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Research Article
Effects of Ethanolic Leaf Extracts of Neem (*Azadirachta indica*) on Oxidative Stability of Palm Oil

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Abstract

Background and Objective: The search for natural alternatives in oil and food preservation is on the increase considering the potential health impact of using synthetic antioxidants. It is in attempt to bridge this gap that this study was designed to test the potency of leaf extract of neem (*Azadirachta indica*) in reducing peroxidation and stabilizing palm oil during storage. **Materials and Methods:** The neem leaf extract was carried out by cold maceration using ethanol as the extraction solvent. The ethanolic leaf extract was then evaluated for chemical composition using GCMS and UV-spectroscopy. Antioxidant activity on the extract was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH); β-carotene linoleic antioxidant activity, oxygen reducing antioxidant capacity (ORAC) and ferric reducing antioxidant power (FRAP) efficacy using standard procedures. Oil samples were prepared and treated with the plant leaf extracts using four different concentrations (100, 200, 400 and 800 mg L^{-1}) on storage for 6 months. Thereafter, chemical properties of the oil samples including peroxide value, anisidine value, iodine value, acid value, saponification value as well as sensory evaluation was also conducted on monthly basis throughout the storage period. **Results:** The results revealed the presence of certain bioactive compounds and their functional groups previously shown to facilitate antioxidant activities. Similarly, the extract had significant antioxidant effect for DPPH (12.5400±0.0173 IC_{50} (%) inhibition); β-carotene linoleic antioxidant activity (43.8233±0.0251 IC_{50} (%) inhibition), ORAC (4.6500±0.0200 μ Mol TE g^{-1}) and FRAP (286.4267±0.0378 μ Mol Fe^{2+} L^{-1}). Oil samples treated with the leaf extracts during storage show significant retardation of oxidation compared to untreated samples. In addition, sensory evaluation on a five-point hedonic scale revealed that all samples were generally acceptable. **Conclusion:** This study has given plausible evidence to support the use of *Azadirachta indica* as a natural antioxidant to prevent vegetable oils oxidation during prolonged storage.

Key words: Palm oil, natural antioxidant, lipid peroxidation, oil stabilization, *Azadirachta indica*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The oil palm (*Elaeis guineensis*) is one of the important economic crops in the tropics. The primary products of the oil palm are palm oil (from the mesocarp) and palm kernel oil obtained from the kernels (seeds), which are derived through different processing techniques. However, edible oils (palm oil inclusive) are highly susceptible to oxidative deterioration and consequent production of undesired flavour during prolonged storage due to multiple environmental and storage conditions[1]. Oil instability is the inability of oil to remain stable due to the presence of acyl lipids such as monomeric, dimeric and oligomeric triacylglycerols and hesterols and phytosterols, which can oxidize on exposure to air or presence of moisture at high temperature to form lipid oxidation products with initial reaction products known as hydroperoxides and later form compounds such as aldehydes, ketones, alcohols and carboxylic acids[2,3]. The overall effect is seen in impaired palatability and avoidable economic loss resulting from changes in chemical composition[4] capable of impairing acceptability. Lipid oxidation depletes important qualities of oils and reduces organoleptic characteristics of the foods they are used to prepare[5]. The abnormal sensory characteristics of oils that have undergone rancidity are paint-like or acrid (burning) odour and abnormal taste. The shelf life of edible oils is greatly dependent on their oxidative stabilities[6] and this affects their applicability for both home and industrial purposes. Upon consumption, rancid oils form harmful free radicals in the body that lead to cellular damage with possible attendant health consequences such as those linked to diabetes and Alzheimer's disease as well as digestive distress and depletion or impairment of absorption of Vitamins B and E in the gastrointestinal tract[6]. To reduce the susceptibility lipid oxidation that may lead to rancidity, edible oil industries use chemicals and synthetic antioxidants in preservation of oils[7]. However, the safety of synthetic additives has been questioned, which has stimulated researches to explore naturally-occurring alternatives with comparable antioxidative properties and health benefits. As previously reported, many natural antioxidants like ginger extract, raspberry leaves, pussy willow extract, olive leaf juice have been used to protect oxidizable constituents of essential oils from oxidation with consequent improvement in shelf life[8,9]. However, natural antioxidants have not been used on a large commercial scale owing to their high input cost, seasonal availability of their sources and a lack of robust scientific and technological evidence in support of their safe, nutritious and sensory parameters. Consequently, a commonly available and easily accessible source of promising phytochemicals is now the interest of many researchers[10]. Few studies have highlighted the medicinal importance of neem leaf and seed[11,12]. However, there is paucity of information and evidence in support of its antioxidant potential, particularly as it relates to oxidative stabilisation of edible oils for extended period of storage. This research work was therefore; designed to explore the antioxidant potential of graded concentrations of neem plant extracts using palm oil as an oxidation substrate on the basis of certain chemical and sensory parameters.

MATERIALS AND METHODS

Place and time: This research was conducted at the Centre for Food Technology and Research Laboratory, Benue State University, Makurdi, Benue State, Nigeria. Implementation was between February-September, 2018.

Sample collection: Mature *Azadirachta indica* (NM) leaves were collected from Makurdi metropolis and were properly identified by a botanist at the Department of Biological Sciences of Benue State University. The leaves were washed under running tap water and air-dried for 3 days and subsequently crushed mechanically to obtain a suitable surface area. The resulting samples were then dried at room temperature in the shade to avoid exposure to sunlight for 2 h before being stored for further analysis. Palm oil sample was collected from Seasons Oil Processing Mill, Abia State, Nigeria and kept in tightly sealed MacCakney bottles prior to storage.

