as the catalyst, while the fuel properties of the biodiesel obtained were determined using ASTM methods. Results showed that the biodiesels obtained from the used vegetable oils using environmental friendly and readily available catalyst, were better substitute with cheap cost of production compared with biodiesel fuel produced from normal vegetable oils. Also, the refining processes showed that the refined oils have gained better industrial application with improved physicochemical properties after each refining stages.

Keywords: transesterification, heterogeneous catalyst, bleaching, alkaline treatment, degumming

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1. Introduction

Sustainability has become an important and guiding principle for modern society and with the increase of anthropogenic waste in our environment; it can be a source of energy, chemicals, catalyst, construction materials and high value functional groups [1]. Waste biomass and waste cooking oil has been increasingly targeted as a renewable feedstock for the production of fuels and to generate catalyst, using waste materials as raw material and catalyst in biodiesel make the production cost effective and environmental friendly [1].

The use of vegetable oils and fats as a frying medium in both shallow and deep-frying mode is an important component in the whole picture of food applications [2]. Frying is a dehydration process usually carried out at a temperature of 160 - 190°C and is an efficient method of heat transfer that allows quick cooking and adds flavour to fried food [3]. Frying is one of the fastest and oldest methods of cooking food and its use dates back to the ancient Egyptians, in around the sixth century BC. The Romans made extensive use of this cooking method and called it 'boiling in oil' [4]. When vegetable oil is used continuously, it begins to smoke, foam, increase in viscosity and become darker [5] which result to disposal of this oil. Most of these used oils are dumped into

running water polluting the ecosystem, increases organic pollution load, forming layers on the surface of water [6] and those dumped on the surface of the earth can leach into the soil and groundwater. Also used oils are sold commercially for animal feeds but European Union has enforced a ban on feeding animals with these mixtures so as to prevent the return of harmful compounds back to food chain through animal meat [7]. Recycling used oils is one of the efficient and economical approaches to solve this environmental problem.

Used vegetable oil contains the desired triglycerides along with undesired substances such as oxidation products, metals (leached into oil from frying pans), free fatty acids, filterable solids, solid triglycerides, pigments, hydrocarbons e.tc. Refining process can be employ to recycle vegetables oils that has undergone continuous frying. Refining remove undesired substance present in vegetable oil so as to make it suitable for edibility and industrial application. This process includes degumming, alkaline treatment, bleaching, and deodorization. Degumming process which is the first refining stage remove hydratable phosphatides, waxes, free fatty acids, e.t.c [8]; alkaline treatment remove pigments, metals, free fatty acids, non-hydratable phosphatides [9]; bleaching treatment remove pigments, oxidation products, metals and soap [10]; deodorization which is the final stage of refining stripped off flavor and odor components, oxidation products, and other volatile products from the

oil [11]. All these stages improve the quality (taste, odour, physico-chemical properties) and shelf life of oils.

Considerable studies have been done on converting used vegetable oils to biodiesel using acid [12], base [13,14], enzyme [15,16] catalyst and two step based catalysis [17] but all these catalyst are cost intensive. Also, wealth of literatures exists on transesterification of triglycerides using calcium-rich materials like eggshell [18,19], snail shell [20,21], shellfish [22,23] e.t.c for biodiesel production. However, there are relatively few studies in literature on conversion of used vegetable oils or raw vegetable oils to biodiesel using potash-rich waste materials such as calcinations of banana peels, plantain peels, and mango peels e.t.c. as catalyst. Exploring the use of calcined fruit peels which is a waste material for catalyst due to their alkaline properties can also lowered the cost of biodiesel production and help in reducing littering of environment with this waste material. Also, there are substantial studies in literature on refining and its effect on conventional [24,25,26,27], and non-conventional vegetable oils [28,29,30] for various purposes but there is paucity of data on study that has explored the refining of used/ waste cooking vegetable oils for edibility, fuel or industrial purposes.

The motivation to this study was predicated on the economical disadvantage of biodiesel due to high cost of production from virgin vegetable oils. Since biodiesel is technically feasible and environmentally acceptable, thus, this study investigated lowering the cost of biodiesel production by exploring used vegetable oils from restaurants as raw material and calcined plantain peels as catalyst for the biodiesel production. Also, refining of used vegetable oils was investigated to know if it can still be reuse for frying, industrial or fuel purposes. Result showed that the degumming and alkaline treatment operations were more effective in removing impurities from the oil; and that the refined oils were not totally safe for consumption based on the

present level of toxicity recorded in this study, but can be used for production of biodiesel and for other industrial applications.

2. Materials and Methods

Materials and Reagents: Plantain peels were obtained from places where plantains are roasted in Ile-Ife, Osun State, Nigeria. Reagents used are of analytical grade and these include potassium hydroxide (Qualikems), potassium iodide (May and Baker limited, England), $\text{Na}_2\text{S}_2\text{O}_3$ (BDH, Poole, England), NaCl (BDH, Poole, England), activated charcoal (May and Baker limited, England), chloroform (BDH, Poole, England), acetic acid (BDH, Poole, England), methanol (BDH, Poole, England), hydrochloric acid ((BDH, Poole, England)

2.1. Sample Collection and Storage

Used vegetable oil samples were fried oils collected from two restaurants in Ile – Ife, Osun State, Nigeria represented as Restaurant 1 (R1) and Restaurant 2 (R2) (Figure 1). They are oils used in frying within a one-week. The samples were collected in plastic containers and stored in a refrigerator until use.

2.2. Preparation of Plantain Peels for Catalyst

Ripe plantain peels obtained were chopped into small pieces, washed using distilled water for about 4-5 times, followed by oven drying for 24 hours at 80°C until the plantain peels were properly dried and crispy. The crispy peels were burnt in open air to charcoal using biodiesel fuel, and the charcoal obtained was turned to powdery form and calcined in a furnace at 700°C for 3 hrs 30 mins. The final product after calcination was sieved to obtain fine powder and stored in a desiccator.

