



Microbiological and Physicochemical Characteristic of Degrading Palm (*Elaeis guineensis*) Kernel and Cashew (*Anacardium occidentale*) Nut Oils

Ariyo Dokun Olanrewaju^{1*}, Ayodele Bosede Adetutu¹
and Fasusi Oluwaseun Adeyinka¹

¹Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: For vegetable oil produce in Nigeria to be competitive in the international market and suitable for downstream applications. The Microbiological and Physicochemical Characteristic of oils are amongst the most important properties that must be studied.

Study Design: The study is design to isolate and identify microorganisms involved in degradation of palm kernel and cashew nut oils and to investigate the physicochemical characteristics of the oil samples during storage.

Place and Duration of Study: The study was conducted between April and September, 2015 at the Microbiology Laboratory of the Federal University of Technology, Akure, Ondo State, Nigeria.

Methodology: Palm kernel and cashew nut seeds were purchased from Oja Oba, Akure, Ondo State. Palm kernel and cashew nut oils were extracted using n-hexane by Soxhlet extractor. The oil samples were stored at room temperature (25°C ± 1) for three months. Microorganisms were isolated from the oil and identified every two weeks of storage. The effect of storage on the physicochemical characteristics (saponification value, peroxide value, acid value and specific gravity) of the oil samples was determined every two weeks of storage.

*Corresponding author: E-mail: lanredariyo@yahoo.com;

Results: The microbial profile of the associated organisms consists of *Bacillus licheniformis*, *Staphylococcus aureus*, *Articosporium inflata*, *Aspergillus flavus*, *A. saprophyticus* and *Penicillium notatum*. All the physicochemical parameters determined were increasing with time except the moisture content. Saponification values (SV) obtained for the palm kernel oil samples is 300 mgKOH/g and cashew oil is 400 mgKOH/g while no significant change was observed in the specific gravity of both oils.

Conclusion: The microbial load of the oil sample was low and the physicochemical property is evidence that the oil can be useful in production of soap and cosmetics.

Keywords: *Microorganisms; physicochemical; palm (Elaeis guineensis) kernel oil; cashew (Anacardium occidentale) nut oil.*

1. INTRODUCTION

Fats and oils whether the source is animal, vegetable or marine in origin represent the highest source of energy per unit weight that man can consume. Apart from being a source of reserved energy, fats deposit insulates the body against loss of heat and protects vital organs against mechanical injury. They are important food source for man, and are also extensively used for nutritional, cosmetic, drug dispersant in therapeutics and industrial purposes and are used for supplying essential fatty acids such as linoleic and arachidonic acids [1].

Report on the nutritive, physico-chemical properties and mineral compositions of the oils extracted from many oil- seeds have been documented [2]. Oils may go rancid and develop an unpleasant odour and flavour if incorrectly stored. The main factors that cause rancidity (in addition to moisture, bacteria and enzymes) are light, heat, air and some types of metals [3]. The storage properties: especially the physicochemical and microbiology of some edible oils stored at room temperature have been investigated [4].

Palm kernel oil is a common cooking ingredient; its increasing use in the commercial food industry throughout the world is buoyed by its lower cost, the high oxidative stability (saturation) of the refined product when used for frying, and its lack of cholesterol and trans fatty acids, both viewed as being heart-healthy attributes [5]. Cashew (*Anacardium occidentale*) belongs to the family Anacardiaceae, which includes many economically, or important tropical and subtropical trees and shrubs. Cashew nut Kernels are pressed to extract an edible vegetable oil of excellent quality [6].

Frying and cooking of the oil can reduce the microbial load to minimum level, but the fact that some individuals consume this product raw is concern as this may result in health problems in such individuals when the microbial load is high. It is against this background this research was carried out with the aim of determining the effect of degradation due to storage on the microbiological and physicochemical qualities of Palm (*Elaeis guineensis*) kernel and Cashew (*Anacardium occidentale*) nut oils. The information will also bring together data to support the economic benefits of the oils.

2. METHODS

2.1 Collection of Samples

Fresh Palm kernel and cashew nut was collected from retailers at Oja Oba Akure, Ondo State Nigeria and transported to the laboratory in a clean bag. They were stored in the laboratory in a clean environment at temperature of 28°C.