Extraction of neem leaf extract: Extraction of leaf extracts was done by cold maceration according to the method described by Hossain *et al.*[11] and Evbuomwan *et al.*[12]. About 500 g of ground leaves were weighed into 4000 mL of ethanol and the solution was macerated for about 24 h with gentle shaking at 360 rpm until the soluble matter had properly dissolved. After extraction, the solution was clarified by filtration under vacuum using Whatman filter paper and the ethanol solvent evaporated completely using a rotary evaporator. The solvent free ethanol crude extract was suspended in diethyl ether to purify the extract after which it was exposed to the atmosphere for a while to ensure elimination of the solvent odour. The extracts obtained were then weighed and refrigerated for further analysis.

Phytochemical screening and quantitation

Determination of total phenolic content: The total phenolic content of the leaf extracts of the samples was determined

by taking 20 µL of the extract in a screw capped 11 mL test tube, together with 1.6 mL distilled water and Folin-Ciocalteu reagent (100 µL). All were mixed with each other, then 300 µL of 20% Na_2CO_3 solution added and well shaken in a shaking water bath at 40°C for 30 min and total phenolic content determined from the standard curve plotted by using gallic acid as standard at 760 nm, according to the method of Anwar *et al.*[13].

Determination of total flavonoid content: The total flavonoid (TF) content of the extracts was quantified according to the method described by Dewanto *et al.*[14] and the results determined as catechin equivalents (mg/100 g of dry weight). At a concentration of 1 mg mL^{-1}, the extracts were diluted with 4 mL of water in a 10 mL volumetric flask. Initially, 0.3 mL of 5% $NaNO_2$ solution was added to each volumetric flask; at 5 min, 0.3 mL of 10% $AlCl_3$ is added and at 6 min, 2 mL of 1.0 mol L^{-1} NaOH was added before 2.4 mL water was added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm.

Determination of tannins: Determination of tannins was carried out by the method described by Wahab and Elabor[15]. About 0.2 g of sample was measured into a 50 mL beaker. About 20 mL of 50% methanol added and covered with paraffin and placed in a water bath at 77-80°C for 1 h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 mL volumetric flask using 50% methanol to rinse. This was then made up to mark with distilled water and thoroughly mixed. About 1 mL of sample extract was then pipette into 50 mL volumetric flask, 20 mL distilled water, 2.5 mL Folin-Denis reagent and 10 mL of 17% Na_2CO_3 then added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min till a bluish-green colouration developed. Standard tannic acid solutions of range 0-10 ppm was treated similarly as 1 mL of sample above. The absorbances of the tannic acid standard solutions as well as samples are read after colour development on a spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated using the equation:

$$\text{Tannin (\%)}\ \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 1000}$$

Gas chromatography-mass spectroscopy analysis: The gas chromatography-mass spectroscopy (GC-MS) analysis was done with SHIMADZU, Japan GCMS-QP2010 PLUS. The GC-MS analysis was undertaken to detect the organic compounds present in *A. indica* ethanolic leaf extract. Each chemical constituent of extract was compared with NIST05s LIB by matching with the mass spectra of published data.

Fourier transform infrared (FT-IR) spectroscopy: A SHIMATZU FTIR-8400S Japan (KBr) spectrophotometer was used for the recording. About 10 mg of dried *A. indica* ethanolic leaf extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope with a Scan range from 400-4000 cm^{-1} with a resolution of 4 cm^{-1}.

Determination of antioxidant activity

DPPH radical scavenging activity (DRSA): Initial DPPH assay on TLC plate was done and then the antioxidant activity of the different crude extracts was evaluated as described by Hossain *et al.*[11] with modification. About 4 mL of each concentration was placed in a working test tube and then DPPH (2,2-diphenyl-1-picrylhydrazyl) (1 mL, 0.1 mM, methanol) added to the test tube and shaken vigorously. After shaking, all the test tubes were allowed to stand at 27°C in a dark place for 45 min. A control sample was prepared according to the same procedure without any extract. The absorbance of the tested samples was then be measured by UV spectrophotometer at the wavelength 517 nm. The antioxidant activity of each sample was expressed in terms of concentration required to inhibit DPPH radical formation by 50% (IC_{50} µg mL^{-1}) and calculated from the log-dose inhibition curve:

$$\text{DPPH (\%)} = \frac{(\text{Ac} - \text{At})}{\text{Ac}} \times 100$$

Where:

Ac : Absorbance of control is the absorbance in absence of standards or extracts

At : Absorbance of sample is the absorbance in presence of standards or extracts

Oxygen radical absorbance capacity (ORAC): The method employed by Girgih *et al.*[16] was used. The samples were dissolved in sodium phosphate buffer (75 mM, pH 7.4) and then mixed with 300 nM fluorescein in a 96-well microplate followed by incubation of the mixture in the dark at 37°C for 15 min (final peptide concentration of 1 mg mL^{-1}). Thereafter, a 50 µL aliquot of 80 mM 2,2^{l}-azobis (2-amidinopropane)

dihydrochloride (AAPH) was added to the mixture and the change in fluorescence due to AAPH-induced oxidation of fluorescein measured at 1 min intervals for 90 min at excitation and emission wavelengths of 485 and 528 nm, respectively, using a fluorescence microplate reader. Different concentrations of Trolox (5-80 μM) was used to prepare a standard curve and the ORAC values of the samples calculated as follows:

$$1+ = \sum_{i=1}^{i=100} \frac{\int i}{\int 0}$$

where, ORAC values were expressed as μM T trolox equivalent (TE) g of sample.

Ferric reducing antioxidant power (FRAP): The method employed by Girgih *et al.*[16] was used. About 250 μL of the sample was dissolved in 0.2 M sodium phosphate buffer at pH 6.6 and blank (250 μL of buffer) was mixed with 250 μL of same buffer followed by addition of 250 μL of 1% (w/v) potassium ferricyanide solution. Thereafter, 250 μL of peptide/TCA mixture was combined with 50 μL of 0.1% (w/v) ferric chloride and 200 μL of double distilled water and allowed to stand at room temperature for 10 min. The solution was then centrifuged at 10,000 rpm and 200 μL of the clear supernatant transferred to a 96-well plate for determination of the absorbance of the supernatant at 700 nm.

