

## ANTIRANCIDITY EFFECTS OF CRUDE EXTRACTS OF *CITRUS SPP* (*Citrus sineensis*, *Citrus limon*, *Citrus aurantifolia*) LEAVES ON THE SHELF LIFE OF PALM OIL

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### ABSTRACT

Rancidification is a process that affects palm oil quality and reduces its nutritional value. The study was aimed at establishing antimicrobial and antirancidity effects of crude extracts of *Citrus sineensis*, *Citrus limon*, *Citrus aurantifolia* leaves on the shelf life of palm oil. Fifteen (15) samples were purchased from local dealers from good shed Umuahia. Five (5) each for treatment 1, 2 and 3 respectively. Treatment one (1) represented the fresh oil sample, treatment two (2) represented five(5) months stored palm oil while treatment three (3) represented a one (1) year stored palm oil sample. The crude extracts of the citrus species: *Citrus aurantifolia*, *Citrus limon*, and *Citrus senensis* were obtained with methanol. The extracts were used in the antirancidity test. Anti-rancidity assay was determined using 1, 1-diphenyl -2-picryl hydroxyl (DPPH) spectrophotometric method. 1ml of DPPH solution in methanol was added to 2ml of sample solution. The mixture was allowed to stand for 30 minutes at room temperature before the absorbance was measured and recorded at 517nm using a spectrophotometer. vitamin (ascorbic acid) dissolved in methanol was used as a positive (+ve) control and absolute methanol used as blank. Gross total values were represented as triplicate MEAN $\pm$ S.E: 58.00  $\pm$  15.99, 69.54  $\pm$  12.93, 77.85  $\pm$  11.65, 87.1  $\pm$  8.06, 94.46  $\pm$  6.41 against 200 $\mu$ g/ml, 400 $\mu$ g/ml, 800 $\mu$ g/ml, 200 $\mu$ g/ml, 1600 $\mu$ g/ml respective titral volumes of oil. From the results the antirancidity results showed no significant difference (P>0.05) at 1600 $\mu$ g/ml of all the extract when compared to ascorbic acid (vitamin C) control treatment. In conclusion the extracts of the citrus species showed antirancidity effect on palm oil stored at different durations.

**Keywords:** Rancidification, *Citrus sineensis*, *Citrus limon*, *Citrus aurantifolia*, Antirancidity

### INTRODUCTION

Palm oil is an essential food rich in fatty acid. Palm oil is obtained from the mesocarp of oil palm (*Elaeis guineensis*) fruit (Sundram *et al.*, 2003). Its consumption in 2016 was estimated at 35 % (Soystat, 2016). Palm oil is an important part of human and animal diet and contributes positively in food processing industry. Its use in the commercial food industry is widespread, owing to its stability and affordability in terms of cost. Sterol, focophenol, pigments and metal ions are also represented (Sundran *et al.*, 2003). The majority of fatty acid is palmitic acid accompanied by oleic acid (Mancini *et al.*, 2015).

One of the important quality parameters in oil refining industry is low level of free fatty acid (FFA) content and oxidative products. However, most of the times palm oil for domestic use is processed through low grade mills which enhances the FFA content and ultimately leads to oxidation during storage. Palm oil quality is usually evaluated on the basis of its rancidity

(indicator of FFA content) and impurities (Tagoe *et al.*, 2012; De Almedie *et al.*, 2013). Infact, the FFA content could serve as an indicator for a good harvest and a good method of extraction. Their presence in palm oil indicates the level of oil degradation during the extraction process. If the FFA content is high, this indicates that the fruits were damaged between harvest and extraction or harvested fruits were rotten (De Almedie, *et al.*, 2013). High values of rancidity due to lipase activity are a reflection of oil quality impairment. Without refining, such oil may be unsuitable for human consumption.

Rancidity is the chemical or microbial decomposition of fats, oils and other lipids. When these processes occur in food, undesirable odours and flavours can result. Rancidification can also affect the nutritional value of food. In the presence of oxygen and /or ultraviolet (UV) radiation, most lipids will break down and degrade, forming several other compounds. Oxygen is eight times more soluble in fats than it is in water; it is this exposure that is the main cause of the autoxidation process, increasing the saturation of the oil (Gordon *et al.*, 1995).