2.2 Extraction of Oil

Palm kernel oil samples were extracted from the crushed sample by Soxhlet extractor using n-hexane as solvent [6,7]. The extraction was carried out according to the procedure of AOAC [8,9]. After every 3 days, in each dessicator, sample of the cashew kernel from the different storage were blended, 25 g of the ground kernel was packed in a filter paper and introduced into porous thimble. In this case, n-hexane was used as the extracting solvent and this was effected for 6 - 7 h. At the end of the extraction, the mixture was concentrated by distilling off the solvent. The oil was again skimmed to further remove traces of water.

2.3 Biodegradation Experiments on Oil Extracts

Oil samples were kept on the bench top for three months in a bottle at 28°C. Microorganisms were isolated on the first day and every two weeks of the three month of extraction.

2.4 Isolation of Microorganisms

2.4.1 Isolation of bacterial

A stock solution of each of the oil samples was made by dissolving one millilitres (1 ml) of each sample in nine millilitres (9 ml) of sterile distilled water already emulsified with 10% v/v of Tween 80 solution. Five – fold serial dilution was made from each stock solution. One millilitre aliquots of the last two dilutions of each sample were inoculated into Nutrient agar in triplicates using the pour plate method. All the plates were incubated at 37°C in an incubator for 24 hours. Colonies were counted using colony counter after 24 hours and results expressed as colony forming units per millilitre [7]. Colonies formed after incubation was sub-cultured on nutrient agar to produce distinct pure cultures of bacteria and these were further studied for identification. The cultures were preserved by inoculating them on agar slant prepared from double strength nutrient agar in McCartney bottles.

2.4.2 Yeast and moulds

A stock solution of each of the samples was made by dissolving one millilitre (1 ml) of each sample in nine millilitres (9 ml) of sterile distilled water already emulsified with 10% v/v of Tween 80 solution. Five – fold serial dilution was made from each stock solution. Aliquots of the last two dilutions of each sample was inoculated on Sabouroud Dextrose Agar (SDA) and incubated at temperature 25 - 28°C in a canister for 7 days. Colonies were counted after 7days and results expressed as colony forming units per milliliter [8]. Spores formed after incubation were sub-cultured on nutrient agar and Potato Dextrose agar to produce distinct pure cultures of bacteria and fungi respectively and these were further studied for identification. The cultures were preserved by inoculating them on agar slant prepared from double strength nutrient agar and Potato Dextrose agar in McCartney bottles.

2.5 Identification of Microbial Isolates

Bacterial isolates were identified by morphological and biochemical tests using

standard procedures. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described [9]. Relevant biochemical tests like Oxidase test, Catalase test, Methyl Red test, Voges-Proskauer test, Urease test and Sugar Fermentation test were carried out as described [10].

Isolated fungi were characterised by macroscopic (physical appearance on agar plates) and microscopic techniques (under light microscope) including colour of aerial and substrate mycelia comparing them with those of known taxa as contained in standard fungal compendium [11].

2.6 Determination of the Physicochemical Characteristics of the Oils

The physicochemical characteristic of the oil samples were determined every two weeks of the three month of extraction. The first week samples served as the Control [12,13].

2.6.1 Determination of peroxide value (PV) of cashew and palm kernel oils

Peroxide value in the oil was determined using the standard method [12] using glacial acetic acid, chloroform: 0.01 M Sodium thiosulphate and potassium iodide (KI) 10% as the major solvents in 1.0 g of oil sample.

2.6.2 Determination of refractive index of extracted cashew and palm kernel oils

This was done using the Abbe refractometer. This equipment was first standardized with water, to a refractive index of 1.33. Thereafter, the meter was cleaned with a cotton wool and a drop of the oil placed on it and the refractive index determined for each sample [12].

2.6.3 Determination of specific gravity of extracted cashew and palm kernel oils

This was done by using the density bottle. A known specific volume of cashew kernel oil was poured into the density bottle and the weight was measured. Water at the same volume was also poured into the density bottle and was weighed. The specific gravity was then determined using a standard method [13].

2.6.4 Determination of acid value (AV) and free fatty acid (FFA) of cashew and palm kernel oils

This was also determined by the standard methods of the American Oil Chemist's Society [12] using diethyl ether, ethanol, phenolphthalein and sodium hydroxide as the reagents.

3. RESULTS

3.1 Bacterial and Fungal Count

The result of the bacterial and fungal counts of palm kernel and cashew nut oils are shown in Figs. 1 and 2. The bacterial count ranged from 2.0×10^4 /ml to 6.0×10^4 /ml and 2.6×10^4 /ml to 8×10^4 /ml in palm kernel and cashew nut oil respectively. The fungal count ranged from 2.2×10^4 /ml to 5.6×10^4 /ml and 2.5×10^4 /ml to 8.0×10^4 /ml in palm kernel and cashew nut oil respectively. The total plate counts of bacteria were higher in palm kernel oil than in cashew nut oil. The bacterial count was at its peak in the 3rd week of storage and the fungal count was at its peak at the 5th week for the palm kernel and cashew nut oil.