β-carotene bleaching antioxidant assay: In this assay the antioxidant capacity of *A. indica* was determined in emulsion by the β-carotene bleaching method of Farag *et al.*[17] consisting in a coupled oxidation of linoleic acid and β-carotene. A stock solution of β-carotene/linoleic acid (Sigma-Aldrich) was prepared as follows. β-carotene (0.5 mg) was dissolved in 1 mL of chloroform (HPLC grade), then 25 μL of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated, then distilled and oxygenated water (100 mL) was added with vigorous shaking. Aliquots (2.5 mL) of the stock solution were transferred to test tubes and 300 mL portions of the extracts (1 g L^{-1} in methanol) were added before incubating for 48 h at room temperature. The antioxidant activity was evaluated by absorbance measurement at 470 nm against a blank containing emulsified linoleic acid without β-carotene.

Preparation of oil samples for storage: The leaf extracts of *A. indica* was added to the palm oil samples in four concentrations of 100, 200, 400 and 800 mg L^{-1}. The acceptable maximum concentration of 100 mg L^{-1} of butylated hydroxyl toluene (BHT) was added to another portion of the oils to act as standard while a sample without extract or BHT was used as the control. All samples were measured into 100 mL polyethylene terephthalate (PET) bottles, cork screwed and labeled as P100 (Palm oil+ 100 mg L^{-1} neem leaf extract), P200 (Palm oil+200 mg L^{-1} neem leaf extract), P400 (Palm oil+400 mg L^{-1} neem leaf extract), P800 (Palm oil+800 mg L^{-1} neem leaf extract), PBHT (Palm oil+100 mg L^{-1} Butylated Hydroxyl Toluene) and PCONT (Palm oil Control without extract). They were then kept in a dark cupboard were samples were taken for analysis each month.

Determination of peroxide value: Peroxide value was determined by iodometric titration which measures the iodine produced from potassium iodide by peroxides present in the oil, according to the method described by Jacobs[18].

Determination of anisidine value: Anisidine values (AV) were determined according to AOAC[19] ISO Method 6885 by measuring the absorbance of p-anisidine at 350 nm on a JENWAY 7315 UV-spectrophotometer.

Determination of acid value: The acid value were determined according to the AOAC[19] official method using 0.5 N potassium hydroxide to titrate 2 g of oil in 50 mL mixture of dietylether and ethanol until a pink colour was obtained.

Determination of iodine value: The Wijs method described by Nadeem *et al.*[20] was employed in determination of iodine value.

Sensory evaluation: Sensory evaluation of oil samples and control were carried out at 25°C in a well illuminated laboratory. Samples of the oils were randomly served to a panel of 25 trained judges and all orders of servings fully randomised. Sensory evaluation was done using a descriptive 5-point Hedonic scale. The rating was 5 very desirable and 1 undesirable for all parameters evaluated, which included colour, appearance, odour, flavor and texture.

Statistical analysis: Data is presented as mean±standard deviation of two replicates and analyzed by one-way ANOVA using statistical products and service solutions (SPSS) software version 21 (SPSS Ltd., USA). Multiple comparisons (*post hoc* Duncan multiple range test) were used to evaluate significant differences of the data at $p \leq 0.05$ confidence limit.

RESULTS

Phytochemical composition of *A. indica* leaf extracts: The results of phytochemical screening and quantitative phytochemical determination of *A. indica* are shown in Table 1 and 2. *Azadirachta indica* showed positive identification for tannins, saponins, alkaloids, phenolics, flavonoids, glycosides and terpenoids with phenolics recording the highest amount (6624.920±4.670) while tannins recorded the least amount of 131.850±0.55 μg mL^{-1}.

Antioxidant activity of *A. indica* leaf extracts using different methods: Results of antioxidant activity of *A. indica* is presented in Table 3 with the extracts showing comparative antioxidant property to its synthetic counterpart.

GC-MS analysis of *A. indica* leaf extracts: The GC-Mass spectra of the ethanolic extracts of Neem leaves is presented in Fig. 1 and the compounds present identified as shown in Table 4. The fragmentation patterns of the mass spectra were compared with those of the known compounds stored in the National Institute of Standards and Technology (NIST) research library.

FTIR analysis of *A. indica* leaf extracts: The FTIR spectra given in Fig. 2 revealed different characteristic peaks corresponding to various functional groups characterizing *A. indica* that may be responsible for the antioxidant properties of the extracts.

Peroxide values of oil samples on storage: The peroxide values of treated oil samples and control are presented in Table 5. Range of peroxide values for treated samples denoted by P100-P800 for concentration of extracts were from 0.695±0.001-0.921±0.000 meq kg^{-1} at first month and 4.266±0.003-7.923±0.004 meq kg^{-1} by end of storage, which

Table 1: Qualitative phytochemical screening results of *A. indica* leaf extracts

Specie name	Tannins	Saponins	Alkaloids	Phenolics	Flavonoids	Steroids	Glycosides	Terpenes	Phlobatannins
A. indica	+	+	+	++	+	-	+	+	+

+: Present, -: Absent

Table 2: Quantitative phytochemical determination *A. indica* leaf extracts

Specie name	Tannins (μg mL^{-1})	Phenolics (μg mL^{-1})	Flavonoids (μg mL^{-1})
A. indica	131.850±0.55	6624.920±4.670	450.224±0.808

Values are mean±standard deviation for two independent determinations

Table 3: Antioxidant activity of *A. indica* leaf extracts

Specie name	DPPH scavenging activity	B-carotene linoleic acid oxidation	ORAC (μ Mol TE g^{-1})	FRAP (μ Mol Fe^{2+} L^{-1})
A. indica	12.5400±0.0173	43.8233±0.0251	4.6500±0.0200	286.4267±0.0378
Standard	82.5333±0.0611	61.0633±0.0404	11.8333±0.0351	314.1267±0.0305