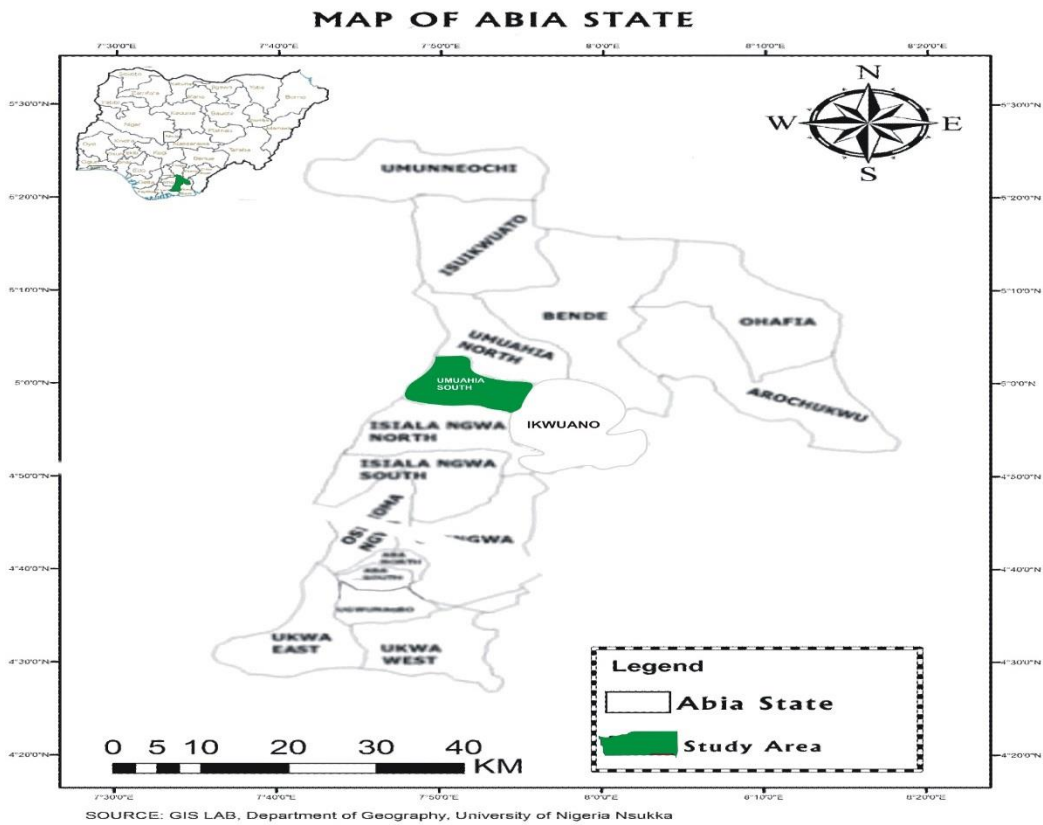
Rancid oil forms harmful free radicals in the body, which are known to cause cellular damage and have been associated with diabetes, Alzheimer's disease and other conditions, they are also known to cause digestive distress and deplete the body's vitamins B and E according to Hartley and Moro (2013). In humans, rancid oil has resulted in several cases of DNA damage, accelerate aging, promote tissue degeneration and foster cancer development.

Edible oil sold in the market comes from various sources and the length of time they stay before being sold varies. Also the condition of storage at home before usage, affects their palatability and edibility. Due to the many application of this vegetable oil in both home and industrial processes, the need to study factors that enhance their quality becomes imperative. The present study was aimed at establishing the anti-rancidity properties of crude extracts of citrus specie leaves to enhance shelf life of palm oil.

