Influence of Packaging Materials and Storage Conditions on the Vitamins A and E Storage Stability of Palm Oil in Nigeria.

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ABSTRACT

Aims: Despite the presence of natural antioxidants contained in palm oil, it is still susceptible to quality deteriorations if not properly stored. This study therefore evaluates the storage stability of vitamins A and E in palm oil in four prominent packaging materials (metal cans, white plastic bottles, glass bottles and pet bottles) used in Nigeria and under three storage conditions [(refrigeration (5°C), closed cupboard (27°C) and direct sunlight (35°C)].

Study design: Freshly produced palm oil was filled in metal cans, white plastic bottles, glass bottles and pet bottles and stored in open, direct sunlight (35 ± 1°C), closed wooden cupboard (27 ± 1°C) and a refrigerator (5 ± 1°C) fo r a period of 120 days The samples were stored in a 4 (packaging materials) x 3 (Temperature) factorial arrangement making 12 treatments for each analysis sampled every 30 days for a period of 120 days. Vitamins A and E contents of palm oil samples were determined at 30 days intervals using ultraviolet spectrometer and high Performance Liquid Chromatography, respectively. Data values of triplicate determinations of vitamins A and E contents obtained from analysis were subjected to analysis of variance (ANOVA) and mean values were separated using Duncan New Multiple Range (DNMR) test using the Statistical Package for Social Sciences (SPSS) version 17.0. The rates of changes in the Vitamins A and E contents over the storage period of 120 days were also determined using Linear Regression analysis.

Place and Duration of Study: Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria between January, 2012 and December 2013

Methodology: Palm oil filled into the four different packaging materials was stored in the three storage conditions for a period of 120 days. Vitamins A and E contents of palm oil samples were determined at 30 days intervals using ultraviolet spectrometer and high Performance Liquid Chromatography, respectively. Data obtained were subjected to Analysis of Variance (ANOVA) to determine the statistical significant differences in the packaging materials and the storage conditions and the interactions between them. Mean values of vitamin A and vitamin E of different packaging methods and storage conditions were separated by Duncan New Multiple Range (DNMR) test to indicate their levels of significant differences. Linear Regression Analysis was also performed to determine the rates of changes in the vitamin A and E with time during storage.

Results: The vitamins A and E content of the samples stored in open, direct sunlight were

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virtually lost at the end of the storage period. For samples stored in sunlight, the vitamin A values in metal cans decreased by 97.45%, in white plastic bottle by 92.19%, in glass bottle by 92.46% and in pet bottle by 93.13% while vitamins E also decreased by 92.31% , 61.54% , 75.48% and 82.05%, respectively. Samples stored at room temperature suffered a higher amount of losses compared to the refrigerated samples. The refrigerated samples recorded only a minimal amount of loss. For the storage in both the sunlight and the dark cupboard and storage under refrigerating temperature of 5 ^{o}C, the order of preference for the packaging materials was white plastic bottle > glass bottle > pet bottle > metal can.

Conclusion: The results obtained from this study have demonstrated that packaging palm oil in white plastic bottle is the best method of preserving palm oil under refrigerating condition and lacquered metal under sunlight and dark cupboard. It has also shown that vitamins A and E degrade faster when palm oil is stored under sunlight and totally unfit for human consumption at the end of the storage period hence, palm oil should be stored in cold, dry places to limit their losses of antioxidant components.

Comment [C3]: Findings from the study should motivate the choice of best packaging materials among the four tested. Decision should be made. The current statement doesn't help enough.

Keywords: Palm oil, packaging, storage stability, vitamin A, vitamin E

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1. INTRODUCTION

Palm oil is orange-red oil with a semi-solid consistency at ambient temperature, which is derived from the fleshy mesocarp of oil palm (*Elaeis guineensis*) fruits. It serves as a major source of dietary fat in Nigeria [1]. Nnadozie *et al.* [2] reported that palm oil has been used mainly for edible purposes; such as cooking oil especially along the coastal and forest regions of West Africa for many years. It is incorporated into blended oils, and a large variety of food products use red palm oil. It is used world-wide as cooking oil, in preparation of margarine and shortening, and also for non-edible purposes in soap and oleo chemical industries. Murthy *et al*. [3] reported that crude palm oil is rich in carotene, a precursor of vitamin A, and in vitamin E (an antioxidant). Red palm oil is one of the most stable vegetable oils; this fact can be attributed to the presence of natural antioxidants and to the balanced ratio of saturated to unsaturated fatty acids. The carotenoids (vitamin A), tocopherols (vitamin E), sterols, phosphatides, triterpenic, and aliphatic alcohols form the minor constituents of palm oil [4]. Though present in less than 1% altogether in palm oil, nevertheless they play a significant role in the stability and refinability of the oil, in addition to increasing the nutritive value of the oil. Crude palm oil contains between 500 and 700 ppm of carotenoids mainly in the forms of α-and β-carotenes, the precursor of vitamin A. Unless extracted prior to refining, these carotenoids are thermally destroyed during the deodorization stage in order to produce the desired colour for refined oil. In crude palm oil, the presence of these carotenoids appears to offer some oxidative protection to the oil through a mechanism where they are oxidized prior to the triglycerides [5; 6].