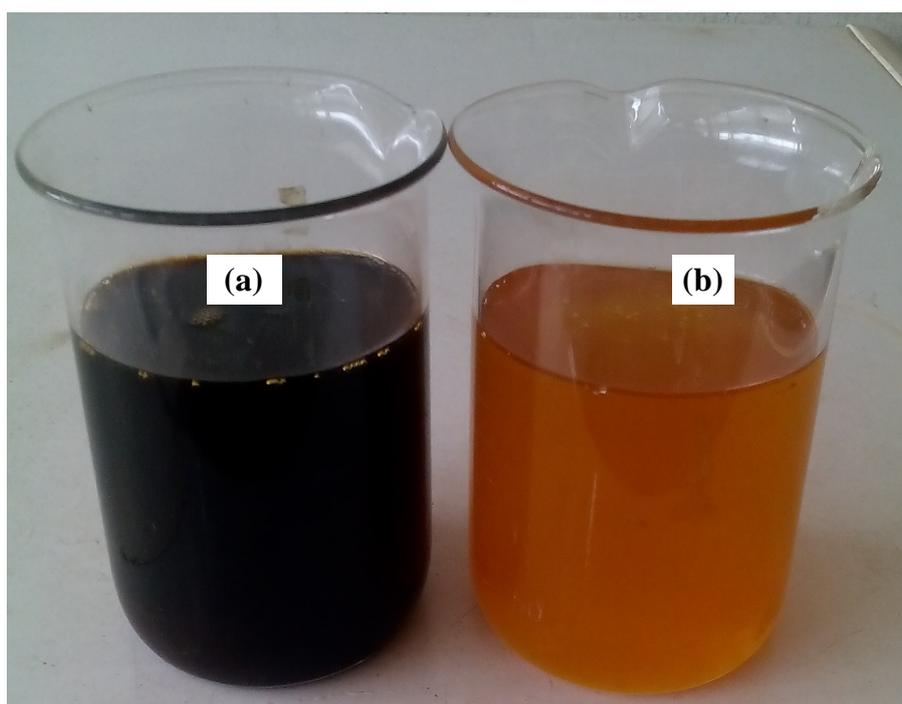


Figure 1. Used vegetable oil obtained from (a) restaurant 1 (R1) and (b) restaurant 2 (R2)

2.3. Transesterification Process

Transesterification of used vegetable oils obtained from Restaurant 1 (R1) and Restaurant 2 (R2) (Figure 1) was carried out with methanol in the presence of KOH, or calcined plantain peels as catalyst. The method recommended by Van Gerpen *et al.* [31] was employed. The transesterification reaction was carried out with a 6:1 methanol to oil molar ratio, 1% KOH or 3% plantain peels catalyst to oil, and 65°C reaction temperature. The percentage biodiesel yield was calculated using the relationship:

$$\% \text{ conversion} = \frac{\text{mass of biodiesel produced}}{\text{mass of oil used}} \times 100.$$

The physico-chemical properties of the biodiesel produced for both the raw and the degummed oils were determined according to American Society for Test and Material—ASTM and Leevijit method [32,33]. The parameters determined were acid value, iodine value, cetane number, kinematic viscosity, density, higher heating value, heating efficiency, pour point, cloud point, smoke point and flash point.

2.4. Refining Process

2.4.1. Degumming Operation

Accurately measured 5 mL of raw oil was mixed with 1 mL of the 300 µg/mL NaCl solution. The oil and NaCl solution mixture was agitated for 60 min at 60°C on magnetic stirrer to render fat-soluble phosphatides insoluble. These insoluble phosphatides were then separated by centrifugation at 1000 rpm for 30 min. During the agitation process, a colloidal mixture beneath the oil layer was formed. This was believed to be a mixture of the fat soluble impurities (phospholipid lecithin complexed with metals) contained earlier in the oil. The oil was separated from the colloidal mixture by decanting [34].

2.4.2. Alkaline Treatment

Accurately measured 100 mL of degummed oil was placed in a beaker and stirred on a magnetic stirrer for 5 minutes. About 15 mL of 2.5 M NaOH was then added and the stirring was continued for 30 minutes. After the agitation, a thick mixture settled at the bottom of the beaker. This was believed to contain a mixture of soaps, metals and the remaining phospholipids. The oil was separated from the mixture by decanting [35].

2.4.3. Bleaching Process

Accurately measured 50 mL of degummed-alkaline treated oil in a beaker was heated to 100°C to remove all the water present. Accurately weighed 0.5 g of activated charcoal was then added and heated up to 130°C for 30 - 45 minutes. The mixture in the beaker was centrifuged at 2500 rpm for 15 minutes and decanted into a clean 250 mL conical flask to obtain clean bleached oil [36].

2.5. Physico-chemical Parameters

The physico-chemical properties of used vegetable oils, and oil obtained from each refining stage were carried out

according to the standard AOAC method [37]. The parameters determined are acid value, iodine value, saponification value, peroxide value, kinematic viscosity, specific gravity, smoke point and surface tension.

2.6. Cytotoxicity Analysis

Brine shrimp eggs were hatched in a shallow rectangular dish filed with artificial sea water. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The eggs were sprinkled into the larger compartment with darkness, while the smaller compartment was illuminated. After 48hrs, the phototropic nauplii were collected from lighted side. Ten brine shrimps were transferred into vial bottles containing 5 mL of artificial sea water and 1 mL of each used and refined oil dissolved in DMSO at concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL in duplicates was added to the mixture in each vial bottles. The vials were maintained under illumination. Survivors were counted after 24 hrs with the aid of magnifying glass. The experiment was carried out in triplicate. LC50 at 95% confidence intervals were determined from the 24 hrs counts using probit analysis method described by Finney [38].

3. Results and Discussion

3.1. Physico-chemical Properties of the Biodiesel

Table 1 presents the physico-chemical parameters of the biodiesel obtained from the used vegetable oils. The biodiesel yields for used oil from R1 and R2 using KOH as catalyst were 86 and 79% respectively, while the yield using plantain peel as catalyst were 84 and 80% respectively (Table 1). The result obtained agreed with the high biodiesel yield reported by Adekunle *et al.* [30] for biodiesel obtained from degummed and raw oils of some non-conventional vegetable oils.

