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Refined Palm Kernel Oil Stabilization Via Addition of Water Chaya Leaf Extract and Butylated Hydroxytoluene

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ABSTRACT

The consumption of rancid edible oils leads to the risk of coronary health diseases. Therefore, this research focuses on the use of Chaya (Cnidoscolus acontifolus) leaves extract to reduce deterioration of refined palm kernel oil stored in a transparent plastic bottle for one year. Fresh chaya leaves were obtained, cut, air-dried, ground and sieved through 40 mm mesh size. The powdery sample was extracted with water at ratio 1:10 for 72 h. Water extract was added at varying concentrations (200–1000 ppm) into refined palm kernel oil (RPKO). RPKO stored with and without 200 ppm butylated hydroxyltoluene (BHT) were also setup (control). Colour and refractive index of oil samples were analysed. Free fatty acid (FFA), acid value (AV) and peroxide value (PV) of RPKO were monitored monthly for twelve months. The colour of RPKO treated with water chaya leaf extract (WCLE) ranged between 10.5 and 14.0 units while the colour was 10.0 units for RPKO without extract (0 ppm) and 200 ppm BHT. The mean values for FFA, AV and PV of RPKO without any additive (0 ppm) were 1.214±0.401%lauric acid, 3.368±1.107 mgKOH/g oil and 9.913±4.183 meqO2./kg oil respectively while the mean values for FFA, AV and PV of RPKO containing 200 ppm BHT were 0.948±0.164% lauric acid, 2.654±0.458 mgKOH/goil and 9.100±3.564 meqO2/Kg oil accordingly. The FFA, AV and PV of RPKO containing 200-1000 ppm WCLE ranged between 0.884±0.137-0.930±0.143% lauric acid, 2.475±0.382-2.535±0.348 mgKOH/g oil and 7.886±2.899– 8.748±3.473 meqO2/kg oil.

It was observed that RPKO containing chaya leaf extracts had lower mean values for FFA, AV and PV than RPKO with and without 200 ppm BHT over one year storage. The research showed that chaya leaf extracts improved the shelf stability of RPKO against hydrolytic and oxidative rancidity than 200 ppm BHT.

Keywords: Antioxidant Activity; Refined Palm Kernel Oil; Water Chaya Leaf Extract; Stability; Storage

Abbreviations: RPKO: Refined Palm Kernel Oil; BHT: Butylated Hydroxyltoluene; FFA: Free Fatty Acid; AV: Acid Value; PV: Peroxide Value; WCLE: Water Chaya Leaf Extract; TBHQ: Tert-Butylated Hydroquinone; BHA: Butylated Hydroxyanisole; WCL: Water Chaya Leaf; DMRT: Duncan Multiple Range Test; OWANOVA: one-way analysis of variance

Introduction

Refined palm kernel oil is one of the edible oils gotten from the kernel of palm fruits. Being an edible oil, its energy value and content of fatty acids make it useful ingredient in foodstuff. Generally during storage, food lipids and oils undergo different chemical reactions such as increase in hydrolytic and oxidative damage; and altering the organoleptic properties via thermolysis that resultantly affect the shelf life, nutritional quality and physiological properties (Gull, et al. [1-4]). Hydrolytic and oxidative stability of vegetable oils are major properties that determine their shelf life and applications (Pereria, et al. [5]). Edible oil containing both saturated and unsaturated fatty acid are susceptible to hydrolytic and oxidative degradation arising from the reaction of water and oxygen with saturated and unsaturated fatty acids respectively. This results in the formation of undesirable compounds such as free fatty acid, hydroperoxides etc. that affect the oil sensory characterization and shelf life (Simona, et al. [6]). It is highly necessary to improve the stability of edible oils against hydrolytic and oxidative rancidity so that their nutritional value and shelf life can be maintained during storage and processing.