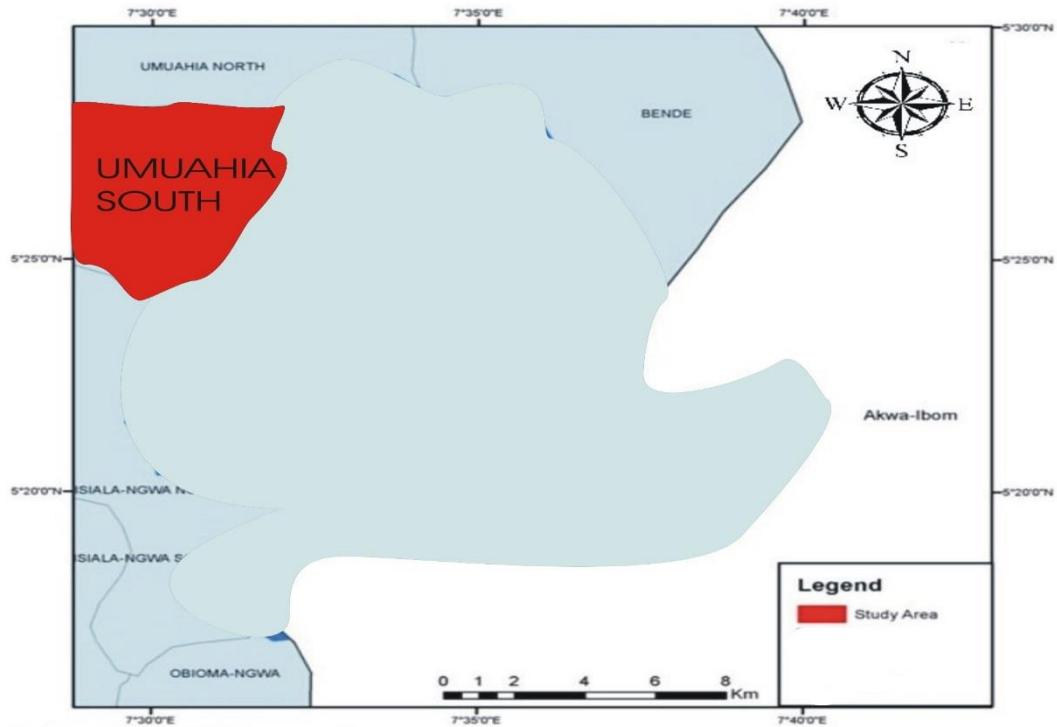
## **MATERIALS AND METHOD**

### **STUDY AREA**

This research was carried out within the geographical location of Umuahia South L.G.A. of Abia State, its headquarters is at Apuimiri in Ubakala. The local government is boarded in the south by Isiala Ngwa North L.G.A, and in the north by Umuahia North L.G.A. Umuahia South has a longitude of 5°.31 and latitude 4°27 and elevation of 55m above the sea level. The climate is tropical and humid all year round. Annual rainfall ranges from 2000mm to 2500mm while the temperature ranges from 22°C to 31°C. The major occupation of the people is farming. The soil in the study area are fertile and the major food crops grown in the area are cassava, yam, maize, cocoyam, local beans, melon and various type of vegetables. Crops grown under plantation are raffia palm, palm tree, plantain and banana. The farm animals reared include sheep, goat, pigs, rabbit, chicken and snail



Map of Abia State showing Ikwuano L.G.A (Study Area)



Map Umuahia south showing the study area



### RESEARCH DESIGN

The research design that was utilized for this study is the experimental design, in an experiment, we deliberately change one or more process variations (or factor) in order to observe the effect the changes have on one or more responses variables. The statically design of experiment (DOE) is an efficient procedure for planning experiment so that the data obtained can be analyzed to yield valid and objective conclusion.

### SAMPLE COLLECTION

Fresh leaves of three different species of Rutaceae family: *Citrus aurantifolio*, *Citrus sinensis* and *Citrus limon* free from any insect infestation, infection and damage were collected from different locations in Umuahia South Local Government Area, Abia State.

### EXTRACTION OF CITRUS LEAVES CRUDE EXTRACT

The extraction of the crude extract from the citrus species leaves was done using methanol as the appropriate solvent. 150g of the leaf powder was weighed and introduced into a 2.5L glass jar of methanol and was covered with the lid until it became air tight. It was allowed to stand for 48hours then the mixture was filtered using Whatmann No. 1 filter paper. The residue was discarded while the filtrate was taken to the water bath in a beaker and concentrated at the temperature below 60<sup>0</sup>C until the entire methanol and moisture were completely expelled, then the crude extract was collected at the base of the beaker for further analysis.