3.2 Occurrences of Microbes Isolated from Palm Kernel and Cashew Oil

Three bacterial isolates *Bacillus licheniformis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and fungal isolates *Articosporium inflata*, *Aspergillus flavus*, *A. saprophyticus*, and *Penicillium notatum* were isolated from both palm

kernel and cashew nut oil samples as shown in Tables 1 and 2. *Staphylococcus aureus*, *Penicillium notatum* and *Aspergillus flavus* had the highest percentage occurrence in both palm kernel and cashew nut oil.

3.3 Isolation and Identification of Microbial Isolates

Fungal (*Articosporium inflata*, *Aspergillus flavus*, *A. saprophyticus*, and *Penicillium notatum*) and three bacterial (*Bacillus licheniformis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) were isolated from the degrading palm kernel and cashew nut oil.

3.4 Physicochemical Properties of Palm Kernel and Cashew Nut Oil

The result for the peroxide value, saponification value, iodine value, refractive index, free fatty acid, acid value, Moisture content and specific gravity of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks are shown in Figs. 3 - 10. The first week served as the control test.

The iodine value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks is shown in Fig. 3. There was a rapid increase in the iodine level of cashew oil from the 1st to 2nd week, after which was a gradual increase. The iodine value of palm kernel oil showed a rapid increase from the 1st week to 10th week of the degradation period. The iodine value was at its peak at the 10th week.