The addition of antioxidants to vegetable oils has been proved to delay the onset of deterioration (Pereria, et al. [5-8,1]). The addition of synthetic antioxidants such as tert-butylated hydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) has been identified to promote DNA damage by binding to nucleic acid causing mutagenic, cancerous and cytotoxic effects (Arawande [7-11]). In attempt to seek for safer antioxidants, researchers have created interest in using natural antioxidants from plant origin such as vegetables, fruit peels, agricultural by-products etc. (Simona, et al. [6,7,12-14]). Chaya is an ever-green vegetable that is botanically known as Cnidoscolus acontifolus. It is a fast-growing perennial shrub. English names for chaya are tree spinach, cabbage star and treadsifly while its French and Latin names are manioc batard and jatropha aconitifolia respectively (Arawande, et al. [15]). The chaya leaf is being used as vegetable and it has been known to be very useful for its medicinal importance in preventing aging, onset of diabetes and arthritis (Akinmoladun, et al. [16]). The focus of this research is to obtain extract of chaya leaves using water as solvent and compare antioxidant activity of the extract at varying concentrations (200–1000 ppm) with 200 ppm butylated hydroxytoluene on refined palm kernel oil stored at ambient temperature for a year.

Materials and Methods

Source of Material

Chaya leaf was obtained from a local farm in Ondo, Southwest Nigeria while the refined palm kernel oil was procured from a vegetable oil processing company located in Akure, Southwest Nigeria. All chemicals were of the analytical grade with the highest purity available (<99.5%) and procured from Sigma Aldrich, USA

Preparation and Extraction of Chaya Leaf

The chaya leaf was cut into smaller pieces for easily drying. The dried leaf was ground using electric blending machine (Solitarire Mixer Grinder VTCL Heavy Duty 750 Watts) and it was sieved through 40 mm mesh size. The powdered sample was extracted with water at ratio 1:10 for 72 h during which it was intermittently shaken on a shaking orbit machine. The resulting mixture was filtered through a 0.45 µm Nylon membrane filter. The extract was desolventised to dryness under reduced pressure at 40 ^{o}C by a rotary evaporator (BUCHI Rotavapor, Model R-124, Germany). The dry extract was stored in a refrigerator (4 ^{0}C) (Bopitiya and Madhujith, 2014; Arawande and Komolafe, 2010).

Addition of Additives and Storage of Refined Palm Kernel Oil

Water extracts of chaya leaf at concentrations of 200 ppm (0.02 g / 100 ml oil) to 1000 ppm (0.10 g / 100 ml oil) was separately added to refined palm kernel oil (RPKO) contained in white transparent plastic bottles of equal capacity and they were thoroughly shaken for proper mixing. RPKO containing 200 ppm BHT (butylated hydroxytoluene) (0.02 g / 100 ml oil) and that which contained no additive (0 ppm (control)) were also set- up. Each container was appropriately labelled and stored in an open place at room temperature ranging from 27 to 33 ^{0}C.

Determination of Physical and Chemical Properties of the Oil

The colour, refractive index, free fatty acid (FFA), acid value (AV) and peroxide value (PV) of each oil sample were monitored monthly using standard methods described by AOCS, 2017 for a period of twelve months.

Determination of Colour

Lovibond Tintometer (model 520) was used to determine the colour of the oil. The oil sample was first filtered through a dry filter paper. The one-inch cell was filled with the filtered oil and placed on the stand in the cabinet in front of the aperture in the Lovibond Tintometer. The eyepiece was fixed and the cabinet was closed. The bulbs were lighted up and the colour slides were set to match with that of the cell. The colour of the oil was calculated thus: (5Red+Yellow-Blue) units.

Determination of Refractive Index

Abbe refractometer was used to determine the refractive index. Few drops of the sample were transferred into the glass slide of the refractometer. Water at 40 ^{0}C was circulated round the glass slide to keep its temperature uniform. The prism was first cleaned using acetone and the oil sample was spread upon the prism after condi-

tioning it to temperature of 40 ^{0}C. Readings were viewed through the eyepiece of the refractometer. The dark portion view was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value recorded as the refractive index.

