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Effect of Methanol and Water Extract of African Lettuce (*Lactuca taraxacifolia*) on Stability of Refined Palm Kernel Oil

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ABSTRACT

African Lettuce extract was evaluated for parameters related to its antioxidant activity during twelve months storage of Refined Palm Kernel Oil (RPKO) in white transparent plastic bottles at room temperature (27°C – 33°C). Extracts of Wild Lettuce were prepared by separately soaking dried, ground and sieved African Lettuce into methanol and water in ratio 1:10 for 72 h. The Methanol African Lettuce Extract (MALE) and Water African Lettuce Extract (WALE) were separately added at varying concentrations (200 ppm to 1000 ppm) to RPKO. Another set of RPKO which contained no additive (0 ppm [control]) and 200 ppm BHT was set up. The colour units and refractive indices of oil samples were immediately determined while Free Fatty Acid (FFA), Acid Value (AV) and Peroxide Value (PV) of RPKO samples were monitored monthly using standard methods for a period of twelve months. The colour of RPKO containing MALE (12.0 – 15.0 units) was higher than RPKO containing WALE (10.5 – 12.0 units) while the colour of RPKO sample containing no additive (0 ppm) was 10.0 units and 11.5 units for RPKO containing 200 ppm BHT. The refractive index of RPKO containing MALE and WALE ranged between 1.454 and 1.455 while that of RPKO containing no additive (0 ppm) and 200 ppm BHT was 1.455. The FFA, AV and PV of RPKO containing MALE and WALE were lower than RPKO containing 200 ppm BHT. The results showed that WALE (Water African Lettuce Extract) is more effective in stabilizing RPKO (hydrolytically and oxidatively) than MALE.

Keywords: African Lettuce, Refined Palm Kernel Oil, Stability, Quality Assessment Test and Methanol.

Introduction

In recent times, there is a drift from the consumption of red palm oil to refined palm kernel oil among the local and low class people in Nigeria (Arawande and Seyifunmi, 2010). During festive periods and ceremonies (naming, graduation, wedding, etc.), a lot of people who are not able to purchase refined soybean and groundnut oils for their cooking go for refined palm kernel oil because it is relatively cheaper and available. As a result of its increased demand, oil merchants purchase it when it is cheaper during March to June and store it for five to eight months before it is later sold when its price has gone up.

Crude palm kernel oil is extracted from palm kernel seeds popularly known as palm kernel which contains about 45 – 50% oil content (Bernardini, 1973). Refining of crude palm kernel oil is done either by chemical or physical processes (Arawande and Seyifunmi, 2010; Bernardini, 1973). The refining of extracted crude palm kernel oil passes through four major stages of processing which include degumming, neutralization, bleaching and deodorization (Ikehoronye and Ngoddy, 1985). Generally, after refining of vegetable oil, there is tendency for the oil to go rancid during handling or storage and this always results in economic losses and quality impairment. Lipid oxidation is influenced by the presence of oxygen, pro-oxidant metals and conditions that activate oxygen such as light and high temperature (Nawar, 1996). Though

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avoiding exposure of edible oils to these conditions could prevent or impede oxidation, this has been found insufficient to control oxidation, and hence the need to use antioxidant arises (Coultrate, 2002; Gunstone and Norris, 1983).

Therefore, in the edible oil processing industry, there is growing interest in finding suitable natural alternatives to synthetic antioxidants such as Butylatedhydroxytoluene (BHT), Butylatedhydroxyanisole (BHA), Propylgallate (PG), Tertiary butylatedhydroquinone (TBHQ) and Citric acid currently in use to prevent lipid deterioration, but are reported not safe (Enrol *et al.*, 2004; Murkovic, 2003; Malecke, 2002; Carrasquerol *et al.*, 1998; Frankel, 1996). The effectiveness of antioxidants of phenolic compounds from some plant sources in retarding lipid deterioration have been reported (Rehab, 2010; Arawande and Komolafe, 2010; Arawande and Abitogun, 2009; Malecke, 2002; Abdalla, 1999; Wanasundara and Shahidi, 1998). Owing to carcinogenicity of synthetic antioxidants, the exploration of safer natural sources of antioxidants continues to gather momentum (Enrol *et al.*, 2004).