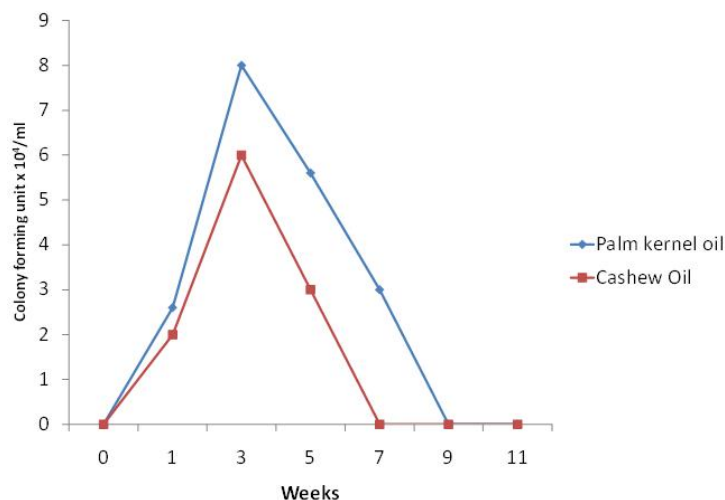


Fig. 1. Total bacteria counts of palm kernel and cashew nut oils

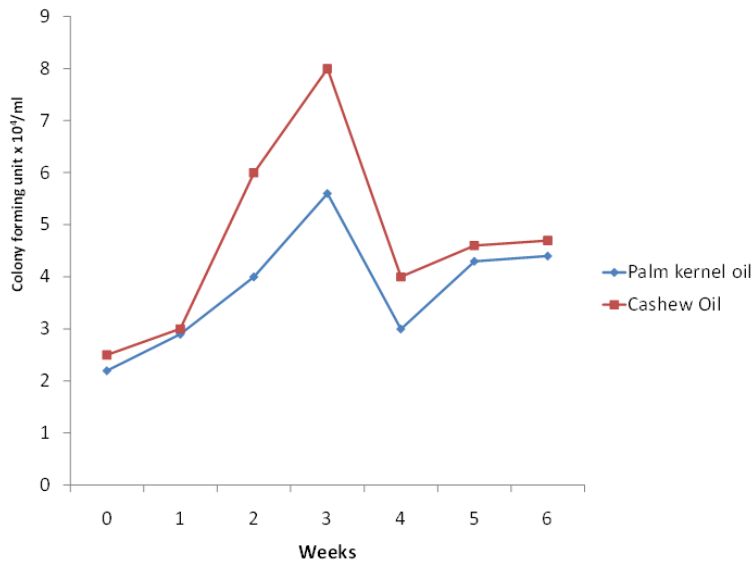


Fig. 2. Total fungal counts of palm kernel and cashew nut oils

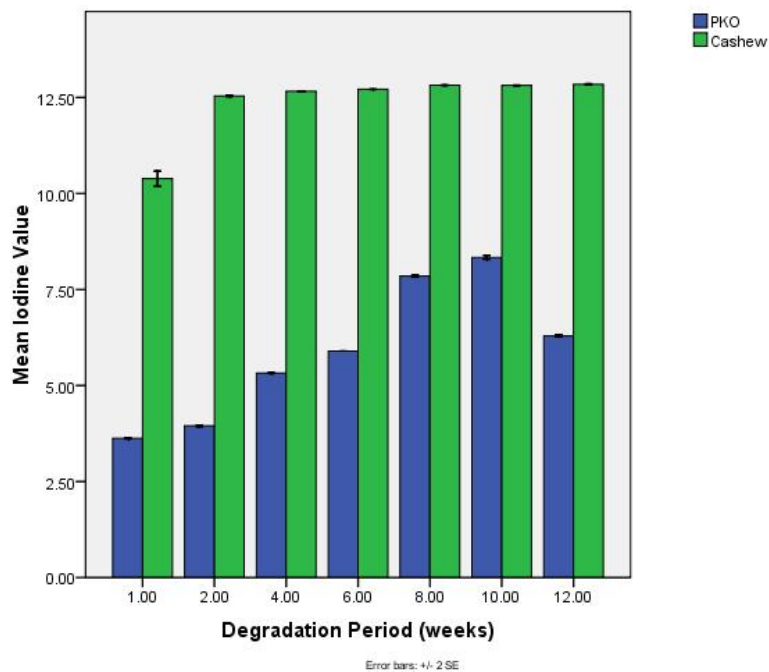


Fig. 3. The iodine value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

The free fatty acid value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks is shown in Fig. 4. The figure revealed that the free fatty acid value of the oil samples increases with

increase in the days of degradation. The free fatty acid of palm kernel oil is revealed to be higher than that of cashew nut oil. There was a rapid increase between the 4th to 6th and 8th to 10th week of the cashew nut oil degradation.

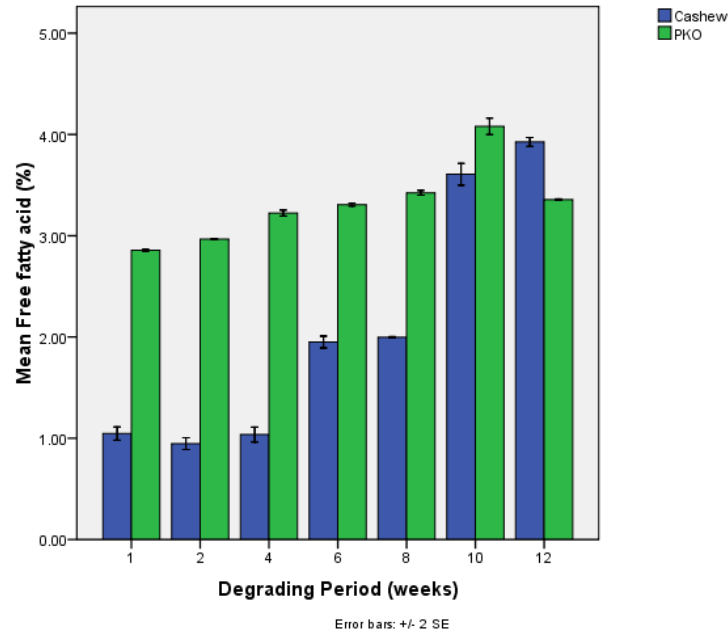


Fig. 4. The Free fatty acid value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