Determination of Free Fatty Acid and Acid Value

Two grams of well mixed sample was accurately weighed into a conical flask; 10 ml of neutralized 95% ethanol and 2 drops of 1% phenolphthalein were added. This was then titrated with 0.1 M KOH solution, shaken constantly until a pink colour persisted for 30s.

$$Free\ fatty\ acid\ \left(\%\ lauric\ acid\right) = \frac{\text{Titre Value } x_1 \text{ Molarity of KOH } x_1\ 20}{\text{Weight of oil sample}}$$

$$Acid\ value\ \left(mg\ KOH\ /\ g\ oil\right) = \frac{\text{Titre Value } x_1 \text{ Molarity of KOH } x_1\ 56.11}{\text{Weight of oil sample}}$$

Determination of Peroxide Value

Two grams of the oil was dissolved in 20 ml of glacial acetic acid: chloroform (3: 2 v/v), 0.5 ml of saturated KI was added to the solution and heated gently. I2 was liberated as the KI reacted with the peroxide. The solution was titrated with standardized 0.1 M sodium thiosulphate using 0.5 ml of 1% starch indicator.

$$Peroxide\ value\ \left(meqO2\ /kg\ oil\right) = \frac{\text{(B-S) } x\text{Molarity of sodium thiosulphate } x1000}{\text{Weight of oil sample}}$$

Where B = blank titre value and S = sample titre value

Statistical Analysis

The results were compared by one-way analysis of variance (one-way ANOVA) to test for significant difference at P<0.05 level. Means of twelve replicates of the group were compared using Duncan Multiple Range Test (DMRT) (SAS, 2002).

Results and discussion

Changes in Colour and Refractive Index of Refined Palm Kernel Oil Stored with Varying Concentration of Water Chaya Leaf Extract and 200 ppm BHT

The changes in colour and refractive index of refined palm kernel oil stored with varying concentration of water chaya leaf extract and 200 ppm BHT is presented in Table 1. The water chaya leaf extract imparted additional colour unit (10.5-14.00 unit in 1" cell) on the refined palm kernel oil. But the colour of refined palm kernel oil without additive as well as with 200 ppm BHT was 10.0 unit in 1" cell. This shows that water extract increased the colour unit of refined palm kernel oil. Colour of edible oils is a physical parameter that influences consumers' acceptability and the lower the colour unit the easier it is for consumers' acceptability. However, most plant extracts do impart colours due to the presence of phytochemicals and chlorophyll that are present in them.

Free Fatty Acid of Refined Palm Kernel Oil Stored with Water Chaya Leaf Extract and BHT for Twelve Months