Crude palm oil contains tocopherols and tocotrienols in the range of 600-1000 ppm. In fact, no other vegetable oil has as much Vitamin E as compared to palm oil [7]. Refined palm oil retains about 50% of these products. Tocopherols (30%) and tocotrienols (70%) isomers are antioxidants and provide some natural oxidative protection to the oil [8]. The combined effects of the properties of the carotenoids, tocopherols, tocotrienols and the 50% unsaturation of the acids confer a higher oxidative stability and anti-carcinogenic activities to palm oil as compared to a lot of other vegetable oils [9]. The low-cholesterol level, together with the anti-thrombotic and anti-carcinogenic properties of some of the carotenoids, tocopherols, and tocotrienols present, add further to the nutritive value of palm oil and palm oil fractions [10; 11]. Nevertheless, palm oil, whether crude or refined is still susceptible to quality deteriorations. Therefore, it is necessary to store it under favourable condition and in appropriate packaging materials [12]. Studies have been carried out on the qualities of palm oil during harvesting, processing and transportation [13; 14] on the effect of packaging materials, of temperature on the storage stability of crude palm oil [15; 16], refined, bleached and deodorized oil [17], and on the effect of irradiation on storage stability of red oil [18]. Many studies have been carried out on the factors of quality of oil during storage [19, 20, and 21]. They have shown that light, oxygen, moisture, and heat affect the quality of oil and that light is an initiator of deterioration of oils [16]. Warner and Mounts [22] suggested that plastic material of PVC or acrinytrile was an alternative to clear glass bottles. Nkpa *et al*. [16] found that lacquered cans were the most suitable over plastic bottles and should be stored in the dark to minimise the hydrolytic and oxidative deterioration of palm oil. They also observed that sunlight and oxygen have adverse effect on the oxidative deterioration of palm oil. It is therefore necessary to preserve oil away from open, direct sunlight and pack it in lacquered metal can or amber and green glass bottles rather than clear plastic bottles. Polyethylene film was found to be unacceptable as a packaging material. Despite all these studies, there is little information on the effect of packaging materials, temperature and sunlight on the carotenoids (vitamin A) and tocopherols (vitamin E) contents of palm oil. Therefore, the objective of this work carried out in Nigeria is to evaluate the influence of packaging materials and storage conditions on vitamins A and E stability in palm oil stored in plastic bottles, cans under sunlight, in dark cupboard at ambient temperature and in a refrigerator.

2. MATERIALS AND METHODS

2.1 Materials

Freshly produced palm oil was purchased from the Aponmu oil mill, Ondo State, Nigeria and prepared according to method described by Nnadozie *et al*. [2], while the packaging materials used for the palm oil storage, which include polyethylene terephthalate (Pet bottles), clear white glass bottles, white plastic bottles and metal cans were purchased at Oba market in Akure, Ondo State, Nigeria. All reagents, hexane: tetrahydrofuran and isopropanol used for this study were of analytical grade and were products of British Drug House Laboratory, England.

Comment [C5]: Readers may like to know what were those reagents.

2.2 Methods

Freshly produced palm oil filled in metal cans, white plastic bottles, glass bottles and pet bottles and stored in open, direct sunlight (35 ± 1℃), closed wooden cupboard (27 ± 1℃) and a refrigerator (5 ± 1℃) for 120 days The packa ging materials were filled with about 700 ml palm oil, such that the head space in each container was about 50 ml. The containers were tightly capped and stored without agitation. One set of containers were stored in open, direct sunlight at temperature of about 35 ± 1℃, w hile an equivalent set of containers (also containing palm oil) were stored in-door in a closed wooden cupboard at room temperature of about 27 ± 1℃. The third set of containers was stored in a refrigerator at about 5 ± 1℃. The samples were stored in a 4 (packaging materials) x 3 (Temperature) factorial arrangement making 12 treatments for each analysis, sampled every 30 days for a period of 120 days. Every container out of the 60 for the whole period, once removed from the storage and used for analysis had to be discarded and not reused. At thirty-day intervals, samples of palm oil in the three sets of samples were removed from storage, shaken vigorously and analyzed for vitamins A and E for a period of one hundred and twenty (120) days. Vitamins A and E contents of palm oil samples were determined at 30 days intervals using ultraviolet spectrometer and high Performance Liquid Chromatography, respectively. Data values of triplicate determinations of vitamins A and E contents obtained from analysis were subjected to analysis of variance (ANOVA) and mean values were separated using Duncan New Multiple Range (DNMR) test using the Statistical Package for Social Sciences (SPSS) version 17.0. The rates of changes in the Vitamins A and E contents over the storage period of 120 days were also determined using Linear Regression analysis.