The heat of combustion or heating value is not specified in the biodiesel standards [32,39]. However, European standard for using biodiesel as heating oil specifies a minimum heating value of 35 MJ/kg [40]. From this study, all the biodiesel obtained from the oils have Higher Heating values (HHV) (45.16 – 45.97 MJ/kg) higher than the specification of 35 MJ/kg for both catalysts. Based on their HHV result, biodiesel from both catalysts can serve as potential supplement or alternative to petroleum diesel.

The flash point (FP) is a measure of the temperature to which a fuel must be heated such that the mixture of vapor and air above the fuel can be ignited. Flash point varies inversely with the fuel's volatility [41]. Flash point of biodiesel from both catalysts was higher (174.0 – 180.0°C) than the minimum standard set by ASTM (130°C) and European Union (120°C). This result suggested that the biodiesels obtained from the used oils will be relatively safe to handle and for storage.

The cloud point (CP) is the temperature at which crystals first start to form in the fuel, while pour point is the measure of the fuel gelling temperature, or the point at which the fuel cannot flow [42]. The cloud point (10.0 –

12.0°C) and pour point (5.0°C) of biodiesel from R1 oil were higher than that of R2 biodiesel (2.0 – 5.0°C; -3.0 to -4.0°C respectively), which means biodiesel from R1 will form gel easily under cold temperature, and would impact relatively negatively on their injection, combustion and energy efficiency.

Cetane number of fuel is a measure of its ignition quality [43]. The result show that all the biodiesels have high cetane number (95.89 – 117.7) than the minimum standard value of 51 established by European standard, and exceed 47 set by ASTM D 6751. High cetane numbers signify only short delays between fuel injection and ignition, and thus ensure good cold start behavior and a smooth run of the engine [44]. From this result, it can be stipulated that biodiesel obtained from both catalyst are good for fuel application.

The kinematics viscosity of biodiesels from R1 and R2 oils using KOH catalyst were 3.71 and 2.95 mm²/s respectively, while that obtained using plantain peels catalyst were 3.02 and 2.01 mm²/s respectively. According to ASTM and European standard for biodiesel, kinematics viscosity must be between 1.9 and 6.5 mm²/s and 3.50 and 5.0 mm²/s respectively. The viscosities of biodiesels obtained in this study using both catalysts were within the set range. The biodiesel obtained using plantain peels catalyst have lower viscosity than biodiesel from KOH catalyst. This mean that the biodiesels from plantain peels catalyst were better, and could have faster injection and ignition properties when used in automobiles as fuel. Since viscosity increases with increase in fatty acid chain [42] and decreases with increase in unsaturation, then the decrease in viscosities of biodiesel obtained using plantain peels as catalyst might be due to the enhanced catalytic efficiency of the biocatalyst during the transesterification reactions, leading to significant decrease in fatty acid chain present in the used oils. Fuel density directly affects fuel performance, and some of the engine properties, such as cetane number, heating value and viscosity are strongly connected to density. The density of the fuel also affects

the quality of atomization and combustion [45]. From this study, densities of biodiesels obtained using plantain peels catalyst were lower than that of KOH catalyst. It can be suggested that biodiesel from plantain peel catalyst would have better fuel performance than the biodiesel from KOH catalyst due to lower density.

Acid value measures the amount of unreacted acids remaining in the finished fuel, and is also an indicator of oxidized fuel [44]. Considering that the presence of free fatty acids influences fuel aging due to hydrolytic cleavage of ester bonds, the European Union and American Standards specify a maximum value of 0.5 mg of KOH/g and 0.8 mg of KOH/g of sample respectively [42]. The acid value of biodiesel obtained from R1 and R2 were 1.40 and 0.28 mg KOH/g respectively for KOH catalyst and 1.96 and 0.84 mg KOH/g for plantain peels catalyst. The acid value obtained for KOH catalyst agreed with the acid value reported (0.29 mg KOH/g) by Nor *et al.*, [46] for biodiesel production from waste cooking oil. Acid values of R1 for both KOH and plantain peels were higher than the recommended standard by ASTM and European Union, this may be due to excessive reuse of the oil, leading to more hydrolytic cleavage of ester bonds. Biodiesel from R2 have acid values within the recommended standard for both the biological and chemical catalysts, even though biodiesel from plantain peels catalyst have higher acid value than that of KOH catalyst, thus suggesting its fuel application.

The iodine value provides information about the degree of unsaturation of the oil which directly affects its stability to oxidation. Iodine value is limited to 120 g I₂/100 g according to the European Union biodiesel standard [39]. The iodine values of biodiesel from R1 and R2 were 50.76 and 30.20 I₂/100g respectively for KOH catalyst, while that of plantain peel catalyst were 40.6 and 20.30 I₂/100g respectively. Iodine value of plantain peels were lower than recommended standard, which means they will be more stable to oxidation, thus, they have moderate unsaturation and they are suitable for fuel application.

Table 1. Physical and chemical properties of biodiesel obtained from used vegetable oils.

Properties	R1 (KOH)	R1 (Plantain peels)	R2 (KOH)	R2 (Plantain peels)
Biodiesel yield (%)	86%	84%	79%	80%
Higher heating value (MJ/kg)	45.97±0.01	45.55±0.00	45.16±0.11	45.39±0.00
Flash point (°C)	178.0±3.0	176.0±5.0	180.0±10.0	174.0±5.0
Smoke point (°C)	70.0±9.0	74.0±3.5	74.0±5.0	86.0±3.0
Cloud point (°C)	12.0±0.5	10.0±0.1	5.0±0.0	2.0±0.0
Pour point (°C)	5.0±0.2	5.0±2.0	-4.0±0.1	-3.0±0.0
Cetane number	117.70±0.02	105.50±0.00	95.89±0.00	101.90±1.10
Kinematic Viscosity at 40°C (mm ² /s)	3.71±0.20	3.02±0.00	2.95±0.10	2.01±0.00
Density at 40°C (g/cm ³)	1.200±0.12	1.103±0.14	0.898±0.14	0.871±0.12
Chemical parameters				
Acid value (mgKOH/g)	1.40±0.00	1.96±0.10	0.28±0.00	0.84±0.00
Saponification value	65.90±2.00	79.90±2.40	96.80±5.00	91.02±3.00
Iodine value (I ₂ /100g)	50.76±0.20	40.60±0.10	30.20±0.10	20.30±0.10
% FFA	0.704±0.12	0.985±0.20	0.141±0.10	0.422±0.51