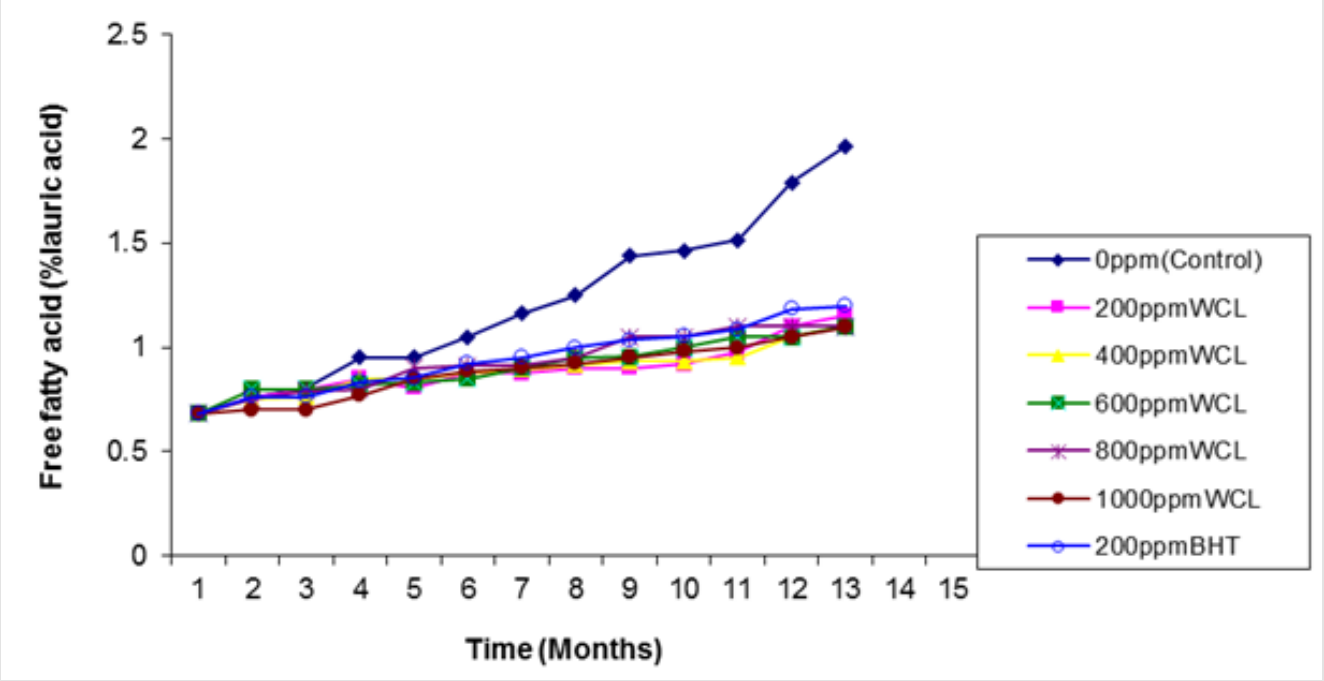

Figure 1: Free fatty acid of refined palm kernel oil stored with water Chaya leaf (WCL) extract and BHT for twelve months.

The trend in the free fatty acid of refined palm kernel oil stored with water chaya leaf extract and BHT for twelve months is shown in (Figure 1). After the first three months of storage, it was noticed that the FFA of RPKO containing no additive (control) had conspicuous highest value of FFA than RPKO stored with varying concentrations (200–1000 ppm) of water chaya leaf extracts and 200 ppm BHT. In the last seven months of storage, RPKO stored with 200 ppm BHT had relatively higher FFA value than RPKO stored with 200–1000 ppm water chaya leaf extract. The overall effect of the varying concentration of water chaya leaf extract on the FFA value of RPKO is well presented in (Table 2).

Table 1: Changes in colour and refractive index of refined palm kernel oil stored with varying concentration of water chaya leaf extract and 200 ppm BHT

Concentration of additive	Colour(units) in 1 inch cell	Refractive index at 400C
0 ppm (No additive)	1R+5Y=10.0	1.460
200 ppm WCLE	1.1R+5Y=10.5	1.460
400 ppm WCLE	1.2R+5Y=11.0	1.460
600 ppm WCLE	1.2R+6Y=12.0	1.460
800 ppm WCLE	1.2R+7Y=13.0	1.460
1000 ppm WCLE	1.4R+7Y=14.0	1.460
200 ppm BHT	1R+5Y=10.0	1.460

Note: WCLE= Water Chaya Leaf Extract, BHT= Butylated hydroxyl toluene; R = Red Slide; Y = Yellow Slide

Table 2: Mean value of some selected quality properties of refined palm kernel oil stored with varying concentration of water chaya leaf extract and 200ppm BHT over a period of twelve months.

Concentration of additive	*Free fatty acid (FFA) (% lauric acid)	*Acid value (AV) (mg KOH/g oil)	*Peroxide value (PV) ($meqO_2$/kg oil)
0 ppm (No additive)	1.214^{c} ±0.401	3.368^{c} ±1.107	9.913^{c}±4.183
200 ppm WCLE	0.892^{ab} ±0.128	2.511^{ab} ±0.352	8.748^{a} ±3.473
400 ppm WCLE	0.888^{ab} ±0.115	2.488^{ab} ±0.322	8.571^{a} ±3.332
600 ppm WCLE	0.907^{a} ±0.123	2.535^{a} ±0.348	8.409^{a} ±3.238
800 ppm WCLE	0.930^{a} ±0.143	2.607^{a} ±0.401	8.059^{a} ±3.024
1000 ppm WCLE	0.884^{a} ±0.137	2.475^{a} ±0.382	7.886^{a} ±2.899
200 ppm BHT	0.948^{b}±0.164	2.654^{b} ±0.458	9.100^{bc}±3.564

Note: Within each column, mean values followed by the same superscript are not significantly different at P ‹ 0.05 level according to Duncan Multiple Range Test (DMRT); *Mean Value of Quality Properties ± Standard Deviation.