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2.3 Analysis

2.3.1 Determination of carotene (vitamin A) content

The carotene content (vitamin A) of the oil samples was determined using a Hitachi ultraviolet spectrometer (Spec U3900 model, Japan). About 1g of the oil sample was dissolved in hexane in a 50 ml volumetric flask. Hexane was used as the blank solution to monitor the baseline. Thereafter, the ultraviolet (UV) absorbance value was measured at 446 nm as described by Choo *et al*. [23]. Using the data obtained, concentrations of the carotene in the samples were calculated, using the following formula

$$[carotene] = \frac{383 \times Absorbance\ (446nm) \times Volume(ml)}{100 \times sample\ weight\ (g)}$$

where;

Carotene = concentration of carotene in units/g

Volume = volume of volumetric flask

383 = diffusion coefficient.

2.3.2 Determination of vitamin E content

About 1.0 g of the palm oil sample was dissolved with hexane in a 1.0 ml vial. The prepared palm oil sample (1.0 ml m^{-1}) was then injected into a High Performance Liquid Chromatography (HPLC) (Waters, Model 2475, USA) system as described by Choo *et al.*[23]. An HPLC with a fluorescence detector (excitation at 295 nm and emission at 325 nm) and a Zorbax analytical silica column (25 cm x 4.6 mm ID, stainless steel, 5 µm) (Aqual Sil Column, USA) was later used to analyse vitamin E. The mobile phase used being hexane: tetrahydrofuran: isopropanol (1000: 60: 4, v/v/v) at a flow rate of 2.3 ml/min. A standard sample with α-tocopherols was also prepared using similar method. Concentration of vitamin E in the palm oil was calibrated using authentic standards.

2.4 Statistical analysis

A 3 (Temperature) x 4 (packaging materials) factorial experiment was used to analyse the result to determine the effects of storage temperatures and packaging materials on the vitamin A and D contents of the oil. All the palm oil samples were in triplicates. The triplicate data values of vitamins A and E contents obtained from analysis were subjected to analysis of variance (ANOVA) for vitamins A and E contents and mean values were separated using Duncan New Multiple Range (DNMR) test using the Statistical Package for Social Sciences (SPSS) version 17.0. The changes in the Vitamins A and E contents over the storage period of 120 days were subjected to Linear Regression analysis and their slopes compared to determine the rate of degradation of both vitamins A and E during storage.

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3. RESULTS AND DISCUSSION

3.1 Effects of Packaging Materials and The Storage Conditions (Refrigeration (5^{o}C), Closed Cupboard (27^{o}C) and Direct Sunlight (35^{o}C) on Vitamin A And Vitamin E

Tables 1 and 2 show the ANOVA result of the 4 x 3 factorial experimental design of the effects of packaging materials (metal can, white plastic bottle, glass bottle, pet bottle) and the storage conditions (refrigeration (5^{o}c), closed cupboard (27^{o}C) and direct sunlight (35^{o}C) on Vitamin A and Vitamin E contents of palm oil stored for 120 days. There was a general decrease in the Vitamin A and E contents with the increase in the storage period ($P < 0.05$). The vitamin A contents decreased from 1308.8 units/g on the first day (day zero) to a range of 18.5 -83.26 units/g., while Vitamin E decreased from 1.56 mg/ml to a range of 0.02 - 0.11 mg/ml. There was also a general decrease in Vitamin A and E contents with increase in the temperature of the storage conditions from 5^{o}C to 35^{o}C ($P < 0.05$). The effect of packaging materials shows that metal can generally had the lowest superscript indicating that at each storage condition vitamins A and E were most degraded in the metal can while least degraded in the white plastic bottles.

Table 1: Effects of packaging materials and storage conditions (5, 27 and 35^{o}C) on the vitamin A contents (units/g) of palm oil under storage for 120 days.