African Lettuce (*Lactuca taraxacifolia*) is a biennial leafy vegetable growing singly or in clusters, on rocky soil, banks, waste places and as a ruderal plant on plains and uplands up to 1000 m above sea level (Burkilli, 1995). Its stems and leaves are rich in a milky bitter sap (lactucarium) which changes colour to yellow at first and then brownish, hardens and dries when in contact with the air (Burkilli, 1995). Its English names are Wild Lettuce, Bitter Lettuce, Opium Lettuce and Poisonous Lettuce. Its French names are *Laitue africaine* and *Lange de vache*. Its local names in Nigeria are *Efo-Yarin*, *Odundun-Odo* in Yoruba; *Namijin dayii*, *Nomen barewa*, *NonanBarya* in Hausa (Burkilli, 1995). The plant is used as food in making salad, soup and sauces. The leaves and stems which contain sap have been used medicinally in curing whooping cough, as lactation stimulant to increase milk yield in cows and to produce multiple birth in goats and sheep (Adebisi, 2000). The various nutritional and

medicinal usage of the plant suggest that it is rich in certain phytochemicals which may be probably exploited in preventing oil rancidity.

The aim of this work is to obtain methanol and water extracts of African Lettuce and to examine the effects of these extracts at varying concentrations on the colour, refractive index, free fatty acid, acid value and peroxide value of refined palm kernel oil stored in white transparent plastic containers with a view of comparing the antioxidative effect of the extract with that of butylatedhydroxytoluene (BHT).

Materials and Methods

Sources of materials

African Lettuce (stems and leaves) was obtained from a farm in Iyere Owo, Ondo State, Nigeria. The refined palm kernel oil was obtained before being fortified with vitamin A at Vegetable Oil Division, JOF Ideal Family Farms Limited, Owo, Ondo State, Nigeria.

Preparation and extraction of African lettuce

The stems and leaves of African Lettuce were rinsed in water, cut into smaller pieces for easy drying. The dried plant parts were ground using the dry milling of an electric blending machine and it was sieved with 40 mm mesh size. The powdery sample was packed into black plastic bags prior to extraction.

Twenty grams of the powdery sample was weighed into two cleaned and dried reagent bottles; and 200 ml of each solvent (methanol and water) was separately added to each bottle and left for 72 h during which it was intermittently shaken on a shaking orbit machine. The mixture was filtered through a 0.45 µm nylon membrane filter. The extracts were evaporated to dryness under reduced pressure at 40°C by a rotary evaporator (Arawande and Komolafe, 2010; Amir *et al.*, 2005).

Addition of additives to refined palm kernel oil

Methanol and water extracts of African Lettuce at concentration of 200 ppm (0.02 g per 100 ml oil), 400 ppm (0.04 g per 100 ml oil), 600 ppm

(0.06 g per 100 ml oil), 800 ppm (0.08g per 100 ml oil), 1000 ppm (0.10 g per 100 ml oil) were 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 (0.02 g per 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°C to 33°C.

Physical and chemical analysis

The colour of the oil sample was determined as described by the AOCS 2004 method using Lovibond Tintometer (Model 520). The refractive index was also determined using Abbe's Refractometer at 40°C (AOCS, 2004). Thereafter, the Free Fatty Acid (FFA), Acid Value (AV) and Peroxide Value (PV) of each oil sample were monitored monthly using standard method of analysis (AOCS, 2004) for a period of twelve months.

Statistical analysis

The results (except colour and refractive index) were compared by one-way analysis of variance (one-way ANOVA) to test for significant difference. Means of the group were compared using Duncan Multiple Range Test (DMRT) (SAS, 2002).

Results and Discussion

Table 1 shows changes in colour and refractive index of refined palm kernel oil stored with varying concentrations of methanol and water extracts of African Lettuce and 200 ppm BHT. Colour and refractive index are very important physical assessment characteristics of edible oils. The colour of oil is a psychological interpretation of a physiological response by the eye and brain to the physical stimulus of light radiation at different wavelengths which influences consumer decision of acceptance or otherwise (Ihekoronye and Ngoddy, 1985). The addition of Methanol African Lettuce Extract (MALE), Water African Lettuce Extract (WALE) and BHT increased the colour units of refined palm kernel oil (RPKO) at varying degrees.