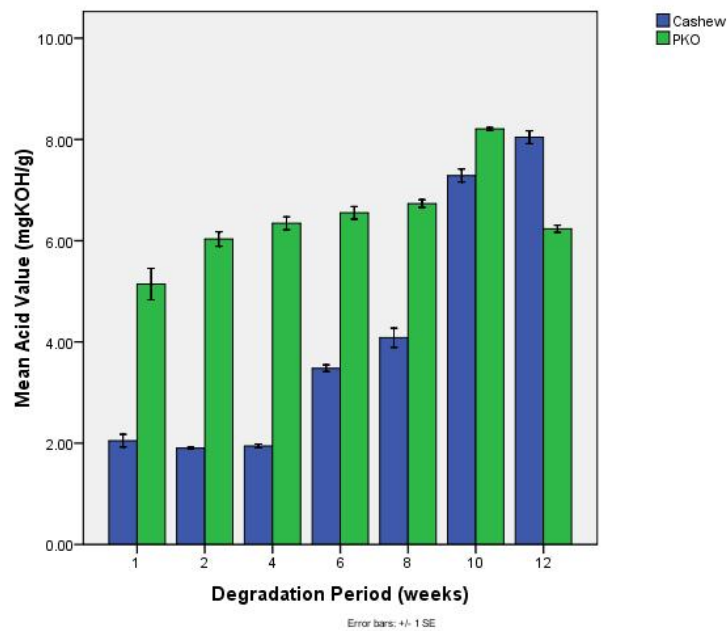


Fig. 5. The acid value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

The acid value of the palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks is shown in Fig. 5. The figure revealed that the acid value of the oil samples increases with increase in days of

degradation. The acid value of the palm kernel oil is revealed to be higher than the acid value of cashew nut oil. There was a rapid increase between the 4th to 6th and 8th to 10th week of cashew nut oil degradation.

Fig. 6 shows the peroxide value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks. The peroxide value increased with increase in days of degradation for both oils. The

Palm kernel oil peroxide value showed a rapid increase while the the cashew oil was gradual. The peroxide value was highest at the 12th week of the degradation for both oils.

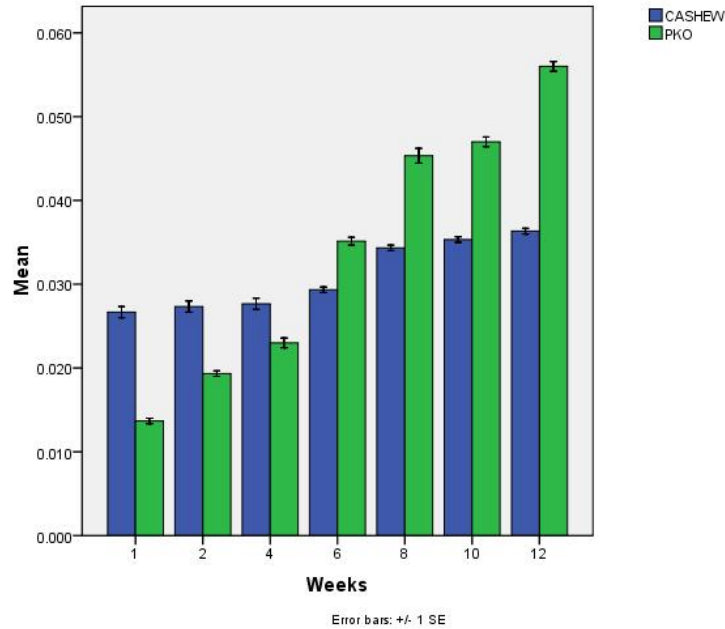


Fig. 6. The peroxide value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

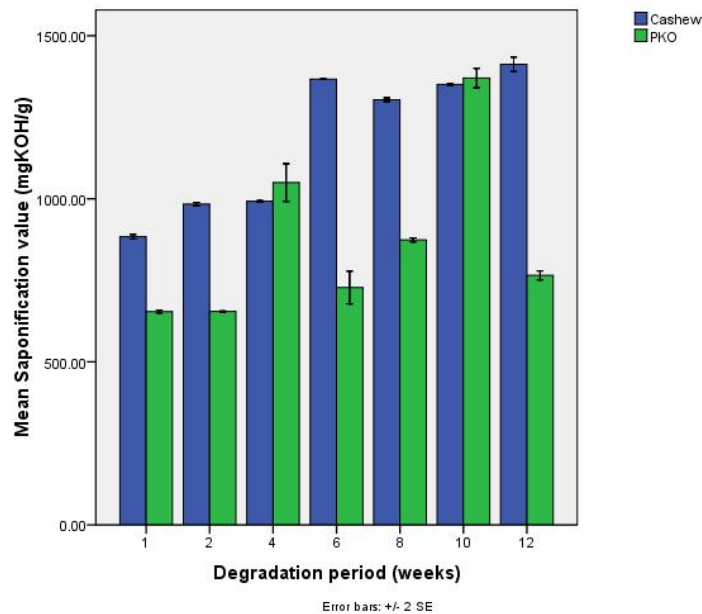


Fig. 7. The saponification value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

The saponification value of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks is shown in Fig. 7. The saponification value increased with increase in the degradation period for the palm

kernel and cashew nut oils but the palm kernel oil saponification value dropped at the 6th week before picking up again. The peak value was revealed to be at the 10th week for palm kernel oil and 12th week for cashew oil.