WCLE= Water Chaya Leaf Extract, BHT= Butylated hydroxy toluene

Acid Value of Refined Palm Kernel Oil Stored with Water Chaya Leaf Extract and BHT for Twelve Months

The trend of acid value of refined palm kernel oil stored with water chaya leaf (WCL) extract and BHT for twelve months is depicted in (Figure 2). After the first three month of storage which served as induction period for the hydrolytic rancidity for refined palm kernel oil, the acid value of refined palm kernel oil without additive (0ppm) increased steadily above the acid value of RPKO stored with varying concentrations (200–1000 ppm) of water chaya leaf extract and 200 ppm BHT. Within the last seven months of storage, RPKO stored with 200 ppm BHT had a gradual increase in acid value over the RPKO stored with 200–1000 ppm of water chaya leaf extract. Water chaya leaf extract at varying concentration impeded the hydrolytic rancidity of refined palm kernel oil than 200 ppm BHT. (Table 2). further explains the effect of water chaya leaf and BHT on the acid value of RPKO for one year storage.

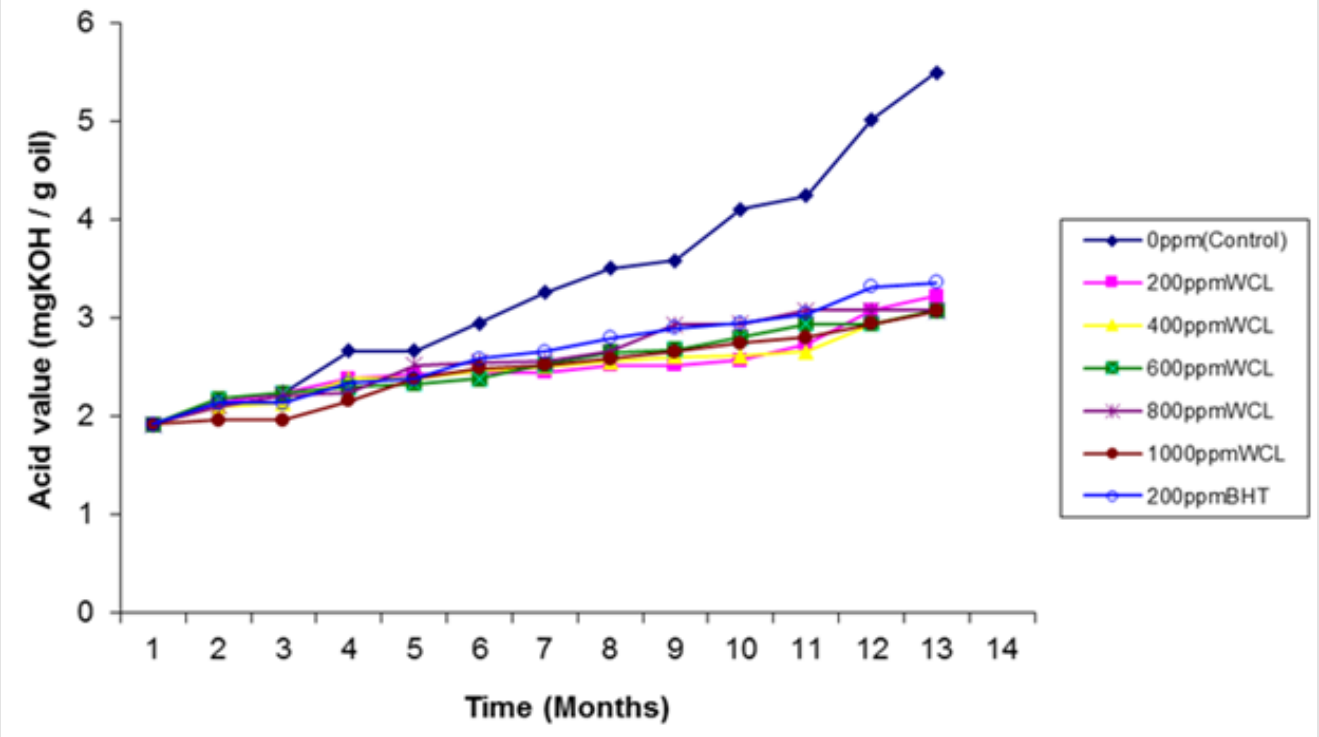

Figure 2: Acid value of refined palm kernel oil stored with water Chaya leaf (WCL) extract BHT for twelve months.