Values are mean±standard deviation for two independent determinations

Table 4: GC-MS result of chemical compounds present in *A. indica* leaf extracts

Peak no.	Retention time	Name of compound	Molecular formulae	Molecular weight (g mol^{-1})	Peak area (%)
1	24.779	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.18	0.80
2	36.883	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.203	3.43
3	37.802	Phytol, acetate	$C_{22}H_{42}O_2$	338.576	1.75
4	38.953	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	456.430	23.34
5	39.735	Phytol	$C_{20}H_{40}O$	296.539	20.29
6	39.966	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.452	19.27
7	40.142	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.538	1.30
8	40.630	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340.592	1.35
9	42.781	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.509	2.70

Table 5: Peroxide values (meq/1000 g) of palm oil treated with *A. indica* leaf extracts

	Months					
Variables	1	2	3	4	5	6
P100	0.921±0.000^{d}	2.132±0.002^{d}	4.123±0.002^{e}	5.816±0.005^{e}	6.510±0.014^{e}	7.923±0.004^{e}
P200	0.729±0.001^{c}	1.565±0.012^{c}	4.013±0.004^{d}	5.145±0.022^{d}	6.361±0.002^{d}	6.748±0.003^{d}
P400	0.721±0.002^{c}	1.531±0.001^{c}	3.655±0.004^{c}	4.870±0.012^{c}	5.356±0.008^{c}	5.482±0.002^{c}
P800	0.695±0.001^{b}	1.078±0.004^{a}	3.457±0.007^{b}	4.581±0.018^{b}	4.039±0.017^{b}	4.266±0.003^{b}
PBHT	0.521±0.001^{a}	1.237±0.002^{b}	2.257±0.004^{a}	2.540±0.007^{a}	3.395±0.098^{a}	3.955±0.006^{a}
PCONT	1.012±0.010^{e}	2.595±0.001^{e}	5.283±0.003^{f}	7.592±0.011^{f}	8.606±0.007^{f}	9.257±0.004^{f}

5: Very desirable, 4: Desirable, 3: Acceptable, 2: Slightly undesirable, 1: Undesirable. Values are Mean±Standard deviation two independent determinations. Values with different superscripts are statistically significant (Duncan multiple range test) at $p \leq 0.05$

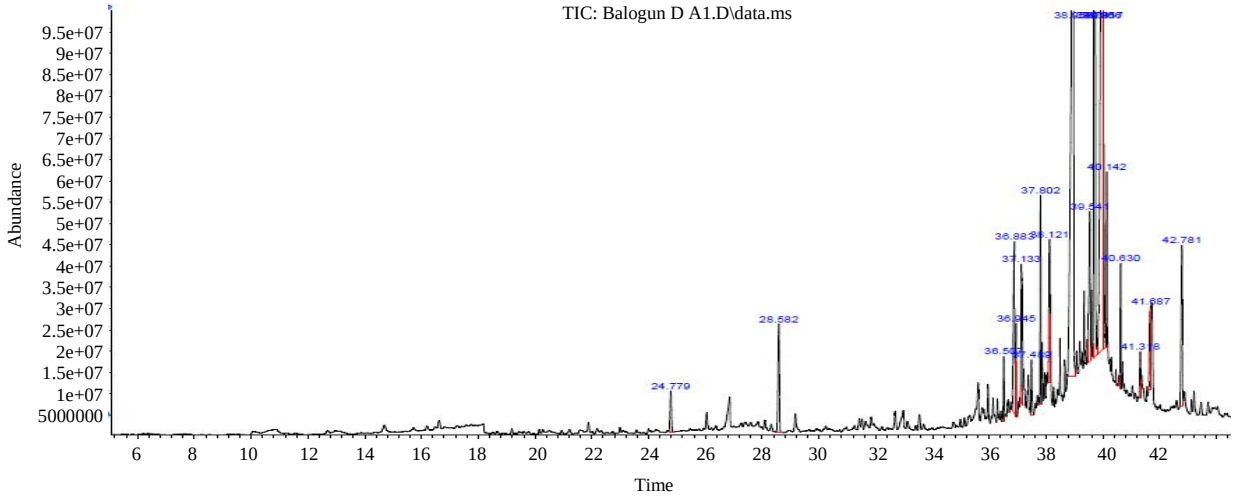

Fig. 1: GC-MS chromatogram of *A. indica* leaf extracts

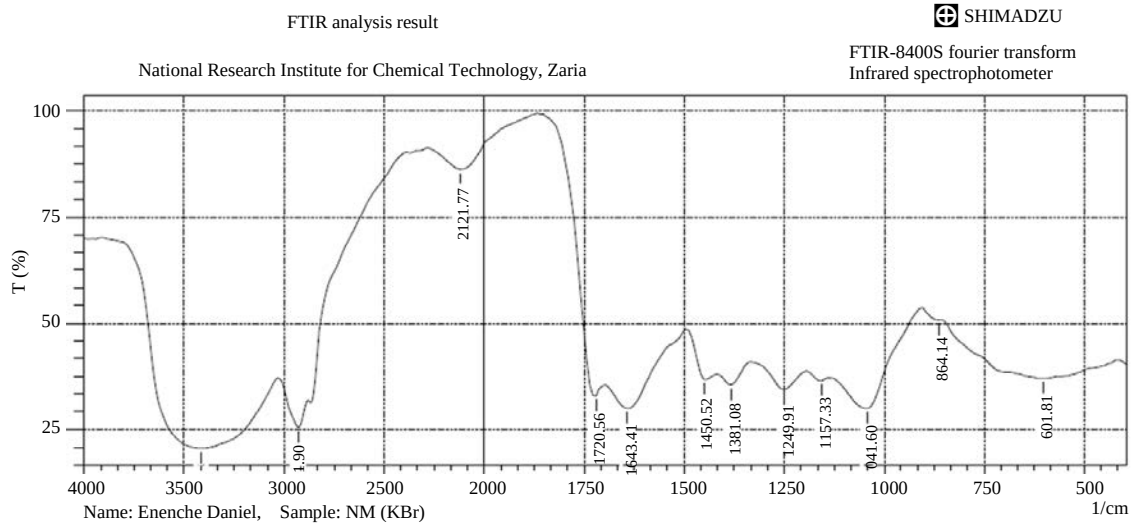

Fig. 2: FT-IR chromatogram of *A. indica* leaf extracts

are comparatively lower than the values of the control group. However, the extract at 800 mg L^{-1} gave a consistently lower values than others.

