RESEARCH ARTICLE

Preliminary phytochemical screening and antioxidant activity of chaya leaf (*Cnidoscolus acontifolus*) extracts on refined palm kernel oil

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ABSTRACT

Fresh chaya leaves were obtained cut, air dried, ground and sieved through 40 mm mesh size. The powdery sample was separately extracted with five different solvents at ratio 1:10 for 72 h. The phytochemical screening of the powdery sample and solvent extracts were carried out. Water and methanol extracts were richer in phytochemicals. Methanol extract was dosed at varying concentrations (200-1000 ppm) into Refined Palm Kernel Oil (RPKO). RPKO stored with and without 200 ppm Butylated Hydroxytoluene (BHT) were also set up as 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 containing Methanol Chaya Leaf Extract (MCLE) was higher (12.0 and 18.0 units) than RPKO with (0 ppm) and 200 ppm BHT. The RPKO containing MCLE had lower mean values for FFA, AV and PV than RPKO with and without 200 ppm BHT over one year storage period. The research showed that MCLE improved the shelf stability of RPKO against hydrolytic and oxidative rancidity than 200 ppm BHT.

Keywords: Chaya leaf extract; phytochemicals; organic preservative; refined palm kernel oil; storage stability

INTRODUCTION

The use of plant extracts as preservatives in food commodities have become very important due to the increase in demand by consumers for safer and less harmful preservatives in food products as a result of the adverse effects of synthetic preservatives. The helpful effects of plant products are primarily attributed to their phytochemical components. Some of these phytochemicals are alkaloids, flavonoids, phenolic acids, terpenoids, steroids, tannins, saponins etc. Phytochemicals are defined as bioactive plant chemicals found in fruits, vegetables, grains and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases [1].

Refined palm kernel oil is one of the vegetable oils obtained from palm kernel seeds. The oil is extracted from the seeds by either expulsion or solvent and thereafter the oil is further processed by subjecting it to degumming, neutralization, bleaching and deodorization in order to have it in refined form which is of better quality and more acceptable by consumers due to its superb organoleptic property [2]. Being edible oil containing both saturated and unsaturated fatty acids, it is prone to deterioration that is termed as hydrolytic and oxidative rancidity. Which limits the usefulness of edible oils. Consumers of rancid oils are liable to health risks such as hypertension, obesity, cardiovascular disorder, thrombosis, atherosclerosis etc. [3]. Consequently, there is need to prevent edible oils from going rancid in order to have a prolong shelf life so that it can be safe for consumption. This necessitates the use of synthetic additives such as propyl gallate, citric acid, butylated hydroxyl anisole, butylated hydroxyl toluene etc. and they have been found effective in delaying the onset of both hydrolytic and oxidative rancidity of vegetable oils. Although it has been also discovered that they are not humanly friendly due to their toxicity, carcinogenicity, mutagenicity and not readily available due to cost. Hence their usage as additives for edible oils is being discouraged in the international market. New trend of research is geared towards using natural sources of plant origin which are safer, cheaper and readily available antioxidants [4].

Chaya (*Cnidoscolus acontifolus*) plant is a perennial shrub which is ever green, available throughout the year. 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. The focus of this research is to obtain extract of chaya leaves using acetone, chloroform, ethyl acetate, methanol and water; and to qualitatively identify the phytochemical constituents of chaya leaves in each solvent extract with a view of establishing the most potent solvent-extract with more bioactive components which can be used for preservation of refined palm kernel oil [5-10].

MATERIALS AND METHODS

Source of materials

Chaya leaf was obtained from a local farm in Owo, South West Nigeria while the refined palm kernel oil was procured from a vegetable oil processing company located in Owo, South West Nigeria. All chemicals were of the analytical grade with the highest purity available (<99.5%) and procured from Sigma Aldrich, USA [11-13].