Table 2. Physico-chemical Properties of unrefined and refined used oils

Sample	Acid Value		% Free Fatty Acid		Peroxide Value	
	R1	R2	R1	R2	R1	R2
Used oil	6.85 ± 0.00	4.49 ± 0.03	3.44 ± 0.00	2.26 ± 0.03	4.00 ± 0.11	14.00 ± 0.01
Degummed oil	5.33 ± 0.01	0.71 ± 0.03	2.68 ± 0.01	0.36 ± 0.03	2.00 ± 0.07	20.00 ± 0.00
Alkaline treated oil	0.58 ± 0.01	0.56 ± 0.00	0.29 ± 0.01	0.28 ± 0.00	2.00 ± 0.01	14.00 ± 0.04
Bleached oil	0.56 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.14 ± 0.01	2.00 ± 0.01	8.00 ± 0.01
Sample	Iodine Value		Saponification Values		Smoke Point	
	R1	R2	R1	R2	R1	R2
Used oil	119.28 ± 0.01	152.28 ± 0.04	149.33 ± 0.03	156.96 ± 0.14	120.00 ± 4.00	110.00 ± 2.00
Degummed oil	83.75 ± 0.12	147.21 ± 0.31	129.13 ± 0.07	134.74 ± 0.09	130.00 ± 2.00	120.00 ± 2.00
Alkaline treated oil	57.57 ± 0.06	139.59 ± 0.15	94.74 ± 0.12	88.31 ± 0.05	142.00 ± 3.00	133.00 ± 3.00
Bleached oil	35.53 ± 0.09	130.46 ± 0.01	79.71 ± 0.10	75.87 ± 0.12	150.00 ± 2.00	180.00 ± 2.00

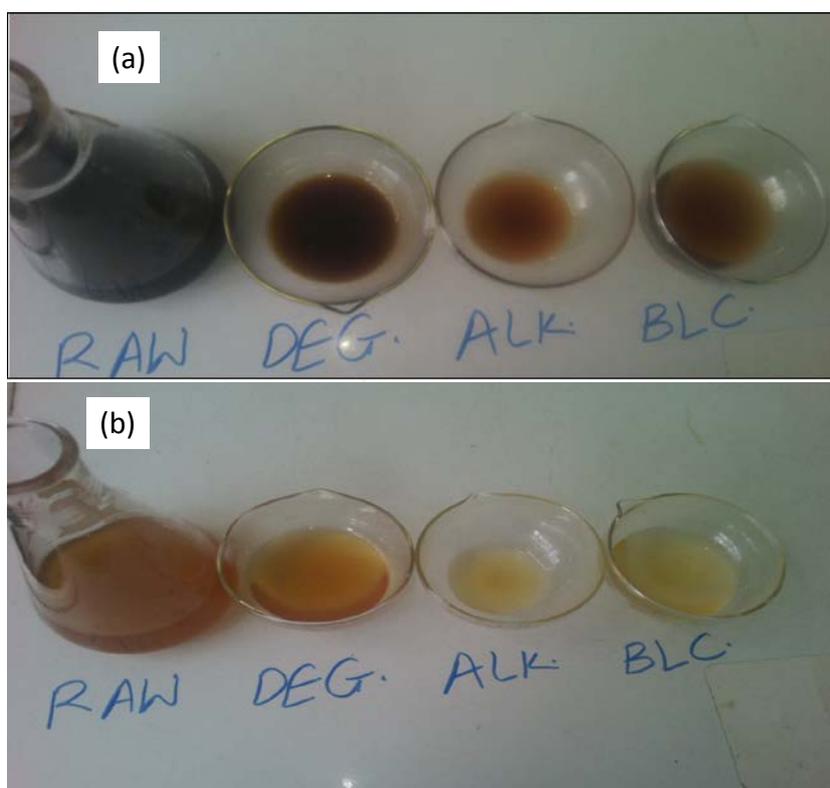


Figure 2. Visible changes in the refined used vegetable oils obtained from (a) restaurant 1 (R1) and (b) restaurant 2 (R2). (Raw= unrefined used oil, DEG = degummed oil, ALK. = alkaline treated oil, BLC. = bleached oil)

3.2. Physico-chemical Properties of the Refined Used Oils

The results of the percentage recovery of the oils after each refining stage for R1 and R2 were: after degumming (81.0, 85.0%); after alkaline treatment (81.0, 72.0%) and after bleaching (63.0, 77.0%) respectively. Figure 2 presents the visible changes that occur during the oil refining process. It was observed that the order of the % recovery of used oil for both R1 and R2 after treatment were degumming > alkaline treatment > bleaching.

However, Table 2 presents the physico-chemical properties of the refined used oils. The acid values obtained for the used oils were high, indicating the presence of large number of free fatty acids in the oil due to continuous hydrolytic cleavage of ester bonds during frying processes. However, there was drastic reduction by about 84% in the acid value of R2 oil due to degumming,

and further 3% and 6% reductions due to alkaline and bleaching respectively treatment (Table 2). Since degumming was meant to remove the hydratable phosphatides in the oil, the raw R2 oil may have well contained mostly free phosphatidic fatty acids (with the –COOH end esterified with a phosphate group). The R1 oil, however only experienced a 22% removal of free fatty acids after degumming; with the major changes occurring after alkaline treatment (a 69% reduction in free fatty acid content and non-hydratable phosphatides). This shows that the oil may mostly contained non-hydratable phosphatides [47]. The extent of frying of the two used oils as depicted by their physical colour appearance (Figure 1) can also be another factor responsible for their degree of responsiveness to the different refining methods employed in this study. The CODEX Alimentarius Commissions [48] holds the maximum acid value of refined edible vegetable oils of 0.6 mgKOH/g oil and a maximum value of 4.8 mg