PERIOD (days)	TEMPE RATURE	METAL CAN	WHITE PLASTIC BOTTLE	GLASS BOTTLE	PET BOTTLE
0	5^{o}C	1308.38^{a}±0.03$^{(a)}$	1308.38^{a}±0.00(a	1308.38^{a}±0.00(a	1308.38^{a}±0.00(a)
	27^{o}C	1308.38^{a}±0.07$^{(a)}$	1308.38^{a}±0.07(a	1308.38^{a}±0.07(a	1308.38^{a}±0.00(a)
	35^{o}C	1308.38^{a}±0.07$^{(a)}$	1308.38^{a}±0.07(a	1308.38^{a}±0.07(a	1308.38^{a}±0.09(a)
30	5^{o}C	1052.10^{a}±0.04$^{(a)}$	1248.00^{a}±0.03(a	1219.30^{a}±0.00(a	1219.43^{a}±0.03(a)
	27^{o}C	906.35^{c}±0.03$^{(b)}$	1111.10^{a}±0.03(b	1109.70^{a}±0.03(b	948.93^{b}±0.02(b)
	35^{o}C	612.89^{d}±0.03$^{(c)}$	972.93^{a}±0.03(c)	814.12^{b}±0.02(c)	728.02^{c}±0.02(c)
60	5^{o}C	843.09^{d}±0.01(a)	1003.13^{a}±0.03(a	991.87^{b}±0.01(a)	945.30^{c}±0.01(a)
	27^{o}C	521.11^{c}±0.02(b)	924.88^{a}±0.01(b)	804.74^{b}±0.04(b)	751.52^{c}±0.01(b)
	35^{o}C	109.70^{d}±0.04(c)	637.04^{a}±0.04(c)	531.18^{b}±0.02(c)	422.21^{c}±0.02(c)
90	5^{o}C	419.02^{d}±0.00(a)	688.13^{a}±0.02(a)	503.25^{b}±0.01(a)	485.04^{b}±0.01(a)a
	27^{o}C	103.49^{d}±0.10(b)	392.85^{a}±0.03(b)	297.64^{b}±0.03(b)	238.19^{c}±0.00(b)
	35^{o}C	52.88^{d}±0.02(c)	204.53^{a}±0.03(c)	111.17^{b}±0.02(c)	73.21^{c}±0.04(c)
120	5^{o}C	105.83^{a}±0.01(a)	272.13^{b}±0.01(a)	198.55^{c}±0.01(a)	137.77^{d}±0.01(a)a
	27^{o}C	33.40^{d}±0.02(b)	102.16^{a}±0.02(b)	98.64^{b}±0.02(b)	89.83^{c}±0.02(b)

35^{o}C	18.52^{d}±0.02(c)	83.26^{a}±0.02(c)	37.74^{b}±0.02(c)	21.12^{c}±0.04(c)
% of loss in 120 days	91.92	79.2	84.83	89.47

Values in a row with different superscript are significantly different (P< 0.05) for the packaging materials (metal can, white plastic bottle, glass bottle, and pet bottle)
Values in parenthesis (-) in a column with different superscript are significantly different (P < 0.05) for storage condition (5, 27 and 35^{o}C).

Table 2: Effects of packaging materials and storage conditions (5, 27 and 35^{o}C) on the vitamin E contents **(mg/ml)** of palm oil under storage for 120 days.

PERIOD (days)	TEMPE RATUR	METAL CAN	WHITE PLASTI C BOTTL E	GLASS BOTTL E	PET BOTTL E
0	5^{o}C	1.56^{a}±0.01(a)	1.56a±0.02^{a}	1.56a±0.03^{a}	1.56a±0.01^{a}
	27^{o}C	1.57^{a}±0.01(a)	1.57a±0.01^{a}	1.57a±0.01^{a}	1.57a±0.01^{a}
	35^{o}C	1.56^{a}±0.01(a)	1.56a±0.01^{a}	1.56a±0.01^{a}	1.56a±0.01^{a}
30	5^{o}C	1.04^{c}±0.01(a)	1.53^{a}±0.00^{a}	1.43^{b}±0.00^{a}	1.51c±0.03^{a}
	27^{o}C	0.93^{d}±0.00$^{(b)}$	1.49^{a}±0.00^{b}	1.36^{b}±0.00^{b}	1.07c±0.00
	35^{o}C	0.67^{d}±0.00$^{(c)}$	0.96^{a}±0.00^{c}	0.85^{b}±0.00^{c}	0.81c±0.00^{c}
60	5^{o}C	0.73^{d}±0.00$^{(a)}$	1.22^{a}±0.00^{a}	1.04^{b}±0.00^{a}	0.99c±0.00^{a}
	27^{o}C	0.64^{d}±0.00$^{(b)}$	1.11^{a}±0.00^{b}	0.93^{b}±0.00^{b}	0.85c±0.00^{b}
	35^{o}C	0.31^{d}±0.00(c)	0.41^{a}±0.00^{c}	0..40^{b}±0.00^{c}	0.36c±0.00^{c}
90	5^{o}C	0.26^{d}±0.00(a)	0.96^{a}±0.00^{a}	0.72^{b}±0.00^{a}	0.44c±0.00^{a}
	27^{o}C	0.18^{d}±0.00$^{(b)}$	0.85^{a}±0.00^{b}	0.52^{b}±0.00^{b}	0.39c±0.00^{b}
	35^{o}C	0.05^{d}±0.00$^{(c)}$	0.12^{a}±0.00^{c}	0.12^{b}±0.00^{c}	0.10c±0.00^{c}
120	5^{o}C	0.14^{d}±0.00$^{(a)}$	0.63^{a}±0.00^{a}	0.42^{b}±0.00^{a}	0.22c±0.00^{a}
	27^{o}C	0.12^{d}±0.00$^{(b)}$	0.60^{a}±0.00^{b}	0.38^{b}±0.00^{b}	0.28c±0.00^{b}
	35^{o}C	0.02^{d}±0.00$^{(c)}$	0.11^{a}±0.00^{c}	0.10^{b}±0.00^{c}	0.02c±0.00^{c}

Values in a row with different superscript are significantly different (P< 0.05) for the packaging materials (metal can, white plastic bottle, glass bottle, and pet bottle)
Values in parenthesis (-) in a column with different superscript are significantly different (P < 0.05) for storage condition (5, 27 and 35^{o}C).