Anisidine value of oil samples on storage: Results for anisidine values of treated oils is reported in Table 6. Anisidine values in treated samples were significantly low and within recommended limits. They were higher than those recorded for samples treated with the synthetic antioxidant but lower than those for the control sample.

Acid values of oil samples on storage: Acid values of treated and untreated oil samples on accelerated storage are presented in Table 7.

Iodine values of oil samples on storage: The iodine values of treated oils are reported in Table 8. A steady but gradual decline in the iodine value was observed for all the samples in a concentration dependent manner.

Saponification values of treated oils: Saponification values of oil samples treated or untreated during the storage period is shown in Table 9.

Sensory evaluation of oil samples treated with extracts of *A. indica*: Results of sensory evaluation conducted by a trained panelists is presented in Table 10. The results indicate that oil samples treated with *A. indica* were generally

Table 6: Anisidine values of palm oil treated with *A. indica* leaf extracts

	Months					
Samples	1	2	3	4	5	6
P100	1.324±0.011^{d}	1.791±0.001^{bc}	2.742±0.299^{d}	4.405±0.199^{e}	5.821±0.092^{d}	6.527±0.021^{de}
P200	1.310±0.002^{d}	1.544±0.0078^{bc}	2.523±0.157^{cd}	4.283±0.053^{de}	5.176±0.098^{bc}	6.295±0.070^{cde}
P400	1.234±0.011^{c}	1.355±0.485^{ab}	2.315±0.168^{cd}	4.005±0.069^{cd}	5.064±0.084^{bc}	6.239±0.036^{cde}
P800	1.111±0.001^{b}	1.658±0.0476^{bc}	1.708±0.203^{ab}	3.650±0.275^{b}	4.961±0.228^{b}	5.894±0.084^{bc}
PBHT	1.002±0.002^{a}	1.316±0.006^{ab}	1.543±0.396^{a}	1.905±0.058^{a}	2.285±0.084^{a}	3.493±0.324^{a}
PCONT	1.491±0.002^{e}	1.813±0.001^{ab}	3.732±0.072^{e}	4.205±0.077^{cde}	6.831±0.106^{e}	7.621±0.233^{f}

5: Very desirable, 4: Desirable, 3: Acceptable, 2: Slightly undesirable, 1: Undesirable. Values are Mean±Standard deviation for two independent determinations. Values with different superscript are statistically significant (Duncan Multiple range test) at $p \leq 0.05$

Table 7: Acid values (meq/1000 g) of palm oil treated with *A. indica* leaf extracts

	Months					
Samples	1	2	3	4	5	6
P100	4.312±0.000^{d}	6.032±0.001^{c}	11.240±0.006^{d}	16.882±0.002^{f}	33.215±0.021^{f}	45.791±0.000^{f}
P200	4.232±0.002^{cd}	6.174±0.000^{cd}	11.198±0.002^{c}	16.205±0.007^{d}	33.177±0.001^{e}	41.646±0.000^{e}
P400	4.206±0.002^{c}	5.711±0.000^{b}	10.961±0.004^{b}	15.706±0.005^{c}	32.939±0.001^{c}	41.306±0.001^{c}
P800	4.024±0.002^{b}	5.540±0.003b	10.634±0.008^{a}	14.835±0.007^{a}	32.724±0.000^{b}	40.581±0.001^{b}
PBHT	3.832±0.001^{a}	4.190±0.001^{a}	10.607±0.006^{a}	15.306±0.021^{b}	31.675±0.000^{a}	37.322±0.001a
PCONT	4.554±0.001^{e}	6.310±0.411^{de}	11.246±0.005^{d}	16.502±0.011^{e}	33.035±0.000^{d}	41.492±0.001^{d}

5: Very desirable, 4: Desirable, 3: Acceptable, 2: Slightly undesirable, 1: Undesirable. Values are Mean±Standard deviation for two independent determinations. Values with different superscripts are statistically significant (Duncan Multiple range test) at $p \leq 0.05$

Table 8: Iodine values (I_2/100 g) of palm oils treated with *A. indica* leaf extracts

	Months					
Samples	1	2	3	4	5	6
P100	54.299±0.000^{ab}	53.142±0.004^{a}	52.430±0.268^{bc}	50.118±0.016^{ab}	47.660±0.039^{c}	43.585±0.053^{b}
P200	54.331±0.031^{b}	53.304±0.010^{b}	52.605±0.346^{bc}	50.424±0.039^{c}	47.681±0.002^{c}	43.878±0.040^{bc}
P400	54.725±0.006^{c}	53.455±0.000^{c}	52.805±0.063^{cd}	50.837±0.024^{d}	47.613±0.004^{bc}	44.385±0.205^{de}
P800	54.952±0.004^{d}	53.748±0.007^{e}	53.065±0.049^{d}	50.914±0.021^{d}	48.650±0.009^{d}	44.640±0.098^{e}
PBHT	55.216±0.021^{e}	53.914±0.000^{f}	53.880±0.084^{e}	53.099±0.000^{e}	50.394±0.044^{e}	48.480±0.225^{f}
PCONT	54.215±0.005^{a}	53.573±0.001^{d}	51.765±0.134^{a}	50.104±0.099^{a}	47.175±0.079^{a}	42.287±0.104^{a}

5: Very desirable, 4: Desirable, 3: Acceptable, 2: Slightly undesirable, 1: Undesirable. Values are Mean±Standard deviation for two independent determinations. Values with different superscripts are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$