KOH/g oil for used cooking oils. Thus, the refined oils have their acid values (R1 = 0.56 mg KOH/g, R2 = 0.28 mg KOH/g) below the maximum value recommended, confirming the efficiency of the refining methods used in regenerating an improved quality oil from their used and deteriorated form. Reduction in acid values observed in this study compared well with the results of Adu *et al.*, [49], Arisa and Lazarus [50], where decrease in acid values of *T. catappa* and *D. edulis* oils after degumming was observed and reported. Also, the percentage free fatty acid content of all the oil samples before (R1 = 3.44%, R2 = 2.26%) and after refining (R1 = 0.28%, R2 = 0.14%) were below 5%, a value above which transesterification reactions of oils lead to the excessive formation of soap during the application of oils in biodiesel production [48]. This result is unique, since a huge reduction in the % fatty acid content of the used oils (R1 = 85.7%, R2 = 93.8%) were successfully recorded after the refining stages. The implication of this result is that apart from suggesting the recycling potential of the used oils, refined oils have gained better domestic and industrial application than the used oils with improved resistance towards oxidative degradation.

The peroxide value gives information about the level of primary oxidation products in a given oil sample [51]. It was observed that the peroxide value of R2 oil increased after degumming, which might be as a result of water and temperature (60 – 70°C) involved during degumming process. Similar result was reported by Idoko *et al* [52] where an increase in peroxide values of palm oil after bleaching and degumming process was observed. But after alkaline and bleaching treatment, the peroxide value decreased. Oils obtained after alkaline treatment of both R1 and R2 have peroxide value lower than the maximum standard for peroxide value by Codex (10 meq O₂/kg) for vegetable oil deterioration. This means that refining has improved the deterioration quality of the oils. Reduction in peroxide value after bleaching treatment had also been reported by Duhan *et al.*, [53] for *C. colocythis* and *P. pinnata* oils.

The iodine value of oil measures the level of unsaturation of the oil and is, therefore a direct indicator of the oxidative stability of the oil. This means an increase in iodine value indicates high susceptibility of oil to oxidative degradation [54]. Reduction in iodine value of both oils was observed after each refining stage which means oils with better oxidative stability were obtained after refining process. The oils can be use as lubricants

because they didn't harden during exposure to air [55]. Reduction of 25.59% and 27.37% of iodine value after refining of *C. colocythis* and *P. pinnata* respectively was reported by Duhan *et al.*, [53].

Saponification values had been reported to be inversely related to the average molecular weight of the fatty acids in the oil fractions [56]. Therefore, the shorter the average chain length (C₄-C₁₂) the higher is the saponification number [57]. Decrease in saponification value by 16.4%, 60% and 30% were observed after degumming, alkaline treatment and bleaching respectively for R2 oil. Similar results were obtained for R1 oil with decrease in saponification value of 18.9%, 44% and 25% due to degumming, alkaline treatment and bleaching respectively. The results show that all the oil samples before and after refining mostly consist of fatty acids with moderately long chain lengths (C₁₆-C₂₀) due to their relatively low saponification values, i.e. they all have values less than 190-209 mg KOH/g, which is the CODEX standard permissible level [48]. Decrease in saponification value was also reported by Idoko *et al* [52] after bleaching palm oil and 13.46 and 13.43% decrease in saponification value was also observed by Duhan *et al.*, [53].

Smoke point is the temperature at which smoke is first seen during the heating of oils [51]. After each process, the smoke point of both R1 and R2 increased, this might be due to the removal of impurities in the oils which may be responsible for low smoke point in raw R1 and R2. Codex standard for smoke point of cooking vegetable oil is minimum of 170°C. Only the oil obtained after bleaching process from R2 had its smoke point higher than the set standard (180°C). This imply that the oils may not be good for frying but they are safe to handle when use as fuel because it will not ignite at low temperature

Specific gravity values are important in the international edible oil trade market with an acceptable limit of 0.899 – 0.920 at room temperature [48]. As shown in Table 3 and Figure 3, decrease in specific gravity was observed after degumming, alkaline treatment and bleaching processes for both oils. This confirms the removal of unwanted substances such as free fatty acids, oxidation products etc. present in the oil. Similar reduction in specific gravity was also obtained for the refining of *Baphia nitida* and *Gliricidia sepium* seed oils by Adewuyi *et al.* [58]. All the refined oils had specific values within the range specified by Codex standard permissible level. The oils have good potentials for application as lubricating oils.

Table 3. Specific gravity and kinematic viscosity values of used and refined oils

Samples	R1			R2		
	30 °C	40 °C	60 °C	30 °C	40 °C	60 °C
Specific gravity						
Raw Oil	0.922 ± 0.25	0.918 ± 0.44	0.917 ± 0.41	0.921 ± 0.23	0.917 ± 0.31	0.914 ± 0.33
After Deg.	0.921 ± 1.01	0.917 ± 0.07	0.915 ± 0.61	0.918 ± 0.18	0.914 ± 0.23	0.911 ± 0.14
After Alk.Tmt.	0.919 ± 0.99	0.916 ± 0.04	0.914 ± 0.27	0.915 ± 0.08	0.913 ± 0.22	0.911 ± 0.72
After Bleaching	0.916 ± 0.54	0.915 ± 0.38	0.913 ± 0.55	0.914 ± 0.17	0.913 ± 0.21	0.909 ± 0.46
Viscosity (Centistoke)						
Raw Oil	90.41 ± 1.04	59.62 ± 2.16	32.13 ± 1.16	59.45 ± 0.29	35.93 ± 0.64	20.31 ± 0.89
After Deg.	79.48 ± 0.25	59.42 ± 2.26	28.92 ± 1.52	54.99 ± 0.60	36.92 ± 0.79	20.18 ± 0.11
After Alk.Tmt.	74.25 ± 1.77	52.25 ± 1.38	26.79 ± 1.04	52.74 ± 0.23	35.41 ± 0.30	19.85 ± 0.52
After Bleaching	72.59 ± 0.15	51.59 ± 1.57	26.63 ± 0.79	51.99 ± 1.63	35.28 ± 1.09	19.26 ± 0.64