### PROCEDURE FOR FREE FATTY ACID (FFA)

About 5g of the oil sample was weighed into a 250ml conical flask, 25ml of di-ethylether with 25ml of alcohol and 1ml of phenolphthalein solution (1%) was added. It was carefully neutralized by titrating with 0.1m NaOH with constant shaking until pink colour which persists for 15 seconds was obtained.

$$\text{Acid value} = \frac{\text{titre (ml)} \times 5.61}{\text{weight in gram of sample used}} \dots\dots\dots 1$$

The FFA value is usually calculated as oleic acid ( 1ml 0.1m sodium hydroxide =0.0282g oleic acid) In which case the acid value =2 X FFA.

$$\text{Therefore FFA} = \frac{\text{Acid value}}{2} \dots\dots\dots 2$$

### PROCEDURE FOR SAPONIFICATION VALUE

About 2g of the oil sample was weighed into a conical flask, 25ml of alcoholic potassium hydroxide solution was also introduced, it was refluxed under a refluxed condenser and the flask in boiling water for 1 hour with frequent shaking. 1ml of phenolphthalein was added and titrated hot, the excess alkali with 0.5m hydrochloric acid. The blank was also carried out at the same time.

$$\text{Saponification value} = \frac{(b-a) \times 28.05}{\text{Weight (g)}} \dots\dots\dots 3$$

Where b = Data value of the blank



a = Data value of the sample

wt = Weight of the sample in gram

### ANTI-RANCIDITY TEST USING 1, 1-DIPHENYL -2-PICRYL HYDROXYL (DPPH) METHOD

Anti-rancidity assay was determined using 1, 1-diphenyl -2-picryl hydroxyl (DPPH) spectrophotometric method 1ml of DPPH solution in methanol was added to 2ml of sample solution. The mixture was allowed to stand for 30 minutes at room temperature before the absorbance was measured and recorded at 517nm using a spectrophotometer. vitamin (ascorbic acid) dissolved in methanol was used as a positive (+ve) control and absolute methanol used as blank. The difference in absorbance between the last samples and control expressed as percentage (%) inhibition was taken as the anti-rancidity activity.

$$\text{Percentage (\%)} I = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times \frac{100}{1} \dots\dots\dots 4$$

### Biochemical test

#### Coagulase test

18 hours old culture was also used for the test, a grease free slide was divided into 2 portions and a drop of water was added to each portion, using an inoculation stick, a portion of each test colony was emulsified in the 2 portions, one portion was labeled test portion while the other was labeled control. On the test portion, a drop of human blood plasma was added, bacteria cells clumping was observed after 5 – 10 minutes indicating coagulase positive test while absence of the clumping indicate coagulase negative –ve (chesbrough,2000)

#### Catalase Test

18 hours old culture was used for the test, Hydrogen Peroxide was placed on the grease free slide and a portion of the test colony was spread over the hydrogen peroxide on the slide using an inoculation stick and observed after 5-10 minutes, colour changes indicate catalase positive test while absence of colour change indicate catalase negative(chesbrough,2000)

### PROCEDURE FOR FREE FATTY ACID (FFA)

About 5g of the oil sample was weighed into a 250ml conical flask, 25ml of di-ethylether with 25ml of alcohol and 1ml of phenolphthalein solution (1%) was added. It was carefully neutralized by titrating with 0.1m NaOH with constant shaking until pink colour which persists for 15 seconds was obtained.

$$\text{Acid value} = \frac{\text{titre (ml)} \times 5.61}{\text{weight in gram of sample used}} \dots\dots\dots 5$$

The FFA value is usually calculated as oleic acid ( 1ml 0.1m sodium hydroxide =0.0282g oleic acid) In which case the acid value =2 X FFA.

$$\text{Therefore FFA} = \frac{\text{Acid value}}{2} \dots\dots\dots 6$$



### PROCEDURE FOR SAPONIFICATION VALUE

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$$\text{Saponification value} = \frac{(b-a) \times 28.05}{\text{Weight (g)}} \dots\dots\dots 7$$