Preparation and extraction of chaya leaf

The chaya leaf was cut into smaller pieces for easy drying. The dried leaf was ground using electric blending machine (Solitarire Mixer Grinder VTCL Heavy Duty 750 Watts) and it was sieved with 40 mm mesh size. The powdered sample was divided into portions, packed in air tight containers labelled appropriately prior to extraction. Each sample was extracted separately with each solvent (acetone, chloroform, ethyl acetate, methanol and 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 extracts were desolventised to dryness under reduced pressure at 40°C by a rotary evaporator (BUCHI Rotavapor, Model R-124, Germany). The dry extract was stored in a refrigerator (4°C) [14].

Qualitative phytochemical screening of chaya leaf extract

Phytochemical screening was carried out on the powdery sample of chaya leaf extracts using standard procedures as described [15-20].

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Addition of additives and storage of refined palm kernel oil

Methanol extract 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 Hydroxyl Toluene) (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°C [21-25].

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 [26-30].

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 [31-35].

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°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 conditioning it to temperature of 40°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 [36-38].

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 30 sec.

$$\text{Free fatty acid (\% Lauric acid)} = \frac{\text{Titre Value x Molarity of KOH x 20}}{\text{Weight of oil sample}}$$

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

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. I_2 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

$$\text{Peroxide value (meq}O_2\text{ /Kg oil)} = \frac{(B-S) \times \text{Molarity of sodium thiosulphate} \times 1000}{\text{Weight of oil sample}}$$

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) [39-45].

RESULTS AND DISCUSSION

Qualitative phytochemical screening chaya leaf extracts

The qualitative phytochemical screening of powdery and solvent-extracts of chaya leaf is presented in Table 1. It was observed that the powdery sample contained all the phytochemicals investigated except alkaloids. Acetone extracted 50% of the phytochemicals (phenol, oxalate, phytate and ascorbic acids). Chloroform extract contained 25% of phytochemical investigated (phytate and ascorbic acids). 62.5% of the bioactive compounds (phenol, oxalate, saponin, phytate and ascorbic acids) were extracted by ethyl acetate Water and methanol extracted 100% of phytochemicals found in the powdery sample [46-50].

Table 1: Qualitative phytochemical screening of powdery and solvent-extracts of chaya leaf.

Constituents	Powdery Sample	Solvent-extracts of powder chaya leaf sample				
		Acetone	Chloroform	Ethylacetate	Methanol	Water
Flavonoids	+	-	-	-	+	+
Carotenoids	+	-	-	-	+	+
Phenol	+	+	-	+	+	+
Oxalate	+	+	-	+	+	+
Tannin	+	-	-	-	+	+
Saponin	+	-	-	+	+	+
Alkaloids	-	-	-	-	-	-
Phytate	+	+	+	+	+	+
Ascorbic acid	+	+	+	+	+	+
% extractable		50	25	62.5	100	100

Flavonoid was present in the powdery sample, water and methanol extracts of chaya leaf. Flavonoids are the largest group of plant phenols and also the most studied one [14]. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks. The flavonoids appear to have played a major role in successful medical treatments in ancient times, and their use has persisted up till now. Most flavonoids occur naturally associated with sugar in conjugated form and may be characterized as monoglycosidic, diglycosidic etc. Flavonoids have gained recent attention because of their broad biological and pharmacological activities. The flavonoids have been reported to exert multiple biological properties including anti-microbial, cytotoxic, anti-inflammatory and anti-tumour activities; but the best-described property of almost every group of flavonoids is the capacity to act as powerful antioxidants [39]. Can protect the human body from the dangerous free radicals and Reactive Oxygen Species (ROS) [51].

Water and methanol extracts of chaya leaf as well as the powdery sample of the leaf contained carotenoids. Carotenoids are considered as the potential natural antioxidant found in fruits and vegetables. They include xanthophyll and carotenes having scavenging of peroxyl radicals. Beta-carotenes are orange-colored carotenoids abundant in yellow-orange and dark green leafy vegetables. Carotenoids are precursor of vitamin A which enhances vision and it functions as both phytochemical and antioxidants [17].