Table 9: Saponification values (mg KOH g^{-1}) for palm oils treated with *A. indica* leaf extracts

	Months					
Samples	1	2	3	4	5	6
P100	198.242±0.213^{b}	200.580±0.466^{a}	203.479±0.481^{b}	215.530±0.410^{cd}	216.085±0.176^{d}	226.665±0.388^{c}
P200	199.125±0.235^{c}	204.250±0.339^{c}	204.515±0.446^{c}	218.745±0.233^{e}	207.325±0.275^{b}	225.390±0.254^{b}
P400	198.054±0.031^{a}	201.600±0.410^{b}	204.505±0.261^{c}	213.345±0.346^{b}	213.360±0.579^{c}	225.555±0.134^{b}
P800	199.545±0.254^{d}	206.360±0.212^{d}	201.412±0.576^{a}	221.515±0.445^{f}	219.085±0.021^{e}	230.545±0.417^{d}
PBHT	200.214±0.036^{e}	205.590±0.381^{d}	211.635±0.134^{d}	211.785±0.318^{a}	213.055±0.063^{c}	224.455±0.318^{a}
PCONT	202.240±0.235^{f}	218.260±0.226^{e}	208.594±0.347^{e}	215.185±0.841^{c}	199.385±0.049^{a}	228.260±0.226^{e}

5: Very desirable, 4: Desirable, 3: Acceptable, 2: Slightly undesirable, 1: Undesirable. Values are Mean±Standard deviation for two independent determinations. Values with different superscript are statistically significant (Duncan multiple range test) at $p \leq 0.05$

Table 10: Sensory attributes of palm oil samples treated with *A. indica* leaf extracts, BHT and control

Samples	Colour	Odour	Appearance	Flavour	Texture	General acceptability
P100	3.8750±1.08781^{cd}	3.3750±0.80623^{a}	3.9375±0.92871^{bc}	3.3125±0.79320^{a}	3.8750±0.95743^{a}	3.7500±0.85635^{b}
P200	3.6250±1.08781^{cd}	3.3125±0.70415^{a}	3.7500±0.85635^{ab}	3.3750±0.71880^{a}	3.8750±0.95743^{a}	3.6875±0.70415^{ab}
P400	3.4375±1.09354^{cd}	3.2500±0.77460^{a}	3.6875±0.87321^{a}	3.1250±0.71880^{a}	3.8125±0.91059^{a}	3.5000±0.73030^{ab}
P800	3.3125±1.19548^{abc}	3.4375±0.72744^{a}	3.6250±0.95743^{a}	3.1250±0.71880^{a}	3.6250±1.02470^{a}	3.3750±0.95743^{ab}
PBHT	4.0000±1.03280^{d}	3.1875±0.83417^{a}	3.9375±0.92871^{bc}	3.3125±0.70415^{a}	3.6875±0.70415^{a}	3.3750±1.02470^{ab}
PCONT	3.8750±1.02470^{cd}	3.3750±0.80623^{a}	4.1875±0.98107^{c}	3.5000±0.89443^{a}	3.8125±0.83417^{a}	3.6250±1.02470^{ab}
ANOVA	0.001	0.976	0.001	0.972	0.531	0.445

Values with different superscripts across the column differ significantly (Duncan Multiple range test) at $p \leq 0.05$

acceptable for the selected parameters with higher scores recorded for samples with low concentration of extract.

DISCUSSION

The phenolic composition as determined in this study is in conformity with other findings, which correlated with extension of oil shelf life[21]. Similarly, the ability of phenolic compounds to enhance oxidative stability of oils has been reported by Baldioli *et al.*[22]. Although the interest in phenolic compounds is related primarily to their antioxidant activities, they also show an important biological activity *in vivo* and may be beneficial in combating diseases arising from exposure to excessive oxygen radical formation exceeding the antioxidant defense capacity of the living system. The values recorded for antioxidant assays carried out on the crude extracts of *A. indica*, which included DPPH radical scavenging assay, β-carotene linoleic antioxidant assay, oxygen reducing antioxidant capacity (ORAC) assay and Ferric reducing antioxidant power (FRAP) assay were comparable to those obtained by Baldioli *et al.*[22]. Although the values are higher than those of the synthetic antioxidant used, the health implications arising from continued use would make the natural alternative a preferable choice. Additionally, there is also the possibility of improving the radical scavenging property of the extracts if it undergoes refinement before usage, which is the focus moving forward in this research area.

The GC-MS data compared favorably with those reported by Prashanth and Krishnaiah[23], revealing the presence of similar bioactive compounds. From the results, it was observed that presence of 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (synonym: Phytol), 9, 12, 15- Octadecatrienoic acid (synonym: Linolenic acid; α-Linolenic acid), 8, 11, 14-Eicosatrienoic acid (Synonym: Homo-γ-linolenic acid), N-Hexadecanoic acid (synonym: Palmitic acid) and Tridecanoic acid (synonym: Tridecylic acid) were the major components in the extract. Phytol is reported to have antioxidant, antiallergic[24], antinociceptive and anti-inflammatory activities[25]. Additionally, recent studies revealed that phytol is an excellent immunostimulant superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity[26]. Palmitic acid is reported to possess antibacterial and cholesterolaemic effects[27]. In addition, palmitic acid and linoleic acid extracted from plants have been shown to possess significant cytotoxic effects in some cancer cell lines including[28] MOLT-4 in addition to the later's inhibitory effect on oxidation. Thus, consumption of palm oil preserved with extracts of *A. indica* containing such active compounds may offer medicinal and antimicrobial benefit as well as nutritional importance. Thus, a combination of this and phenolic compounds found in *A. indica* should give confidence to the use of this plant as suitable antioxidant. The FT-IR data obtained in this study was similar to those reported by Ibrahim and Sani[29]. This may have been responsible for the beneficial chemical properties exhibited by the crude extract of the plant leaf in treated palm oil samples. However, further studies would be required to elucidate the structural identification of these constituents and the mechanisms of their actions in bequeathing antioxidant properties to the plant leaf and possible contribution to phytoremediation as a panacea for environmental degradation occasioned by oil spillage.