Peroxide Value of Refined Palm Kernel Oil Stored with Water Chaya Leaf Extract and BHT for Twelve Months

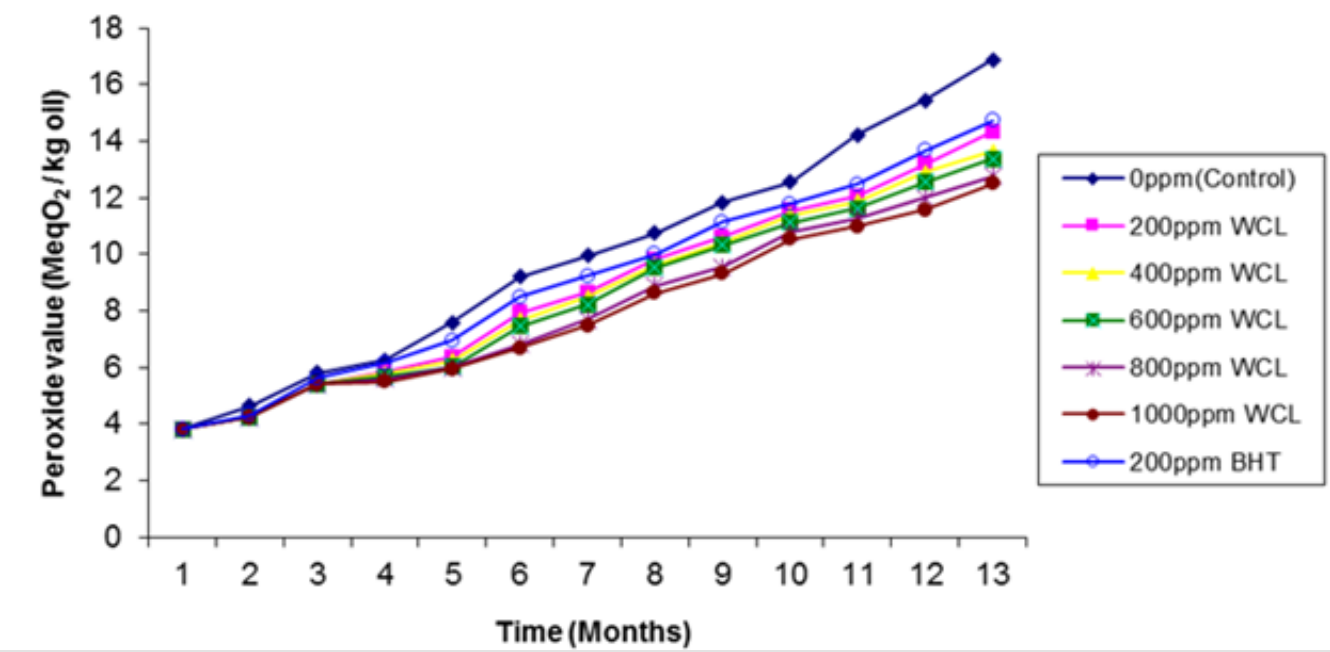

Figure 3: Peroxide value of refined palm kernel oil stored with water Chaya leaf (WCL) extract and BHT for twelve months.

The trend in peroxide value of refined palm kernel oil stored with water chaya leaf (WCL) extract and 200 pp BHT for twelve months is shown in (Figure 3). The trend of the plot of the peroxide value in (Figure 3) is very similar to the trend observed in relative increase in peroxide value of treated sunflower oil samples under accelerated storage (Iqbal, et al. [3]). The peroxide values of the treated and untreated RPKO were very close in the first three months of storage. The peroxide value of untreated RPKO was distinctly higher than the treated RPKO in the last nine months of storage. The peroxide value of RPKO treated with 200 ppm BHT was relatively higher than the RPKO treated with varying concentrations (200–1000 ppm) of water chaya leaf extract. Within the last nine months of storage, the peroxide values of RPKO stored with varying concentrations (200–1000 ppm) of water chaya leaf extract were lower as the concentration of the additive increased. The stabilization of RPKO was highest with water chaya leaf extract and the extent of stabilization increased as the concentration of the extract increased in the oil.