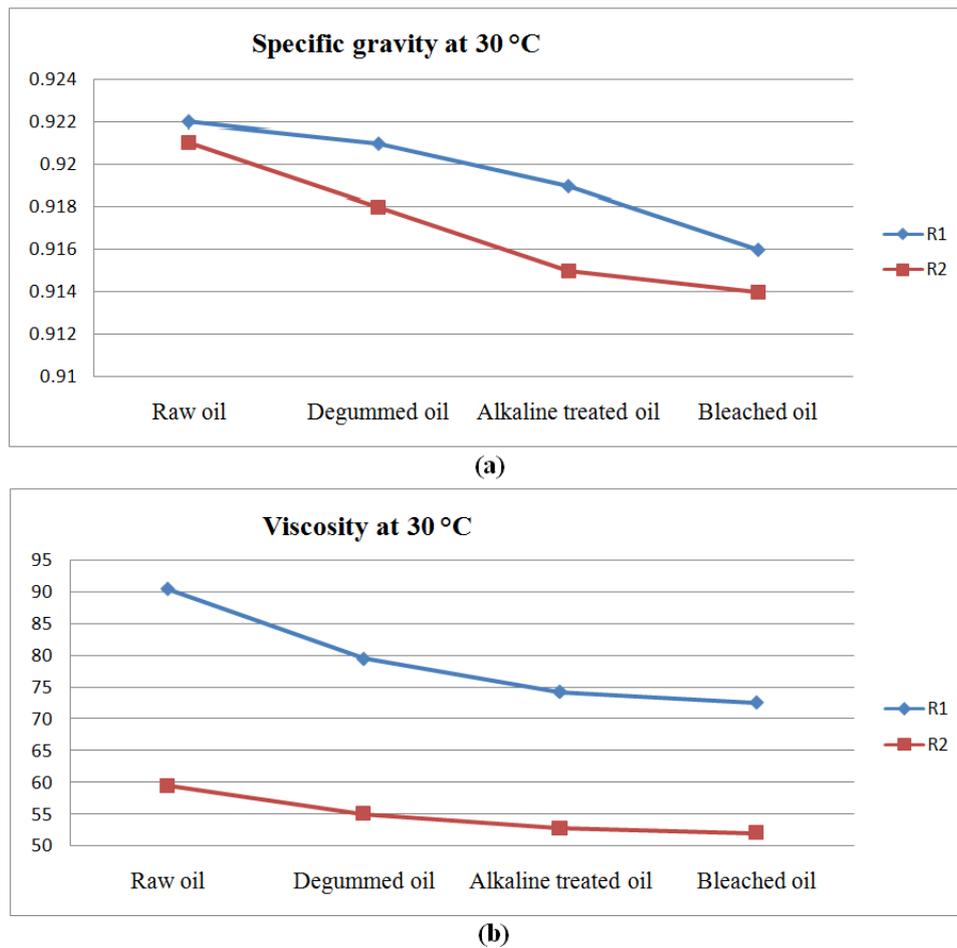


Figure 3. (a) Specific gravity and (b) Viscosity of unrefined and refined used oils

Viscosity is a measure of the resistance of a fluid to deform under shear stress [59]. It is commonly perceived as thickness, or resistance to pouring. Decrease in viscosity of oil was observed after each refining process in both R1 and R2 (Table 3). Ryan *et al.* [60] reported that the viscosity of vegetable oils is influenced mainly by fatty acid composition and quantity of contaminants. Therefore, it may be postulated that removal of impurities from the oil as a result of each refining stage led to the reduction in viscosity.

3.3. Cytotoxicity of Oil Samples

The results of the toxicity of the oil samples are shown in Table 4a. The brine shrimp results in this study are interpreted in terms of the lethal concentration at 50 (LC₅₀), which is a measure of the toxicity of the surrounding medium that will kill half of the population of a specific test organism as follows: LC₅₀ <1.0 µg/ml – highly toxic; LC₅₀ 1.0–10.0 µg/ml – toxic; LC₅₀ 10.0–30.0 µg/ml – moderately toxic; LC₅₀ >30 <100 µg/ml – mildly toxic, and > 100 µg/ml as non - toxic [61]. The results showed the lowest toxicity for the alkaline-treated R2 oil with an LC₅₀ value of 89.37 (Table 4a), which indicates mild toxicity. Other oils in this category were the raw, degummed and bleached R2 oils. The R1 oil, on the other hand, had a much higher level of toxicity with LC₅₀ values of 3.82, 6.55 and 9.50 for the raw, degummed and alkaline-treated oil respectively (Table 4a), indicating a much higher level of degradation (presence of more toxic

substances) due to continuous use. Only the bleached R1 oil, however, showed a mild toxicity with an LC₅₀ value of 38.44.

High toxicity of R1 oil than R2 oil might be as a result of excess continuous use of the oil which may have led to formation of toxic compounds. The toxicity of R1 oil decrease after each refining step but the bleached oil experienced improved mild toxicity. Both R1 and R2 oils resulted in mild toxicity after refining. It can therefore be concluded that the R2 oil, if treated further, could pose little or no health effect when consumed as a much lesser level of toxicity would be obtained. Though, further work on animal toxicity will be needed to validate this suggestion. The R1 oil, on the other hand will be more suitable for soap making and paint production. However, a more rigorous refining process could improve its toxicity levels.

Table 4b presents data on the Brine Shrimp Lethality Assay. The numbers of shrimps affected by each oil sample at different concentrations after 24 hours of introduction were presented. Generally, it can be concluded that the R1 oil is more toxic as more of the shrimps were dead after 24 hours.