A measure of oxidative stability of the oil samples was determined through the measurement of peroxide values, anisidine value as well as iodine values. The range of peroxide values for treated samples (P100-P800) was comparable to the reports of Frank *et al.*[30] but lower than those reported by Amata and Ozuor[31], which may be due to the application of different plant extracts and techniques of extraction. The peroxide values recorded for samples treated with synthetic antioxidant (PBHT) were lower than the samples treated with the plant extract but higher than the control samples, indicating the potential of the plant extract to improve the oil quality upon storage. Generally, change in peroxide values was in a concentration-dependent manner and the values for all samples correspond to normal CODEX 210 values[32], which recommended a maximum PV of 15 meq O_2 kg^{-1}. Anisidine values for samples treated with the extracts were lower than those for the control sample indicating that the extract had significant effects in reducing anisidine value of palm oils upon storage. Again, it is observed that the anisidine value increased slightly but steadily as storage time extended and indicated that anisidine value reduced as the concentration of extracts increased. According to White[33], an acceptable AV for well-refined oils is between 1 and 10 mmol kg^{-1}, whereas oils with high levels of polyunsaturated fatty acids might have higher levels even when fresh. Acid values showed a gradual decline in values as concentration of *A. indica* increased similar to reports by Van der Merwe *et al.*[34] who observed a gradual and then sharp increase in free fatty acid in oil stored over 52 weeks. The trend shown in iodine value establishes a relationship between extract efficacy and oil saturation. In general, the greater the degree of unsaturation, the more readily the oil or fat becomes rancid. This trend was similarly observed for saponification value, which was found to be higher than previous studies[35,36]. Saponification values fell within range set by both national and international regulatory agencies. Since hydrolysis depletes esters, this

accounts for why there is a decrease in the saponification value of oil samples in agreement with the findings of Akubor and Ogu[37].

Results of sensory evaluation showed that sample treatment with *A. indica* leaf extracts were generally acceptable. However, in terms of appearance and colour, control sample was most desirable. The presence of green pigments in treated oil samples may have impacted on the appearance and colouration of the oil, which may affect their high rating on the scale.

CONCLUSION

This study showed that ethanolic extracts of *A. indica* has constituents that have been severally reported to have antioxidant properties including phytol and palmitic acid and other important phytochemicals. This assertion was confirmed by the antioxidant assays, which showed significant radical scavenging ability for the extracts as well as oxygen reducing antioxidant power with lower IC_{50} doses compared to standard antioxidants. In addition, the results also clearly indicated that the oxidative stability of palm oil significantly ($p<0.05$) improved by the application of *A. indica* ethanolic extracts in the order P800 P400 P200 P100, making the plant leaf a promising alternative for synthetic antioxidants for oil preservation. The encouraging sensory acceptability also affirms that palm oil treated with *A. indica* are marketable with good economic potential.

SIGNIFICANCE STATEMENT

As concerns for use of synthetic chemicals such as preservatives and other additives continue to grow, the need to embrace healthier alternatives becomes more imperative. This study discovered that *A. indica* leaf extracts contain valuable health-promoting phytochemicals and possess antioxidant ability in preventing oil spoilage, thus documenting important contributions to evidence in support of natural preservatives. This discovery is key to streamlining key suspects that would enable other researchers explore further details on the candidate plant.

REFERENCES

1. Ajani, E.N., E.A. Onwubuya and H.U. Nwalieji, 2012. Assessment of oil palm production and processing among rural women in Enugu North agricultural zone of Enugu State, Nigeria. Int. J. Agric. Sci., 2: 322-329.
2. Tabee, E., 2008. Lipid and phytosterol oxidation in vegetable oils and fried potato products. Ph.D. Thesis, Department of Food Science, Faculty of Natural Resources and Agricultural Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.
3. Azeez, O.T., K.O. Ejeta, E.O. Frank and N.E. Gerald, 2013. Effects of antioxidants on the oxidative stability of vegetable oil at elevated temperature. Int. J. Applied Sci. Technol., 3: 107-115.
4. Coelho, D.F., L.O. Pereira-Lancha, D.S. Chaves, D. Diwan and R. Ferraz *et al*., 2011. Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity and the role of exercise on these parameters. Braz. J. Med. Biol. Res., 44: 966-972.
5. Aluyor, E.O. and M. Ori-Jesu, 2008. The use of antioxidants in vegetable oils-A review. Afr. J. Biotechnol., 7: 4836-4842.
6. Wang, J., D. Wu and W.C. Shen, 2002. Structure-activity relationship of reversibly lipidized peptides: Studies of fatty acid-desmopressin conjugates. Pharmaceut. Res., 19: 609-614.
7. Fan, L. and N.A.M. Eskin, 2015. The Use of Antioxidants in the Preservation of Edible Oils. In: Handbook of Antioxidants for Food Preservation, Shahidi, F. (Ed.). Chapter 15, Woodhead Publishing, USA., ISBN-13: 978-1-78242-089-7, pp: 373-388.
8. Iqbal, S. and M.I. Bhanger, 2007. Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chem., 100: 246-254.
9. Farag, R.S., E.A. Mahmoud and A.M. Basuny, 2007. Use crude olive leaf juice as a natural antioxidant for the stability of sunflower oil during heating. Int. J. Food Technol., 42: 107-115.
10. Hameed, A., S.A. Hussain, J. Yang, M.U. Ijaz, Q. Liu, H.A.R. Suleria and Y. Song, 2017. Antioxidants potential of the filamentous fungi (*Mucor circinelloides*). Nutrients, Vol. 9, No. 10. 10.3390/nu9101101.
11. Hossain, M.A., W.A.S. Al-Toubi, A.M. Weli, Q.A. Al-Riyami and J.N. Al-Sabahi, 2013. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. J. Taibah Univ. Sci., 7: 181-188.
12. Evbuomwan, B.O., I. Felix-Achor and C.C. Opute, 2015. Extraction and characterization of oil from neem seeds, leaves and barks. Eur. Int. J. Sci. Technol., 4: 1-7.
13. Anwar, F., A. Siddiq, S. Iqbal and M.R. Asi, 2007. Stabilization of sunflower oil with *Moringa oleifera* leaves under ambient storage. J. Food Lipids, 14: 35-49.
14. Dewanto, V., X. Wu, K.K. Adom and R.H. Liu, 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem., 50: 3010-3014.
15. Wahab, O.M. and Q.C. Elabor, 2016. Variation in the phytochemical constituents of seeds, mature and immature leaves of *Moringa oleifera* Lam. growing in five local government areas of Oyo State, Nigeria. J. Nat. Sci. Res., 6: 54-60.