Where b = Data value of the blank

a = Data value of the sample

wt = Weight of the sample in gram

### PROCEDURE FOR SPECIFIC GRAVITY/ RELATIVE DENSITY

This was done gravimetrically by using density bottle, analytical balance and oven. 50ml density bottle was washed and dried in an oven with its lid, it was allowed to cool to room temperature, it was weighed with the lid and filled with the oil sample covered with the lid. The spillover was cleared and dried with tissue paper, reweighed W<sub>2</sub>. The density of the sample was calculated as:

$$\text{Density} = \frac{W_2 - W_1}{50} \dots\dots\dots 8$$

### PROCEDURE FOR DETERMINATION OF IODINE VALUE

The oil sample was poured into a small beaker a small rod was added and weighed out a suitable quality of the sample by difference into a dry glass-stoppered bottle of about 250ml capacity. The approximate weight in gram of the oil was taken. 10ml of carbon tetrachloride was added to the oil or melted fat and dissolve. 20ml of Wiji's solution was added and the stopper was inserted (previously moistened with potassium iodine solution) and allowed to stand in the dark for 30 minutes. 15ml of potassium iodide solution (10% and 100ml of water was added, mixed and titrated with 0.1m thiosulphate solution using starch as indicator just before the end point (Titration = aml) a blank was carried out the same time commencing with 10ml of carbon tetrachloride (Titration = bml). The iodine value was calculated using.

$$\text{Iodine} = \frac{(b-a) \times 1.269}{\text{weight in gram of sample}} \dots\dots\dots 9$$

### **PROCEDURE FOR DETERMINATION OF PEROXIDE VALUE**

1g of oil sample was weighed out into a density boiling tube and while still liquid 1g of potassium iodide powdered and 20ml of solvent mixture (2vol. flaccid acetic acid has 1vol. of chlorofoin) was added. The test was placed in boiling water so that the liquid boils within 30 seconds are allowed to boil vigorously for not more than 30 seconds. The content was quickly poured into a flask containing 20ml of potassium iodide solution (5%). The test tube was weighed twice with 25ml water and titrated with 0.002m sodiumthiosulphate solution using starch. The peroxide value was calculated.

### **PROCEDURE FOR DETERMINATION OF pH**

The oil sample was introduced into a 100ml beaker. It was taken to a pre-calibrated unicon pH meter on 4.0 and 7.0 pH, the electrode was introduced into the beaker containing the sample and allowed to stand until the cursor displays the reading and is taken as the pH value.

### **PROCEDURE FOR MOISTURE CONTENT DETERMINATION (OVEN AIR METHOD)**

The empty moisture dish was weighed (which has previously been washed, dried and cooled).Then feeding stuff was properly mixed which has been milled to pass through 1mm sieve thoroughly and transfer 2-5g into the weighted moisture dish. The weighed moisture dish with sample was placed in an oven to dry at 100<sup>0</sup>c for 6 hours or 16 to 24hours for samples that do not compose on long period of drying. The dish with sample was removed from oven, cooled in a desiccator and reweighed. It was dried for another 1hour, cool and weigh until constant weight has been achieved (when the different between 2 consecutive weighing do not differ by more than 2mg for 5g sample)

Calculation

$$\frac{\text{weight of sample before drying+dish}-\text{weight of sample+dish after drying}}{\text{wieght of sample taken}} \times \frac{100}{1}=\% \text{Moisture} \dots\dots \text{Eqn (11)}$$

$$100-\% \text{moisture} +\% \text{Dry Matter/Total Solids}=\frac{\text{Weight of dried sample}}{\text{weight of sample taken}} \times \frac{100}{1}$$

### **PROCEDURE FOR COLOUR DETERMINATION**

All the test samples were dissolved in n-hexane in a ratio of 1/50 vol/vol to obtain faint colour then read with red wavelength in a photo-electric colorimeter.