Table 4a. LC₅₀ of Cytotoxicity for oil samples

Sample	R1	R2
Raw oil	3.82	45.03
Degummed oil	6.55	57.15
Alkaline treated oil	9.50	89.37
Bleached oil	38.44	72.38

Table 4b. Toxicity level of oil samples at different concentrations ($\mu\text{g/mL}$)

Sample	CONCENTRATION ($\mu\text{g/mL}$)														
	100			50			25			12.5			6.25		
	Exp.	Aff.	%M	Exp.	Aff.	%M	Exp.	Aff.	%M	Exp.	Aff.	%M	Exp.	Aff.	%M
R1															
R	20	9	45.0	20	8	40.0	20	7	35.0	20	7	35.0	20	6	30.0
D	20	8	40.0	20	7	35.0	20	6	30.0	20	6	30.0	20	5	25.0
A	20	6	30.0	20	6	30.0	20	6	30.0	20	6	30.0	20	5	25.0
B	20	5	25.0	20	5	25.0	20	5	25.0	20	4	20.0	20	4	20.0
R2															
R	20	8	40.0	20	6	30.0	20	5	25.0	20	5	25.0	20	5	25.0
D	20	6	30.0	20	6	30.0	20	5	25.0	20	4	20.0	20	4	20.0
A	20	5	25.0	20	5	25.0	20	4	20.0	20	4	20.0	20	4	20.0
B	20	5	25.0	20	5	25.0	20	3	15.0	20	3	15.0	20	2	10.0

Key: Exp = Exposed, Aff = Affected, %M = percentage mortality, **R1** = Oil from restaurant 1, **R2** = Oil from restaurant 2, **R** = Raw oil, **D** = degummed oil, **A** = alkaline-treated oil, **B** = bleached oil.

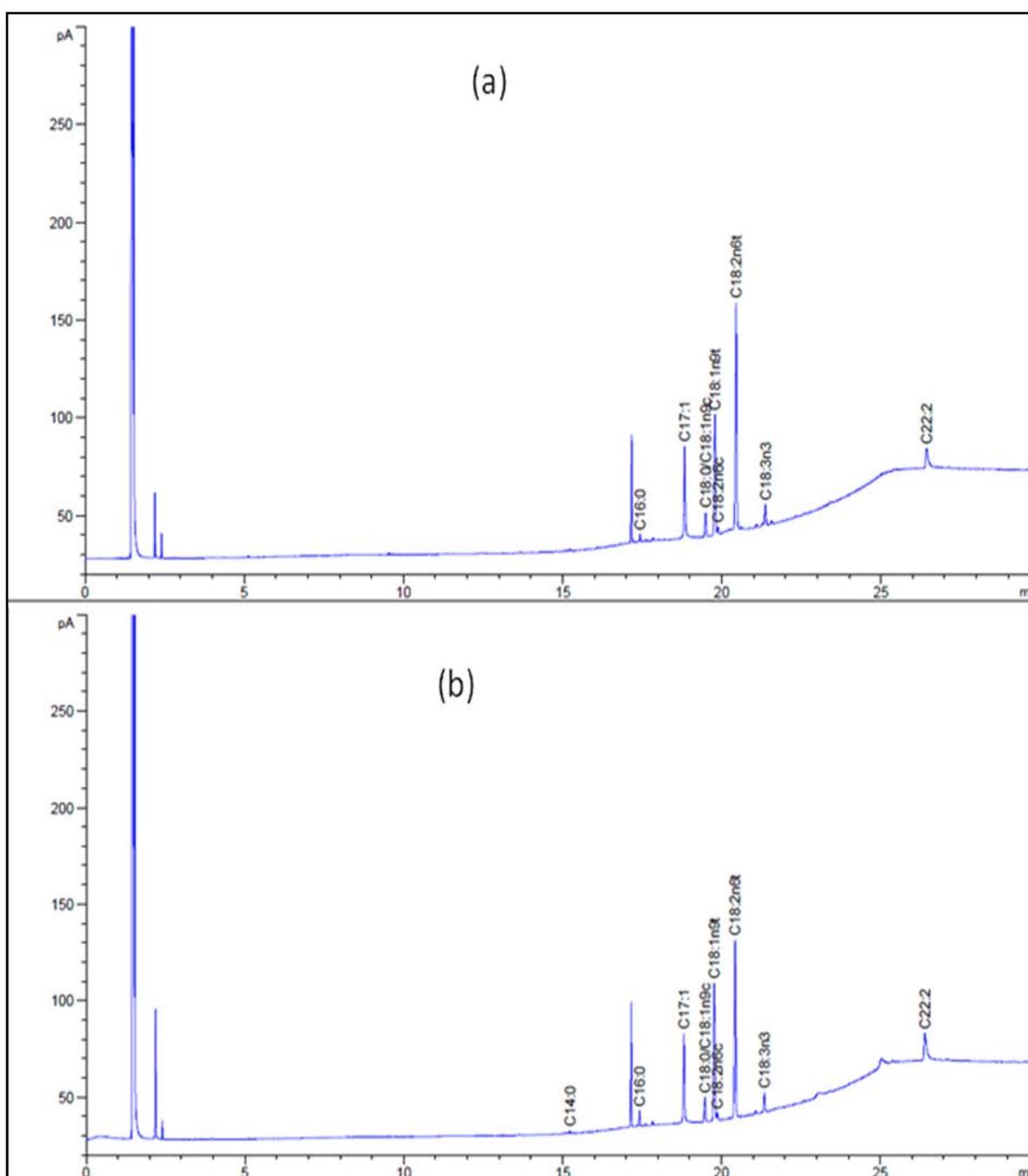


Figure 4. GC-FID spectrum showing the fatty acid composition of (a) used vegetable oil and (b) refined oil

Table 5. Comparison of % fatty acid composition of refined and unrefined R2 oils

Important fatty acids	Unrefined Oil (%)	Refined Oil (%)	CODEX STANDARDS (%)
C16:0 (palmitic acid)	1.13	2.37	8-14
C18:1n9t (elaidic acid)	14.36	17.61	35-69
C18:0 (stearic acid) and C18:1n9c (oleic acid)	0.70	0.75	
C18:2n6c (linoleic acid)	0.54	0.63	12-45
C18:2n6t (linolelaidic acid)	52.63	42.57	
C18:3n3 (linolenic acid)	5.98	6.18	1-5
C22:2 (cis-13,16-docosadienoic acid)	7.76	12.08	0.3-3