16. Girgih, A.T., D. Chao, L. Lin, R. He, S. Jung and R.E. Aluko, 2015. Enzymatic protein hydrolysates from high pressure-pretreated isolated pea proteins have better antioxidant properties than similar hydrolysates produced from heat pretreatment. Food Chem., 188: 510-516.
17. Farag, R.S., A.Z.M.A. Badei, F.M. Hewedi and G.S.A. El-Baroty, 1989. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. J. Am. Oil Chem. Soc., 66: 792-799.
18. Jacobs, M.B., 1999. The Chemical Analysis of Foods and Food Products. 3rd Edn., Litton Educational Publishing Inc., USA., ISBN-13: 978-8123906430.
19. AOAC., 2000. Official Methods of Analysis. 17th Edn., Association of Official Analytical Chemist, Washington, DC., USA.
20. Nadeem, M., M. Abdullah, I. Hussain, S. Inayat, A. Javid and Y. Zahoor, 2013. Antioxidant potential of *Moringa oleifera* leaf extract for the stabilisation of butter at refrigeration temperature. Czech. J. Food Sci., 31: 332-339.
21. Gallego, M., M. Skowyra, M. Gordon, N. Azman and M. Almajano, 2017. Effect of leaves of *Caesalpinia decapetala* on oxidative stability of oil-in-water emulsions. Antioxidants, Vol. 6, No. 1. 10.3390/antiox6010019.
22. Baldioli, M., M. Servili, G. Perretti and G. Montedoro, 1996. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. J. Am. Oil Chem. Soc., 73: 1589-1593.
23. Prashanth, G.K. and G.M. Krishnaiah, 2014. Chemical composition of the leaves of *Azadirachta indica* Linn (Neem). Int. J. Adv. Eng. Technol. Manage. Applied Sci., 1: 21-31.
24. Santos, C., M. Salvadori, V. Mota, L. Costa and A. de Almeida *et al*., 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci. J., Vol. 2013. 10.1155/2013/949452.
25. Ryu, K.R., J.Y. Choi, S. Chung and D.H. Kim, 2011. Anti-scratching behavioral effect of the essential oil and phytol isolated from *Artemisia princeps* Pamp. in mice. Planta Medica, 77: 22-26.
26. Lim, S.Y., M. Meyer, R.A. Kjonaas and S.K. Ghosh, 2006. Phytol-based novel adjuvants in vaccine formulation: 1. Assessment of safety and efficacy during stimulation of humoral and cell-mediated immune responses. J. Immune Based Ther. Vaccines, Vol. 4. 10.1186/1476-8518-4-6.
27. French, M.A., K. Sundram and M.T. Clandinin, 2002. Cholesterolaemic effect of palmitic acid in relation to other dietary fatty acids. Asia Pac. J. Clin. Nutr., 11: S401-S407.
28. Harada, H., U. Yamashita, H. Kurihara, F. Fukushi, J. Kawabata and Y. Kamei, 2002. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res., 22: 2587-2590.
29. Ibrahim, M.B. and S. Sani, 2015. Neem (*Azadirachta indica*) leaves for removal of organic pollutants. J. Geosci. Environ. Protect., 3: 1-9.
30. Frank, N.E.G., M.M.E. Albert, D.E.E. Laverdure and K. Paul, 2011. Assessment of the quality of crude palm oil from smallholders in Cameroon. J. Stored Prod. Postharvest Res., 2: 52-58.
31. Amata, I.A. and E. Ozuor, 2013. The effect of different processing methods on the quality of Crude Palm Oil (CPO) in Delta North agricultural zone of Delta State Nigeria. Int. J. Agric. Biosci., 2: 116-119.
32. Codex Alimentarius Commission, 2005. Codex standard for named vegetable oils (CODEX-STAN 210-1999). Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome, Italy.
33. White, P.J., 1995. Conjugated Diene, Anisidine Value and Carbonyl Value Analysis. In: Methods to Access Quality and Stability of Oils and Fat-Containing Foods, Warner, K. and N.A.M. Eskin (Eds.). Chapter 9, AOCS Press, Champaign, IL., USA., ISBN-13: 9780935315585, pp: 159-178.
34. Van der Merwe, G.H., L.M. du Plessis and J.R. Taylor, 2004. Changes in chemical quality indices during long-term storage of palm-olein oil under heated storage and transport-type conditions. J. Sci. Food Agric., 84: 52-58.
35. Akinola, F.F., O.O. Oguntibeju, A.W. Adisa and O.S. Owojuyigbe, 2010. Physico-chemical properties of palm oil from different palm oil local factories in Nigeria. J. Food Agric. Environ., 8: 264-269.
36. Onwuka, G.I. and B.I. Akaerue 2006. Evaluation of the quality of palm oil produced by different methods of processing. Res. Biol. Sci., 1: 16-19.
37. Akubor, P. and G.I. Ogu, 2012. Quality attributes of fresh palm oils produced from selected communities around Anyigba, Kogi state, Nigeria. Niger. J. Nutr. Sci., 33: 12-17.