The powdery sample and all the solvent extracts except chloroform contained phenol. Phenolic compounds represent the largest category of phytochemicals and they are most widely distributed in the plant kingdom. Phenols are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) group is bonded directly to an aromatic hydrocarbon group. Phenol (C_6H_5OH) is considered the simplest class of this group of natural compounds. Being a secondary metabolite, they have an important role as defence compounds and they exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes.

Tannin was detected in the powdery sample, water and methanol extracts of chaya leaf. Tannins are an important group of secondary plant metabolites that were originally used in the leather production industry in the tanning of animal hides. Tannins are generally defined as soluble, astringent complex phenolic substances of plant origin used in tanning of animal skins or precipitation of proteins. Due to their documented bioactivities and successful clinical trials, tannins are pivotal to the nutraceutical and pharmaceutical industries. Tannins are polyphenols with molecular weights between 500 and 3000 Da. The chemical structure of tannic acid depends on the plant species producing the compound and their biological activity.

Alkaloids are not detected in the powdery sample as well as the solvent extracts of chaya leaf. Alkaloids have been defined as a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms [50]. Alkaloids constitute a structurally diverse array of natural products and these compounds have a wide range of biological activities. Many have important pharmaceutical uses. Plants are regarded as the oldest source of alkaloids [30]. Alkaloids are often classified according to their molecular skeleton and their botanical origin.

Saponin is detected in the powdery sample, ethylacetate, water and methanol extract of chaya leaf extract. Many saponins are known to be anti-microbial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defense systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants. Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body.

Phytate and ascorbic acid were present in the powdery sample and solvent extracts of chaya leaf. Phytate has therapeutics uses as phytonutrient, providing an antioxidant effect. It has a strong binding affinity to important minerals such as calcium, magnesium, iron and zinc; and such it becomes insoluble precipitate and will be non-absorbed in the intestine. The mineral binding properties of phytate prevent colon cancer by reducing oxidative stress in the intestinal tract. Ascorbic acid is a reductone sugar acid with antioxidant properties which helps to protect the body against cancer and other degenerative diseases such as arthritis and type II diabetes mellitus. Ascorbic acid is also known to strengthen the body's immune system. The water soluble compounds of sodium, potassium and calcium salts of ascorbic acid are commonly used as antioxidant food additives but these compounds cannot protect fats oxidation except the fat soluble ester of ascorbic acid with long chain fatty acids (ascorbyl palmitate or ascorbly stearate) is used.

Changes in colour and refractive index of refined palm kernel oil stored with varying concentration of methanol chaya leaf extract and 200 ppm BHT.

The changes in colour and refractive index of refined palm kernel oil stored with varying concentration of methanol chaya leaf extract and 200 ppm BHT is depicted in Table 2. The methanol chaya leaf extract imparted colour (12.0-18.0 unit in 1" cell) to the refined palm kernel oil. The colour of refined palm kernel oil without additive as well as with 200 ppm BHT is 10.0 units in 1" cell. This shows that methanol extract increased the colour unit of refined palm kernel oil. The colour unit of edible oil is very important quality parameters that influence consumer choice. The less the colour unit of edible oils the more attractive is the oil to the consumers. However, none of the plant additives had more than 3R (red slide) on the oil as stipulated by Standards Organization of Nigeria (SON). Therefore the colour unit of refined palm kernel oil containing varying concentrations of methanol chaya leaf is within the acceptable standard setup by Standards Organization of Nigeria. The refractive index of refined palm kernel oil stored with varying concentration (200–1000 ppm) of methanol chaya leaf extract ranged between 1.470 and 1.472 and the refractive index of RPKO with 200 ppm BHT and that of the control was 1.460. Refractive index of edible oils is a physical parameter which determines the purity level of oils. It is an index of adulteration of edible oils. The recommended value of refractive index of refined palm kernel oil ranged from 1.450 to 1.480 and the values obtained are within the recommended acceptable values specified by Standards Organization of Nigeria (SON 2000).