3.1.1 Effect of packaging materials and open, direct sunlight (35 ± 1℃)

Tables 3 and 4, extracts from Tables 1 and 2, show the effect of open, direct sunlight (35 ± 1℃) on the nutritional qualities of palm oil store d in different containers for a period of 120 days. Vitamins A and E contents decreased as the storage days increase for all samples (P < 0.05). The vitamin A contents of the oil stored in the metal can, white plastic bottle, glass

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bottle, pet bottle decreased significantly by 98.61%, 93.64%, 97.12% and 98.39%, respectively while those of vitamin E decreased in the same packaging materials by 98.72%, 92.95% , 93.55% and 98.72% respectively within 120 days of storage.

Table 3: Effect of packaging materials on the Vitamins A contents of palm oil stored in open, direct sunlight (35±1℃) for 120 days.

Period (days)	Vitamin A(units/g)			
	Metal can	White plastic bottle	Glass bottle	Pet bottle
0	1308.38 ± 0.47^{a}	1308.38 ± 0.67^{a}	1308.38 ± 0.57^{a}	1308.38 ± 0.79^{a}
30	612.88±0.03^{d}	972.93±0.03^{a}	814.11±0.02^{b}	728.01±0.02^{c}
60	109.7±0.04^{d}	637.04±0.04^{a}	531.19±0.02^{b}	422.20±0.02^{c}
90	52.88±0.02^{d}	204.53±0.03^{a}	111.17±0.02^{b}	73.21±0.04^{c}
120	18.52±0.02^{d}	83.24±0.02^{a}	37.73±0.02^{b}	21.12±0.04^{c}
% Loss in 120 days	98.61	93.64	97.12	98.39

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Values in each column with different superscripts are significantly different ($p<0.05$)

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Table 4: Effect of packaging materials on the Vitamins E contents of palm oil stored in open, direct sunlight (35±1℃) for 120 days

Period (days)	Vitamin E(mg/ml)			
	Metal	White plastic bottle	Glass bottle	Pet bottle
0	1.56 ±0.01^{a}	1.56 ± 0.02^{a}	1.56 ± 0.03^{a}	1.56 ± 0.01^{a}
30	0.67 ± 0.00^{d}	0.96 ± 0.00^{a}	0.85 ± 0.00^{b}	0.81 ± 0.00^{c}
60	0.31 ± 0.00^{d}	0.41 ± 0.00^{a}	0..40 ± 0.00^{b}	0.36 ± 0.00^{c}
90	0.05 ± 0.00^{d}	0.12 ± 0.00^{a}	0.12 ± 0.00^{b}	0.10 ± 0.00^{c}
120	0.02 ± 0.00^{d}	0.11 ± 0.00^{a}	0.10 ± 0.00^{b}	0.0 ± 0.00^{c}
% Loss in 120 days	98.72	92.95	93.55	98.72

Values in each row with different superscripts are significantly different ($p<0.05$).