3.4. Effect of Refining on the Fatty Acid Composition of Used and Refined Oils

Figure 4 is the fatty acid spectra of the unrefined and refined used oils of R2 after the refining processes (degumming, alkaline treatment and bleaching), analysed using Gas Chromatography- Flame Ionization Detector (GC-FID). Generally, the fatty acids present in the used vegetable oils showed some % increase after refining (Table 5). About double the amount of palmitoleic acid (C16:0) was observed in the refined R2 oil as compared to its unrefined oil (Table 5). Also, a slightly higher amount of the cis stearic acid acids (C18:0), oleic acid (C18:1n9c) and linoleic acid (C18:2n6c) was observed for the refined oil. Increase in the amount of oleic acid and linoleic acid of some oils after refining process was also reported by Duhan *et al.* [53] and Anil *et al.* [62]. Finally, the refined oil showed a reduced level of the trans acid C18:2n6t (Linolelaidic acid) and a much greater amount of C22:2 (cis-13, 16 -docosadienoic acid) compared to the unrefined oil. This result demonstrated the efficiency of the refining process in the recycling of used vegetable oils for potential applications in cosmetics, soap, paint and energy (biodiesel) industries. This significant positive impact of the refining processes employed in this study on the used oils could be further enhanced if more rigorous conditions, and in conjunction with other refining process were explored.

4. Conclusion

The study showed that used vegetable oils can be a potential source of biodiesel production and an alternative to the conventional used petroleum diesel. It was also observed that the use of environmental friendly catalyst that is cheap and readily available could subsequently be employed since it gives biodiesel properties that compares favourably with that obtained using base homogeneous catalyst. Also, degumming process and alkaline treatment showed the most effective output in reducing the free fatty acids and oxidation products present in the used oils which resulted in oils with improved properties for emulsification, biodiesel production and other industrial applications.

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Supplementary

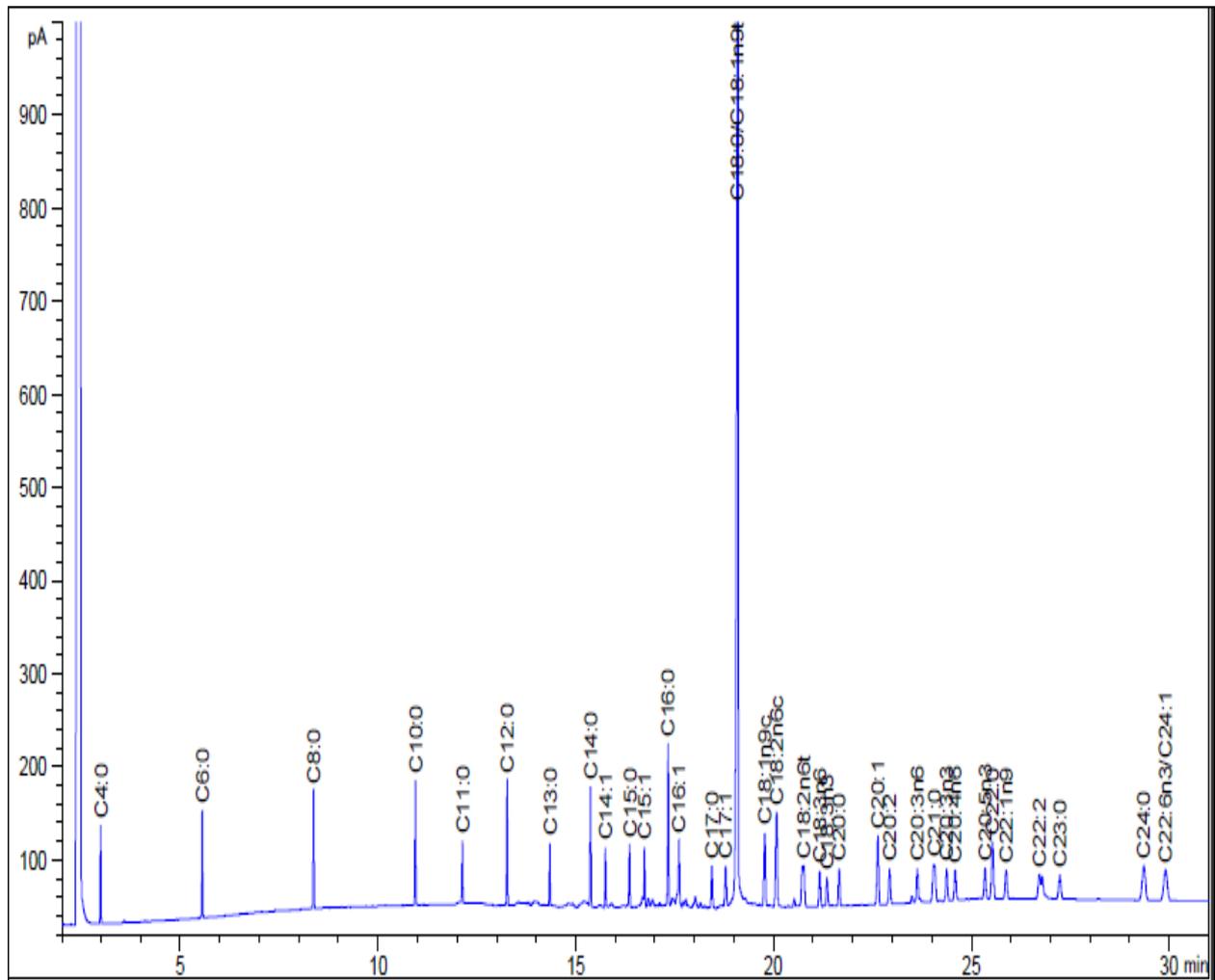


Figure S1. GC-FID spectrum for standards fatty acids