Table 2: Changes in colour and refractive index of refined palm kernel oil stored with varying concentration of methanol chaya leaf extract and 200 ppm BHT.

Concentration of additive	Colour(units) in 1 inch cell	Refractive index at 40°C
0 ppm (No additive)	1 R+5 Y=10.0	1.460
200 ppm MCLE	1.2 R+6 Y=12.0	1.470
400 ppm MCLE	1.4 R+7 Y=14.0	1.471
600 ppm MCLE	1.4 R+9 Y=16.0	1.471
800 ppm MCLE	1.6 R+10 Y=18.0	1.472
1000 ppm MCLE	1.6 R+10 Y=18.0	1.472
200 ppm BHT	1 R+5 Y=10.0	1.460

Free fatty acid of refined palm kernel oil stored with Methanol Chaya Leaf (MCL) extract and BHT for twelve months

The free fatty acid of refined palm kernel oil stored with Methanol Chaya Leaf (MCL) extract and BHT for twelve months is presented in Figure 1. The trend showed that there was no significant increase in free fatty acid of refined palm kernel oil stored with and without additives in the first three months of storage. And this indicates the induction period of the hydrolytic rancidity of the oil. After the three months of storage, there was steady increase in the free fatty acid trend in the refined palm kernel oil stored with plant additive and 200 ppm BHT. Whereas there was sudden increase in the free fatty acid of refined palm kernel oil stored without additives after the first three months of storage. It was conspicuously noticed that the additives reduced the free fatty acid of refined palm kernel oil over the twelve months of storage (Figure 1). Free fatty acid of edible oils is one of the quality parameters that measure the extent of hydrolytic rancidity. The lower the free fatty acid of an edible oil the better the quality of the oil. Therefore the refined palm kernel oil stored with methanol chaya leaf extract improved the quality of the oil by reducing the degree of hydrolytic rancidity of the oil. The MCL extract performed better than 200 ppm BHT in combating hydrolytic rancidity of refined palm kernel oil. The extent of the effect of the varying concentrations of the methanol chaya leaf extract on the free fatty acid of refined palm kernel oil is further explained.

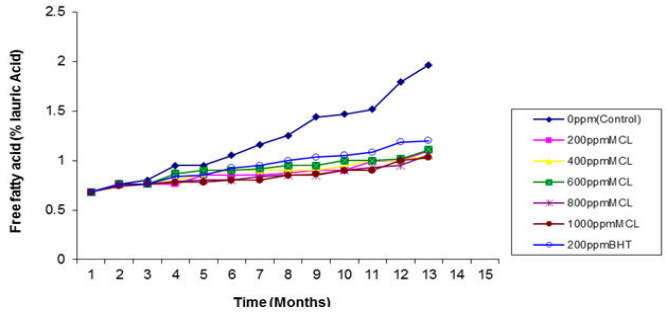

Figure 1: free fatty acid or refined palm oil stored with Methanol Chaya Leaf (MCL) extract and BHT for twelve months.

Acid value of refined palm kernel oil stored with methanol chaya leaf (MCL) extract and BHT for twelve months