Vitamins A and E values obtained under direct sunlight (27^{o}C) in all the packaging materials showed faster decrease in values (P < 0.05). These vitamins are natural occurring bioactive compounds which have high affinity towards heat and light [25]. Usually, normal room temperature itself is enough to cause the compound to degrade [26]. It is observed that palm oil samples stored under open, direct sunlight almost lost all their carotene content except the sample in the white plastic bottle which has a slight higher value compared to others at the end of the 120 days storage period with 93.64%, decrease. This may be attributed to the fact that the white plastic bottle resisted the penetration of sunlight and heat to some extent compared to other containers used under this storage condition, since heat and sunlight are the main factors that cause the disintegration of these bioactive compounds [27]. However this is contrary to the findings of Nkpa *et al*.[16] who stored freshly produced Nigerian crude palm oil in Lacquered metal cans, green glass bottles, amber glass bottles, clear glass bottles and clear plastic bottles in open, direct sunlight (40 ± 1℃) and in the dark (27 ± 1℃ over a period of 98 days. They observed that crude palm oil packaged in plastic bottles and clear glass bottles recorded higher total oxidation values (peroxide, free fatty acid and anisidine values) than oils packaged in either lacquered metal cans or amber and green glass bottles. They also observed that Lacquered metal cans gave the greatest protection against oxidation. Oxidation proceeded faster in cases where the packaging materials were stored in open, direct sunlight. Agbaire [13] observed that the carotene level of palm oil decreases with the time of storage in palm oil sold in some major markets in Delta State, southern Nigeria. The vitamin E content of the palm oil samples stored under open, direct sunlight were almost lost in all the packaging materials. This is in conformity with the work of Nnadozie *et al.* [2] who reported that heat and sunlight greatly affected vitamins composition of palm oil. Chandrasekaram et al. [15] also observed that carotenes suffer minimal loss in its concentration because oxidation of vitamin E could have started before carotenes and thus protected the carotenes from deterioration. Rodriguez-Amaya [28] and Aletor *et al.* [29] demonstrated that traditionally extracted palm oils retained more β-carotene than mechanically processed oils because palm fruits processed in a traditional manner were not exposed to high temperatures. Rodriguez-Amaya [28] observed that during the processing techniques employed in Bahia, Brazil, palm seeds are exposed to sunlight and sterilized long after harvest, resulting in prolonged heating of the crude oil and greater fluctuations in impurity levels. Under these conditions, carotenoids oxidation was more pronounced. Obahiagbon [21] observed that the low tocopherols values in the palm oils produced from small scale mills in Ovia - North East local government area of Edo state in Nigeria was certainly due to the prolonged and uncontrolled heating of the oils. Therefore exposing palm oil to open, direct sunlight resulted in a faster reduction of the Vitamin A and E contents and the reduction was fastest in the sample stored in the metal can while slowest in the sample in the white plastic bottle.

3.2 Effect of closed, dark condition (closed dark cupboard, 27 ±1℃ ^{o}C) on vitamin A and vitamin E

Tables 5 and 6 show the effect of storage of palm oil in closed, dark cupboard (27 ± 1℃) on the vitamin A and vitamin E contents in different containers for a period of 120 days The contents of vitamins A and E in palm oil decreased as storage days increased (P < 0.05) The vitamin A values of the sample stored in the metal can decreased by 97.45%, white plastic bottle by 92.19%, glass bottle by 92.46% and pet bottle by 93.13% respectively while they similarly decreased by 92.31% , 61.54% , 75.48% and 82.05% respectively. Chandrasekaram *et al*. [15] observed that samples stored at room temperature suffered a higher loss compared to the refrigerated sample and that vitamins A and E in the sample stored at room temperature degraded considerably compared to their concentrations at the start of the study.

Table 5: Vitamins A contents of palm oil stored in a closed wooden cupboard (27 ± 1℃)

Period (days)	Vitamin A(units/g)			
	Metal can	White plastic bottle	Glass bottle	Pet bottle
0	130838 ± 0.47^{a}	1308.38 ± 0.67^{a}	1308.38 ± 0.57^{a}	1308.38 ± 0.79^{a}
30	906.35 ± 0.03^{d}	1111.18 ± 0.03^{a}	1109.71 ± 0.03^{b}	948.93 ± 0.02^{c}
60	521.11 ± 0.02^{d}	924.87 ± 0.01^{a}	804.73 ± 0.04^{b}	751.51 ± 0.04^{c}
90	103.49 ± 0.10^{d}	392.85 ± 0.03^{a}	297.63 ± 0.03^{b}	238.19 ± 0.02^{c}
120	33.40 ± 0.02^{d}	102.15 ± 0.02^{a}	98.63 ± 0.02^{b}	89.85 ± 0.04^{c}
% Loss in 120 days	97.45	92.19	92.46	93.13

Values in each row with different superscripts are significantly different ($p < 0.05$).

Table 6: Vitamins E contents of palm oil stored in a closed wooden cupboard (27±1℃)

Period (days)	Vitamin E(mg/ml)			
	Metal can	White plastic bottle	Glass bottle	Pet bottle
0	1.56 ± 0.01^{a}	1.56±0.02^{a}	1.56±0.03^{a}	1.56±0.01^{a}
30	0.93 ± 0.00^{d}	1.49±0.00^{a}	1.36±0.00^{b}	1.07±0.00^{c}
60	0.64 ± 0.00^{d}	1.11±0.00^{a}	0.93±0.00^{b}	0.85±0.00^{c}
90	0.18 ± 0.00^{d}	0.85±0.00$_{a}$	0.52±0.00^{b}	0.39±0.00^{c}
120	0.12 ± 0.00^{d}	0.60±0.00^{a}	0.38±0.00^{b}	0.28±0.00^{c}
% Loss in 120 days	92.31	61.54	75.48	82.05

Values in each row with different superscripts are significantly different ($p < 0.05$).