Blank= Absorbance 0.0

Transmitter 100%

## RESULTS

**TABLE 1: Antirancidity test result of citrus spp leaves against palm oil sample.**

	200µg/ml	400µg/ml	800µg/ml	1200µg/ml	1600µg/ml
<i>C. aurantifolia</i>	42.71 ± 0.13 <sup>c</sup>	60.21 ± 0.52 <sup>c</sup>	72.55 ± 0.15 <sup>c</sup>	82.06 ± 0.07 <sup>c</sup>	94.74 ± 0.12 <sup>c</sup>
<i>C. limon</i>	43.96 ± 0.76 <sup>c</sup>	57.89 ± 0.18 <sup>d</sup>	68.84 ± 0.03 <sup>d</sup>	79.00 ± 0.00 <sup>d</sup>	84.62 ± 0.03 <sup>d</sup>
<i>C. sinesin</i>	68.86 ± 0.23 <sup>b</sup>	71.64 ± 0.62 <sup>b</sup>	79.62 ± 0.03 <sup>b</sup>	88.71 ± 0.11 <sup>b</sup>	98.47 ± 0.06 <sup>b</sup>
Vitamin C	76.49 ± 2.89 <sup>a</sup>	88.44 ± 0.08 <sup>a</sup>	94.41 ± 0.29 <sup>a</sup>	98.66 ± 0.06 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
<b>Gross mean</b>	<b>58.00 ± 15.99</b>	<b>69.54 ± 12.93</b>	<b>77.85 ± 11.65</b>	<b>87.1 ± 8.06</b>	<b>94.46 ± 6.41</b>

Values are represented as triplicate MEAN±S.E

### ANTIRANCIDITY TEST OF CITRUS LEAF EXTRACTS AGAINST ISOLATES

The table 4 below represents the anti-rancidity test of citrus specie leaf extract agent microbial isolates. From the table below the anti-rancidity that was carried out at different concentration ranging from 200µg/ml - 1600µg/ml. this was done against a control which is vitamin C. Although the control being vitamin C had a higher antirancidity effect on the sample, the citrus species all showed a more effective anti-rancidity on sample at the highest concentration being 1600µg/ml. From the table below, the result showed that the higher the concentration of the extract the more effective its results will be.

**Table 2: Free fatty acid test result of palm oil samples over a given period.**

Values are represented as triplicate MEAN±S.D

Period	X <sub>1</sub>	X <sub>2</sub>	Mean + SD
Fresh	11.42meq	11.42meq	<b>11.42 ± 0.00<sub>c</sub></b>
5 months	43.98meq	44.90meq	<b>44.44 ± 0.65<sub>a</sub></b>
1 year	28.93meq	29.02meq	<b>28.98 ± 14.78</b>
<b>Gross mean</b>			<b>28.27 ± 14.78</b>

**TABLE 3: Physico-chemical analysis of certain parameters**

Parameters	Period of time			
	Fresh	5 months	1 year	Standard value
Specific gravity/	0.93g/ml	0.946g/ml	0.928g/ml	0.898 – 0.907
Relative density				
Iodine Value	1.5989	2.835	3.3145	4.5 – 5.5
Peroxide value	9.961geq	26.773geq	28.306geq	10.00
pH value	5.0	3.9	4.2	Nil
Saponification value	89.047	110.308	113.104	185 – 205
Viscosity	77.43Cs	62.55Cs	71 – 77Cs	
Colour	0.86	0.55	1.06	
Moisture content	0.21	0.29	0.35	0.2%

## DISCUSSION

Present days consumers are more conscious about their health and demand food that is fresh, this leads to search for antioxidants from natural sources. In this connection, citrus species are larvish sources of polyphenolic compounds that possess strong antioxidant and antimicrobial capacity and can be potentially used to substitute the synthetic antioxidants from food products and confer some additional health effects. The quantity of extracted antioxidant compounds of plant sources solely depends on the efficiency of solvents and the methods used for extraction. The data obtained shows that a gradual increase in the antioxidants potentials in the 3 selected species of citrus plant extract were with increase in the aqueous component of organic solvent. It has been observed that polyphenolic compounds are responsible for antioxidant and antibacterial activity of plant materials and are highly effective radical scavengers (Pan *et al.*, 2008). These antioxidant activities are associated with redox properties. The phenol moiety hydroxyl group on aromatic rings enables them to react as reducing agents, singlet oxygen quenchers and hydrogen donors (Chua *et al.*, 2008).