The acid value of refined palm kernel oil stored with methanol chaya leaf (MCL) extract and BHT for twelve months is depicted in Figure 2. Only that the numerical value of acid value of refined palm kernel oil is higher than the numerical value of free fatty acid. The trend showed that there was no significant increase in acid value of refined palm kernel oil stored with and without additives in the first three months of storage. And this indicates the induction period of the hydrolytic rancidity of the oil. After the three months of storage, there was steady increase in the acid value trend in the refined palm kernel oil stored with plant additive and 200 ppm BHT. Whereas there was sudden increase in the acid value of refined palm kernel oil stored without additives after the first three months of storage. It was obvious that the additives reduced the acid value of refined palm kernel oil over the twelve months of storage. Acid value of edible oils is one of the quality parameters that measure the extent of hydrolytic rancidity. The lower the acid value of edible oil the better the quality of the oil. Therefore the refined palm kernel oil stored with methanol chaya leaf extract enhanced the quality of the oil by reducing the degree of hydrolytic rancidity of the oil. The MCL extract performed better than 200 ppm BHT in combating hydrolytic rancidity of refined palm kernel oil. The effect of the varying concentrations of the methanol chaya leaf extract on the acid value of refined palm kernel oil is further explicated in Table 3.

Table 3: Effect of methanol chaya leaf extract and 200 ppm BHT on some selected properties of refined palm kernel oil stored 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)	$1.214^{c} \pm 0.401$	$3.368^{c} \pm 1.107$	$9.913^{c} \pm 4.183$
200 ppm MCLE	$0.867^{b} \pm 0.116$	$2.428^{b} \pm 0.324$	$8.138^{b} \pm 4.173$
400 ppm MCLE	$0.881^{a} \pm 0.107$	$2.468^{a} \pm 0.298$	$7.808^{a} \pm 3.869$
600 ppm MCLE	$0.909^{ab} \pm 0.119$	$2.546^{ab} \pm 0.331$	$7.443^{a} \pm 3.684$
800 ppm MCLE	$0.843^{ab} \pm 0.097$	$2.363^{ab} \pm 0.270$	$7.265^{a} \pm 2.452$
1000 ppm MCLE	$0.838^{a} \pm 0.100$	$2.346^{a} \pm 0.281$	$7.205^{a} \pm 3.422$
200 ppm BHT	0.948b ± 0.164	$2.654^{b} \pm 0.458$	$9.100^{bc} \pm 3.564$

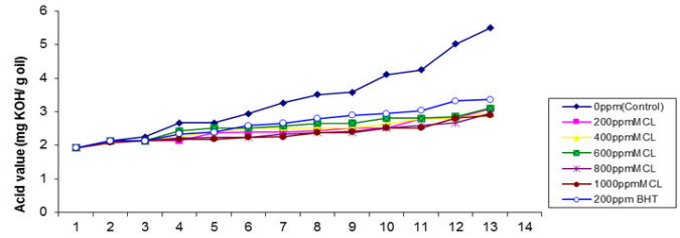

Figure 2: Acid value or refined palm kernel oil stored with Methanol Chaya Leaf (MCL) extract and BHT for twelve months.

Peroxide value of refined palm kernel oil stored with Methanol Chaya Leaf (MCL) extract and 200 ppm BHT for twelve months

The trend in peroxide value of refined palm kernel oil stored with Methanol Chaya Leaf (MCL) extract and 200 ppm BHT for twelve months is presented in Figure 3. It was observed that there was no significant difference in the peroxide values of the refined palm kernel oil stored with and without additives in the first three months of storage. However, the peroxide value of RPKO without additives (0 ppm) increased steadily more than the peroxide value of RPKO stored with additives in the last five months of storage. Within the last five months of storage, the RPKO stored with 200 ppm had higher peroxide value than RPKO stored with varying concentrations (200–1000 ppm) of methanol chaya leaf extract. Peroxide value of edible oil is a quality characteristic that measures the degree of oxidative rancidity of oil. The lower the peroxide values of oil the better the quality of the oil. Treatment that gives the oil low peroxide value enhances the quality of the oil by impeding the occurrence of oxidative rancidity of the oil. The RPKO stored with varying concentrations of methanol chaya leaf extract had relatively lower peroxide value than RPKO stored with 200 ppm BHT.