Vitamins A and E which were investigated under this storage condition with all the packaging materials showed considerable decrease in values as the storage period advanced. It was also observed that palm oil samples stored in the white plastic bottle had higher values of vitamins A and E compared to other containers at the end of the storage period ($P < 0.05$). Heat is the main factor that causes the degradation of these bioactive compounds, and losses up to 70% of deterioration at room temperature [25]. Carotenes suffer minimal loss in its concentration compared to vitamin E; this could be because oxidation of vitamin E starts before carotenes (vitamin A) and thus protected the carotenes from deterioration [15]. It is observed from this study that the vitamin E content of the palm oil samples stored at closed, dark condition were less degraded than those stored in open, direct sunlight. On the

Comment [C14]: Less degraded

contrary, the refrigerated samples recorded only a minimal loss not exceeding 10% of the initial concentrations.

3.4 Effect of refrigerating condition (5 ± 1 ℃) on the Vitamins A and E. contents of palm oil

Tables 1, 2 and Figures 1 and 2 show the effects of refrigerating condition (5 ± 1℃) on contents of vitamin A and vitamin E of palm oil stored in different containers for 120 days. All the nutritional qualities investigated decreased in values throughout the storage period. The vitamin A values of the sample stored in the metal can, glass bottle and pet bottle decreased by 91.92%, 79.20%, 84.83% and 89.47% respectively while the vitamin E values also decreased by 93.59%, 59.62%, 72.90%, and 85.90%, respectively. Decrease in vitamins A and E in the refrigerated samples followed the same trend as with other storage methods, but to a lesser degree. From the results obtained it can be seen that palm oil samples in the refrigerator retained more of their carotene, while sample in the white plastic bottle shows higher retention capability compared to the other methods at the end of the storage period. This was also confirmed by the study of Chandrasekaram *et al*. [15] who observed that refrigerated samples recorded only a minimal amount of loss not exceeding 10% of the initial concentration. The phytonutrients in the refrigerated samples however experience nearly no degradation. This may be attributed to the fact that the white plastic bottle resists the penetration of initiator of oxidative rancidity to some extent coupled with the favourable cold storage temperature compared to other containers used [31].

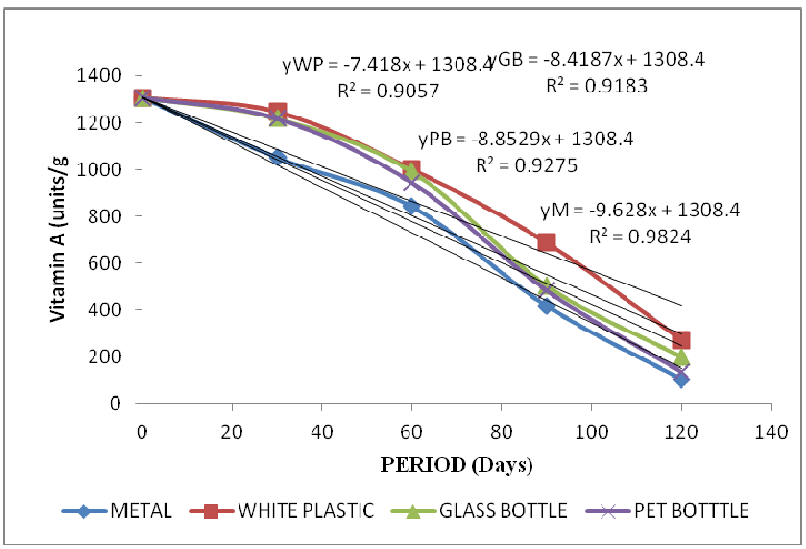

Figure 1: Changes in Vitamin A contents of Palm oil over storage at refrigerating condition (5 ± 1℃)

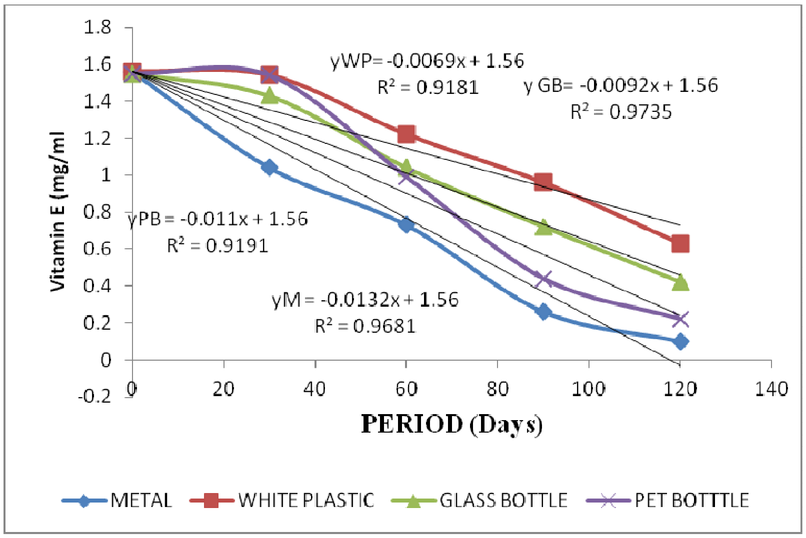

Figure 2: Changes in Vitamin E contents of Palm oil over storage at refrigerating condition (5 ± 1℃)

3.3 Comparative effect of the storage conditions and packaging on the rate of loss of vitamin A and E contents

Table 7 shows the comparison of the rate of reduction (k) among the three storage conditions of open, direct sunlight, the dark cupboard and refrigerating condition using the four packaging materials (metal can, white plastic bottle, pet bottle and glass bottle).