Mean Value of Some Selected Quality Properties of Refined Palm Kernel Oil Stored with Varying Concentration of Water Chaya Leaf Extract and 200ppm BHT Over a Period of Twelve Months

The mean value of some selected quality properties of refined palm kernel oil stored with varying concentration of water chaya leaf extract and 200ppm BHT over a period of twelve months is presented in (Table 2). The selected quality properties of RPKO are free fatty acid, acid value and peroxide value. The free fatty acid content of lipid is a measure of lipid hydrolysis and the FFA is formed as a result of hydrolysis of triglyceride in oil (Abd-Allah, et al. [7]) while acid value (AV) is the weight of KOH in milligram needed to neutralize the organic acids present in 1 g of fat or oil and it is a measure of free fatty acid present in the fat or oil (Gbenga-Fabusiwa, et al. [17]). The mean values of free fatty acid and acid value of the oil samples followed the same trend. The FFA and AV of RPKO treated with additives were lower that RPKO stored with no additive. The mean value of FFA of RPKO stored with 200–1000 ppm water chaya leaf extract (WCLE) ranged between 0.888±0.115 to 0.930±0.143 % lauric acid and the AV of RPKO stored with 200–1000 ppm WCLE ranged between 2.488±0.322 to 2.607±0.401 mg KOH/g oil. The mean values of FFA and AV of RPKO stored with 600 to 1000 ppm WCLE were not significantly difference ($p < 0.05$).

There was also no significant difference ($p<0.05$) in the mean values of FFA and AV of RPKO stored with 200 ppm and 400 ppm WCLE. The mean value of FFA and AV of RPKO stored with varying concentrations (200 – 1000 ppm) of WCLE was lower than the mean of value of FFA and AV of RPKO stored with 200 ppm BHT. The FFA and AV are quality characteristics that both measure the degree of hydrolytic stability of fat and oil. FFA and AV are common parameters in the specification of fats and oils. It was reported that the lower the values of FFA and AV of edible oils the better the quality of the oil and any treatment or additives that produced low FFA and AV in edible oils gave the oil hydrolytic stability (Arawande, Aderibigbe, et al. [9,17]). It was obvious that water chaya leaf extract was more effective than 200 ppm BHT in stabilizing RPKO against hydrolytic deterioration. Peroxide value (PV) measures the concentration of peroxides and hydroperoxides formed during the initial stages of oil oxidation and it is a good indicator of the primary (Abd-Allah, et al. [1,3,7,18]). The mean values of PVs of RPKO stored with 200–1000 ppm water chaya leaf extract (WCLE) was between 7.886±2.899-8.748±3.473 meqO2/kg oil while the mean values of PVs of RPKO without additive (0 ppm) and with 200 ppm BHT were 9.913±4.183 and 9.100±3.564 meqO2/kg oil respectively.

The mean values of PVs of RPKO decreased as the concentration of WCLE increased in the oil sample. There was no significant difference ($p<0.05$) in mean values of PVs of RPKO treated with varying concentrations of WCLE. However, there was significant difference ($p<0.05$) in mean values of PVs of RPKO treated with WCLE, 200 ppm BHT and control (0 ppm). During the twelve months of storage, it was observed that the mean values of PVs of RPKO stored with 200 – 1000 ppm WCLE were lower than the mean values of PVs of RPKO stored with 200 ppm BHT. The addition of the natural antioxidant extracted with water from chaya leaf to RPKO resulted in an increase in the oxidative stability of the oil [19,20].

Conclusion

The addition of water chaya leaf extracts to refined palm kernel oil enhanced the oil's stability against hydrolytic and oxidative rancidity of the oil but it imparted addition colour unit to the oil. However, further research needs to be conducted in bleaching the extract so that it can enhance the colour of refined palm kernel oil. This research work has confirmed that chaya leaf extract is a potent source of natural antioxidant and it can be used as alternative to synthetic antioxidant to improve the shelf-life refined palm kernel oil.

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