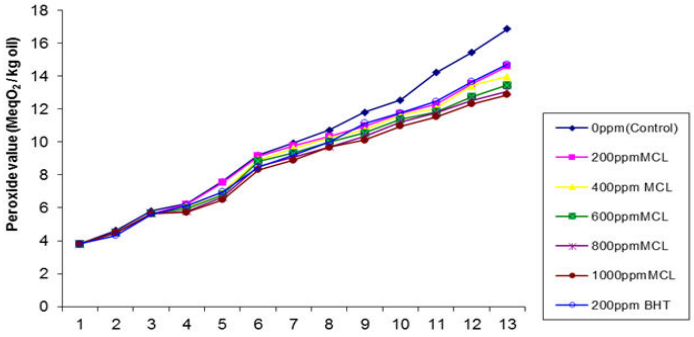

Figure 3: peroxide value of refined kernel oil stored methanol Chaya leaf (MCL) extract and BHT for twelve months.

Effect of methanol chaya leaf extract and 200 ppm BHT on some selected properties of refined palm kernel oil stored over a period of twelve months

The mean values of some selected quality properties of refined palm kernel oil stored with varying concentration of methanol chaya leaf extract and 200 ppm BHT over a period of twelve months is presented in Table 3. The selected quality properties 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 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 [18].

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 with no additive. The FFA of RPKO stored with 200–1000 ppm Methanol Chaya Leaf Extract (MCLE) ranged between 0.867 to 0.909% lauric acid while the AV of RPKO containing 200–1000 ppm methanol chaya leaf extract ranged between 2.428 to 2.546 mg KOH/g oil. The mean value of FFA and AV of RPKO stored with 400 ppm and 1000 ppm were not significantly difference at $p<0.05$. The mean value of FFA and AV of RPKO stored with 600 ppm and 800 ppm were not significantly difference ($p<0.05$) but there was significant difference ($p<0.05$) for the mean value of RPKO stored with 200 ppm MCLE. There was significant difference ($p<0.05$) in mean values of FFA and AV of RPKO stored with varying concentration of MCLE, 200 ppm BHT and 0 ppm (RPKO with no additive).

The highest mean value of FFA and AV was found with oil sample stored without additive and next to this was RPKO stored with 200 ppm BHT while the FFA and AV of RPKO stored with varying concentration of MCLE had the lowest values. 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 oil 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 [18]. It was obvious that chaya leaf extract was more effective than 200 ppm BHT in stabilizing RPKO against hydrolytic deterioration.

Peroxide Value (PV) is the measure of peroxide and hydroperoxides formed during initial oxidation of fats and oils and is used as an indicator to determine the oil oxidative rancidity [2]. The mean values of PVs of RPKO containing varying concentrations (200–1000 ppm) methanol chaya leaf extract ranged between 7.205-8.138 meqO_2/kg oil and the mean value of PVs of RPKO decreased as the concentration of MCLE increased in the oil sample. There was no significant difference ($p<0.05$) in the mean values of PVs of RPKO containing 400 to 1000 ppm MCLE.

Although there was significant difference ($p<0.05$) in the mean value of PV of RPKO containing 200 ppm MCLE. The mean values of PVs of RPKO stored with 200–1000 ppm MCLE were lower than that of the mean value of RPKO stored with 200 ppm BHT. 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 meqO_2/kg oil respectively. A significant difference ($p<0.05$) in mean values of PV was observed between RPKO without additive (control), RPKO with MCLE and RPKO with 200 ppm BHT. The addition of MCLE at varying concentrations to RPKO has a great retarding effect on primary oxidation of the RPKO comparable to 200 ppm BHT.

CONCLUSION

The extractive value of water and methanol is very high. Water and methanol are more effective among other solvents examined in obtaining bioactive ingredients from chaya leaves. Methanol and water extracts of chaya leaves would be potent antioxidants hence useful as additives in foods especially in lipids (fats and oils). The addition of methanol 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 of refined palm kernel oil.

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