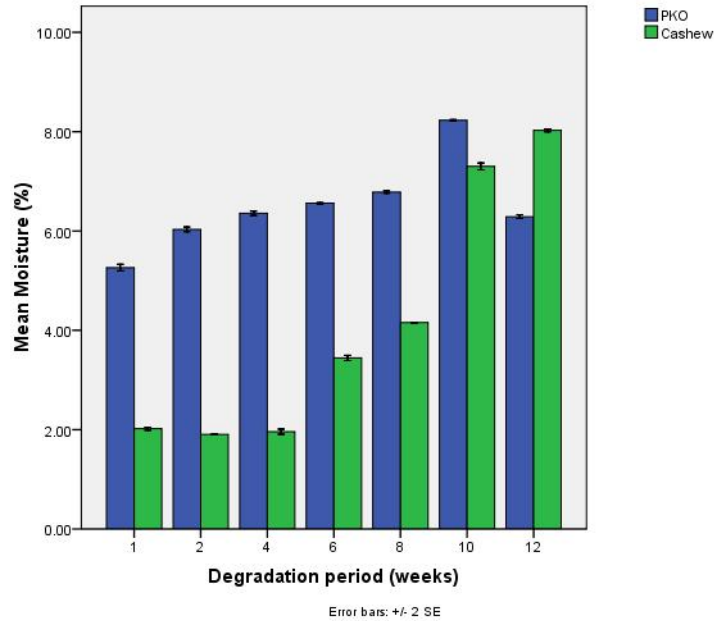


Fig. 8. The Moisture content of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

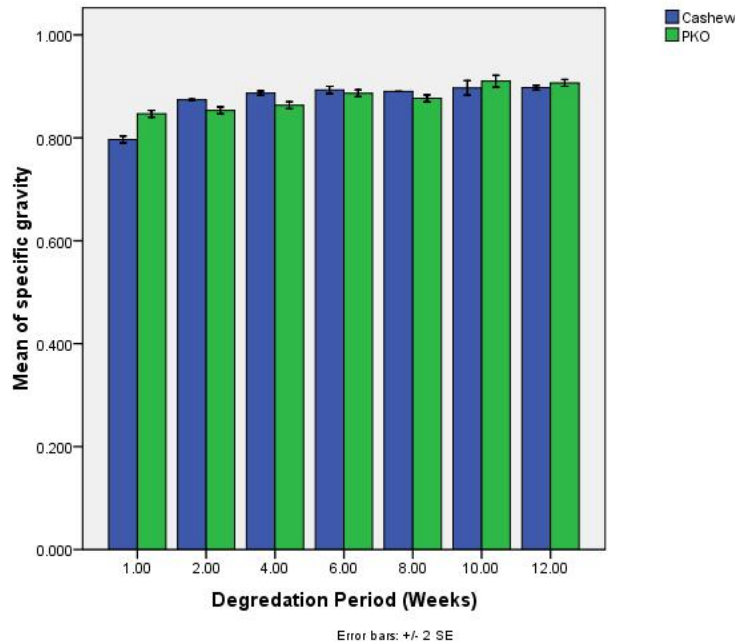


Fig. 9. Specific gravity content of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

Moisture content of the oil samples are show in Fig. 8. The value reduced gradually in both oils as the degradation periods increases. There was no difference in the moisture content of the

cashew oil on the week 10th and 12th of degradation period. The cashew nut oil shows a higher moisture content than the palm kernel oil.

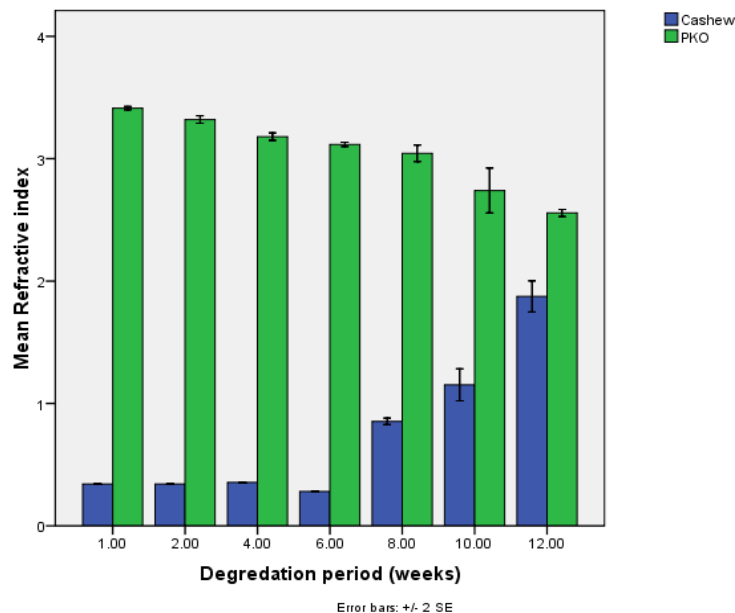


Fig. 10. The refractive index of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks

Table 1. Occurrences of microbes isolated from palm kernel oil

	Weeks						
	0	1 st	3 rd	5 th	7 th	9 th	11 th
Bacteria							
<i>Bacillus licheniformis</i>	-	-	-	+	+	-	-
<i>Staphylococcus aureus</i>	-	+	+	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	+	-	-	-	-	-
Fungi							
<i>Articosporium inflate</i>	+	+	+	-	-	-	-
<i>Aspegillus flavus</i>	+	+	+	+	+	+	+
<i>Aspergillus saprophyticus</i>	-	-	-	+	+	+	-
<i>Penicillium notatum</i>	-	+	+	+	+	+	-

Key: + = positive and - = negative

Table 2. Occurrences of microbes isolated from cashew nut oil

	Weeks						
	0	2 nd	4 th	6 th	8 th	10 th	12 th
Bacteria							
<i>Bacillus licheniformis</i>	-	-	+	+	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	-	-	-
Fungi							
<i>Articosporium inflate</i>	-	+	+	-	-	-	-
<i>Aspegillus flavus</i>	-	+	+	-	+	+	+
<i>Aspergillus saprophyticus</i>	-	-	-	+	+	+	+
<i>Penicillium notatum</i>	+	-	+	-	+	+	+

Key: + = positive and - = negative

The specific gravity of palm kernel and cashew nut oil is shown in Fig. 9. The increase in the degradation period shows no effect in the specific gravity of both oils. There was no significant difference in the specific gravity of both oils.

Fig. 10 shows that the values of refractive index of palm kernel and cashew nut oils subjected to spontaneous degradation over a period of 12 weeks. The values reduced in palm kernel oil but increases in the cashew nut oil with increase in the weeks of the degradation. The decrease in the refractive index of the palm kernel oil was gradual while the cashew oil showed no significant change in the first month of the degradation before showing a rapid increase in value.

4. DISCUSSION

The bacterial load of the extracted palm kernel and cashew nut oil were within the minimum acceptable microbiological level required by the Nigerian Agency for Food and Drug Administration (NAFDAC), which stipulated that the maximum allowable number of organisms in a sample unit of oil should not be more than 2×10^4 with acceptable microbiological level of 10^4 /ml. The microbial load obtained agrees with the reported findings [14]. Some of the microorganisms isolated, even though their microbial load was not high, can cause health problems in individuals who consume the product without heat processing. *Aspergillus* spp. and *Penicillium* spp associated with nut are known to have strain that can produce toxic metabolites. Thus they pose a potential hazard to consumer health [15].

The slight increase in bacterial and fungal count with the length of storage is in agreement with the earlier literature report [16]. The cashew nut oil had a higher fungal count compared with the palm kernel oil, an observation that contrasts the bacterial count. The bacteria isolated from the oil samples were *Bacillus licheniformis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and fungal isolates are *Articosporium inflata*, *Aspergillus flavus*, *A. saprophyticus*, and *Penicillium notatum*. The circumstantial evidence for the involvement of bacteria in the deterioration of stored cashew nuts is the ability of the spores of *Bacillus* to resist desiccation and this allows their survival in dried products [17]. The microflora of the stored oil is similar to that reported for some stored Nigerian foodstuffs [16].

Levels of unsaturation in the palm kernel and cashew nut oils were different since they registered approximately different iodine values 11.19 for cashew and 4.28 for PKO. There was no significant difference ($P < 0.05$) in the iodine value (IV) of the cashew oil stored while the PKO showed significant differences ($P < 0.05$). As at the 10th week the iodine value of PKO raised to 8. Iodine value indicates high unsaturation of fats and oils and low iodine value oils are more saturated with fewer double-bonds [18]. The iodine values obtained were within the standard range of 45 – 53 Wij's as recommended [19]. However, there are indications that as the oil undergoes storage, the oil samples may become highly unsaturated and therefore susceptible to oxidation. The addition of antioxidants may be necessary to prolong the storage stability of the oils. Accumulation of excessive iodine in the body could lead to development of goiter and the enlargement of the thyroid gland [20]. The iodine value is lower than typical iodine values obtained for coconut oil (25-40), palm oil (37-54), olive oil (75-95) and peanut oil (85-100).