DPPH assay is routinely used for screening antioxidant activity of natural compounds. Basic principle behind this assay is reducing DPPH free radical in antioxidant presence which donates hydrogen. It is a very sensitive method with the ability to detect scavenging compounds even at low concentration and can accommodate many samples within a short period of duration DPPH is a stable violet colored free radical with nitrogen atom present at the centre which

when reduced changes to yellow colour due to the presence of hydrogen or electron density ability of antioxidants present in extracts. *Citrus sinensis* showed the highest DPPH radical scavenging activity in methanol extract.

The results of the present study regarding DPPH assay were in accordance with the finding of Thakur, *et al.* (2012). They probed the antioxidant, antibacterial and anti-inflammatory activities of ethanolic extract of Storage conditions of refined deodorized palm oil (RDPO) have an effect on the quality and safety of the products (Thakur, *et al.*, 2013, Ullah *et al.*, 2003). These results are in agreement with the previous findings of Umerie *et al.* (2004). They explored the efficacy of the use of *Ficus exasperata* leaves to improve the quality and stability of palm oil. They concluded that use of Ficus leaves enhanced the storage stability of palm oil. Wanget *et al.* (2008) determined the effect of synthetic and natural antioxidants on the oxidative stability of palm-based diesel and noticed that both antioxidants exposed beneficial effects in preventing the oxidation of palm diesel. Zhang, *et al.* (2010) explored the effect of natural and synthetic antioxidants on shelf life stability of sunflower oil. They investigated the peroxide values, panisidine value, free fatty acids, thiobarbituric acid-reactive substances and concluded that the natural antioxidants are better in reducing the oxidation damage to the oil as compared to synthetic one. The results of current research are in accordance with the findings of Umeri *et al.* (2004). They explored the potential of natural antioxidants from potato peels in improving the oxidative stability of soybean oil and inferred that potato peel extract exhibited strong antioxidant capacity which is comparable to synthetic antioxidants BHA and BHT. Aleman *et al.* (2010) concluded that garlic extract is a potent antioxidant and can be employed to reduce lipid oxidation in food products.

Both natural and synthetic antioxidants may inhibit or postpone the process of fat oxidation. An antioxidant can play important role in the prevention of oxidation by acting as reducing agent, free radical scavenger, chelator and as singlet oxygen scavenger (Ebrahimabadi, *et al.*, 2010). A number of different antioxidants are being registered as synthetic antioxidants yet few are acceptable as food additive by regulation due to toxicity and other allied harmful effects. Natural antioxidants are readily acceptable to modern consumers as being ideal food additives with good free radical scavenging activity. Moreover, they are safer and healthier as compared to synthetic ones (Meghwal and Goswami, 2012).

Citrus spices are one of the sources of effective antioxidants and enhance the efficacy of other important antioxidant molecules. It contains several potent antioxidants and is most important spice used for curtailing oxidative stress (Jayaprakasha *et al.*, 2006). The antioxidant potential of citrus is attributed to array of flavonoid compounds that it contains.

Recent studies have observed the role of synthetic antioxidants as carcinogens (Gohari *et al.*, 2010). Hence the search of effective, non-toxic, natural compounds with antioxidative ability has been strengthened in recent years (Gupta *et al.*, 2006).

Plants and their products have been utilized extensively throughout history for medical treatment of disease condition. Numerous studies have been reported on the extraction of various natural products to ascertain their antimicrobial potential. (Nita *et al.*, 2002). Medicinal plants are often good sources of antimicrobial compounds. Majority of medicinal plants are utilized for raw drugs extraction and possess variation in their medicinal properties. (Mahomud *et al.*, 2007). *Citrus aurantifolio* at a concentration of 100µg/ml and 500µg/ml was effective against *Enterococcus* spp. for *Staphylococcus aureus*, only *Citrus sinensis* was effective at a concentrating volume of 100µg/ml and 50µg/ml. The antimicrobial activity of *Citrus maviman*

and *C. sinensis* has been reported (Singh et al., 2010). However, leaf extracts could show more antimicrobial activity against microbial strains or with further modification in the extraction protocols.