Table 7: Comparative effect of packaging and storage conditions on the rate of reduction in the Vitamins A.

	Open Sunlight (35^oC)	Dark Cupboard (27^o)	Refrigerating condition (5^oC)
Metal can	-13.354	-11.879	-9.628
White plastic bottle	-10.989	-9.484	-7.418
Pet bottle	-12.453	-10.62	-8.4187

Glass bottle	-11.825	-10.086	-8.8529

Values in each column with different superscripts are significantly different ($P < .05$).

Effect of Packaging: Comparing the packages it was found out that the reduction in the samples stored in the metal cans was fastest (-13.354, -11.879, -9.628) followed by that of the glass bottle (-12.453, -10.62, -8.4187), pet bottle (-11.825, -10.086, -8.8529) while the reduction was slowest in samples stored in the white plastic bottle (-10.989, -9.484, -7.418) in all the three storage conditions. The trend was similar for Vitamin E content of the oil. This shows that white plastic bottle preserved best the Vitamin A and E contents of palm oil under the three storage conditions while the Metal can gave the least preservation. Most of the vitamins were almost lost in the sample stored in the cans..

Effect of Storage conditions (5°, 27° and 35°C): Comparing the rates of reduction (k) of the palm oil in the three storage conditions (Table 7), it was observed that the rates of reduction were fastest in the samples stored under direct open sunlight (-13.354, -10.989, -12.453, -11.825,) followed by those in dark cupboard (-11.879, -9.484, -10.62, -10.086) while the rate of reduction was slowest in samples stored under the refrigerating condition (-9.628, -7.418, -8.4187, -8.8529). This must have been due to the action of the sun rays directly on the exposed sample in the glass bottle. A similar results was obtained from studies carried out by Favaro *et al*. [30] who measured stability of soy bean oil stored in open cans in the dark as well as open cans exposed to light for 10 hours a day after three months, Favaro *et al*.[30] found only 48% of original vitamin A level in the opened cans exposed to light and 76% in opened cans kept in the dark. In a similar calculation based on linear deterioration, after 30 days, 92% of vitamin A would be recovered from cans left in the dark and 83% from the cans exposed to light though deterioration is not linear but logarithmic, with most vitamin A deterioration at the end of the time period, but this calculation provides a conservative estimate [32]. Billions et al [33] observed that vitamins A and E were of good stability for 20 days; the final concentrations ranged from 75% to 100% of initial concentrations whatever the conditions studied. There was no significant difference of action between all containers and that the presence or absence of lipids and trace elements in admixtures stored at 4℃ or ambient temperature makes no difference. With exposure to sunlight, vitamin losses were 100% at 3 hours for vitamin A and 50% for vitamin K_1; vitamin E concentrations were unchanged after 12 hours of experiment. The presence of lipids or type of container did not appear to enhance protection from direct sunlight. Nkpa et al. [16] also observed that oxidation proceeded faster in packaging materials stored in direct sunlight; they advised that direct exposure of palm oil to direct sunlight in the markets should be discouraged

4. CONCLUSION

Samples stored under open, direct sunlight (35oC) and at room temperature (27oC) suffered a huge amount of loss of vitamins A and E given their concentration at the beginning of the study. On the contrary, refrigerated samples recorded only a minimal amount of loss not exceeding 10% of the initial concentration. Packaging palm oil in white plastic bottle was found to be the best method of preserving palm oil under the three storage conditions, while the metal can gave the least preservation of the vitamins. The order of preference for the packaging materials are therefore white plastic bottle > glass bottle > pet bottle > metal can. It has also been shown that the rates of reduction in the vitamins A and E were higher in samples stored under sunlight while there was minimal reduction in samples stored under refrigerates condition, hence, palm oil should packaged in white plastic bottles and stored in cold, non humid and dry environment to prevent the degradation of antioxidant components.

COMPETING INTERESTS

"We declare that no competing interests exist."

AUTHORS' CONTRIBUTIONS

Oluwalana IB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Toriola BO carried out the chemical analysis and wrote the first draft, Oluwamukomi MO reviewed, managed the literature searches and carried out subsequent correction during review and OR Karim ran the SPSS and Linear regression analyses. All authors read and approved the final manuscript."

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Comment [C15]: It is right to get the date of publication at the end?

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