The value of the free fatty acids (FFA) obtained from this study (Fig. 4) may be due to decomposition of glycerides by the fungi. It was stated that glycerides in oil can be decomposed by lipase or other actions and that decomposition may be accelerated by light heat [20]. The palm kernel oil (2.8 mg KOH/g) shows a higher FFA value than cashew nut oil (1 KOH/g) value which may be due to the variation in the moisture contents or extraction process. The value of FFA in this research is lower than the maximum free fatty acid content of 3.5mgKOH/g of oil specified by SON [19]. There was a significant difference ($P < 0.05$) in the FFA of the stored oils. The value for the PKO was at its peak on the 10th week with a value of 4KOH/g while the cashew oil was 3.8KOH/g at the 12th week. Since the storage of the oils increased the FFA value a little above the standard, it's advisable for the oil to undergo refining processes to improve its quality for industrial purposes.

Results obtained from this work indicated that the acid value of the oil corresponds to low levels of free fatty acids present in the oil, which also suggested low levels of hydrolytic and lipolytic activities in the oils. Acid value represents free fatty acid content due to enzymatic activity and is usually indicative of spoilage. Acid value is used as an indicator for edibility of oil and suitability for use in the paint industry.

The peroxide value (PV) determines the extent to which the oil has undergone rancidity, thus it could be used as an indication of the quality and stability of fats and oils [21]. The PV obtained in this study PKO (1.5 meq/Kg) and cashew nut oil (2.8 meq/Kg) is lower than standard value of 10 meq/Kg specified by SON [19]. It does not agree with the results [22] that rancidity often becomes noticeable when peroxide value is between 20 and 40 mEq/kg. Rancidity is an indication of deterioration of fats and oils. The Peroxide value of palm kernel oil obtained in this study is lower than that of cashew nut oil at the initial stage but there was a significant difference ($P < 0.05$) in the PV of PKO, the value later rose to 5.8 meq/Kg at the 12th week while the cashew nut was 3.5 meq/Kg. The peroxide value detected in this analysis is a good property which gives more resistant to oxidation, with better shelf- life.

Saponification values (SV) obtained for the palm kernel oil samples is 300 mgKOH/g and cashew oil is 400 mgKOH/g. The values are higher than saponification value of coconut oil 253 mgKOH/g and butter fat 225 mgKOH/g [23] that high saponification value indicates high proportion of lower fatty acids. This quality in the oil quantifies its use in soap production. The saponification value of the cashew increased and later dropped as a result of storage while the stored PKO showed an increase in value with significant difference ($P < 0.05$).

There is a significant ($p < 0.05$) difference between moisture content of cashew nut oil and PKO. The cashew nut oil had a moisture content of 9.5% while the PKO had a moisture content of 0.7%. However, they both have a high moisture contents above the standard levels. This could possibly be due to the precision of the methods used. Even though significant differences occur between the samples and storage periods for moisture and impurity, these differences do not show any trend. For example, it is not clear whether moisture content is influenced by the length of storage of the oil or the type of fruits used to process the oil.

Specific gravity is the heaviness of a substance compared to that of water, and it is expressed without units. The specific gravity obtained for all oil samples are less than 1.0 when measured at 30°C [23]. There was no significant difference between the values obtained for the oil. There was no indication that the specific gravity is influenced by the length of storage. Corresponding values obtained for the oils are

less than that reported for racemosa seed oil (4.947) by Amoo and Moza [24] but the values can be compared with that reported for cotton seed (0.9202), coconut oil and sunflower seed [23] by this value the oil is less dense than water.

5. CONCLUSION

The quality of degrading Palm (*Elaeis guineensis*) kernel and Cashew (*Anacardium occidentale*) nut oils provides information on the physicochemical properties of the stored palm kernel and cashew nut Oils. The high saponification value is evidence that the oil can be useful in production of soap and cosmetics. The high peroxide value and free fatty acid indicates that the oil samples have undergone some level of deterioration. Fungi were predominant organisms isolated and a few bacteria from stored palm kernel and cashew nut oil at a different storage period. Some of the microorganisms isolated, even though their microbial load was not high, can cause health problems in individuals who consume the product without heat processing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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