The prevalence of antibiotic resistance is a continual problem due to the evolution of a potent defense mechanism against antibiotics. Therefore, it is necessary to exploit and develop a novel inhibitory agent against those bacteria (Cabello, 2006). Plants and plant products have been used extensively throughout history to treat medical problems.

The relative density/ specific gravity are used to determine the adulteration of the palm oil sample with impurities such as water, sludge or alcohol. From the result obtained, the mean of the collected palm oil samples values for the respective storage period (90.928 – 0.946g/ml) against 0.904g/ml) are above the standard moisture content is a confirmatory check on the dryness of the oil or fat sample. A palm oil sample has a maximum moisture content of 0.2% m/m. This is the standard value for a quality palm oil in the course of this study. It was realized that the moisture content were slightly higher than the standard. This might be due to adulteration within. This could possibly have occurred during the procession stage of the oil (Abdulkadir and Jimoh, 2013).

On the other hand, since fats and oils are triglycerides the FFA should be relatively low in a highly graded lipid sample. In addition free fatty acid, acid phosphates and amino acids can contribute to acidity FFA as described as the percentage by weight of a specified fatty acid (oleic acid). Where the FFA is expected as oleic acid the acid value for all the intents and purposes is double the FFA figure (Milnidsky and Gabriel, 1982).

The standard FFA value is nil for palm oil in the vain the results obtained in the analysis showed that the sample is having a high FFA value of (11.42 – 28.98meq) deviating from the standard value. This in order words indicates a high level of unesterified long chain fatty acids in all the samples which was increasing over storage time. FFA detects the level of unesterified fatty acid in lipid sample to define its quality. It also estimates the amount of oil lost during refining steps designed to remove fatty acid in the production of refined bleached decolourized palm oil. Hence high fatty acid content means a poorly refined oil breakdown after storage or use: if the fatty acid liberated is volatile then its FFA would be a measure of hydrolytic rancidity. The high Free Fatty Acid (FFA) observed from the various palm oil samples following increased storage duration is an indication that rancidity had set in (Chuku *et al.*, 2002).

The iodine value measures the degree of unsaturation ( $c = c$ ) in relation to the amount of fat or oil. It is defined as the gram of iodine absorbed per 100g sample: Hence the higher the iodine value, the greater the degree of unsaturation. It is used to characterize fat and oil to follow hydrogenation process during refining and so as indicator of lipid oxidation. It is an important parameter which suggests the extent to which the lipid sample can be prone to oxidation and thus becomes rancid. The iodine value obtained from the samples ranges from (1.589 – 3.3145) which is far below the standard value of iodine value (4.5 – 5.5) indicating the oil not rancid.

Peroxide value measures the degree of lipid oxidation in fats and oil but not its stability. Peroxide value is a transient product of oxidation. A low value represents early or advanced oxidation, which can be distinguished with storage time. It is difficult to obtain sufficient quantities from foods low in fat but this method is highly empirical and its modifications may alter the results but despite these setbacks peroxide value is one of the most common test for lipids oxidation (Nielsen, 2002). It checks the presence of unsaturation from the results



obtained the values obtained after 5 months and 1 year storage had a high peroxide value which was far above the standard and peroxide value indicating that the oil samples weren't rancid but the freshly prepared oil had a value of 9.96geq which is slightly below the standard value but at the same time the oil is not rancid because it differs by 0.1geq. From the result obtained in terms of colour, the colour increases as the storage period increases due to some chemical activities occurring overtime. The viscosities were within the range of 62.556 – 77.43CsC. Viscosity measures the degree of thickness, stickiness or semi-fluid consistency of a sample due to internal fraction.

The pH value of the samples tend to be acid with the sample stored for 5months having the highest acid concentration of 3.9, followed by that of 1 year and then the freshly prepared samples.

Saponification value is used to determine the saponification number of oil which is an index of the average molecular weight of the triacylglyceride in the sample. It is a very vital factor in soap production. The smaller the saponification value, the higher or longer the average fatty acid chain length. Although adulteration of oil with unsaponification matter can result in drop in saponification value (Nielsen, 2002). From the result obtained the samples are having saponification value within the range of standard i.e. 185 – 205mg/KOH/g of oil

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