The colour units increased as the concentration of the extracts increased. RPKO containing 200 ppm to 1000 ppm MALE had colour units ranging from 12.0 to 15.0 units compared to between 10.5 and 15.0 units for RPKO containing 200 ppm to 1000 ppm WALE. RPKO containing 200 ppm BHT had colour of 11.5 units while RPKO without additive (0 ppm [control]) had colour of 10.0 units. The lower the colour unit, the more acceptable and attractive the palm kernel oil, therefore at low concentration, the WALE is superior to MALE in terms of colour characteristic of the oil. Refractive index of edible oil is a measure of the extent of edible oil adulteration or purity (Cocks and Rede, 1966). The addition of WALE and MALE at varying concentration to RPKO did not reflect that the oil was adulterated and it had almost the same refractive index with RPKO containing 200 ppm BHT. RPKO containing 200 ppm to 1000 ppm MALE had refractive index of 1.455 while it was between 1.455 and 1.454 for RPKO containing 200 ppm to 100 ppm WALE. RPKO which contained no additive had refractive index of 1.455.

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

Concentration of additive	Colour (Units)	Refractive index
0 ppm (No additive)	1R + 5Y = 10.0	1.455
200 ppm MALE	1.2R + 6Y = 12.0	1.455
400 ppm MALE	1.2R + 6Y = 12.0	1.455
600 ppm MALE	1.3 + 7Y = 13.5	1.455
800 ppm MALE	1.4 + 7Y = 14.0	1.455
1000 ppm MALE	1.4R + 8Y = 15.0	1.455
200 ppm WALE	1.1R + 5Y = 10.5	1.455
400 ppm WALE	1.1 + 6Y = 11.0	1.455
600 ppm WALE	1.2R + 6Y = 12.0	1.454
800 ppm WALE	1.4R + 6Y = 13.0	1.454
1000 ppm WALE	1.4R + 7Y = 14.0	1.454
200 ppm BHT	1.3R + 5Y = 11.5	1.455

MALE = Methanol African Lettuce Extract; WALE = Water African Lettuce Extract, BHT= Butylated Hydroxyl Toluene
R = Red Slide; Y = Yellow Slide

Figure 1 shows Free Fatty Acid (FFA) of RPKO stored with Methanol African Lettuce (MALE) and Butylatedhydroxytoluene (BHT) for twelve months. It was observed that RPKO containing 200 ppm to 1000 ppm MALE had lower FFA values than oil sample containing 200 ppm BHT. As the concentration of the extract increases, the FFA of RPKO gradually decreases. The FFA of oil containing MALE was lower than oil which contained no additive at all (0 ppm [control]).

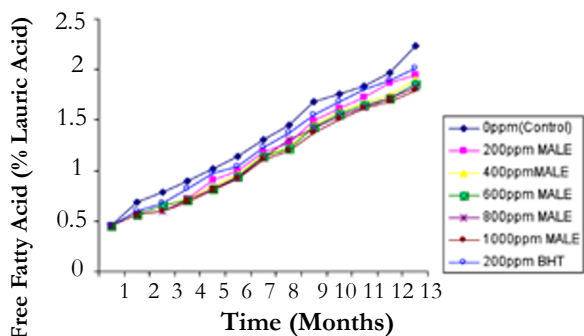


Fig. 1: Free fatty acid of refined palm kernel oil stored with methanol African lettuce extract (MALE) and BHT for twelve months

Figure 2 depicts Free Fatty Acid (FFA) of RPKO stored with Water African Lettuce (WALE) and Butylatedhydroxytoluene (BHT) for twelve months. The FFA of oil sample which contained no additive was higher than oil sample that contained WALE. The FFA of oil containing WALE was lower than the FFA of oil containing 200 ppm BHT. The FFA of the oil sample containing WALE slightly decreased as the concentration of WALE in the oil increased.

The Acid Value (AV) of Refined Palm Kernel Oil stored with Methanol African Lettuce Extract (MALE) and BHT for twelve months is shown in Figure 3. The trend observed is similar to that of Figure 1 above, only that the acid values obtained were higher than that of free fatty acid. All the varying concentrations of MALE were effective

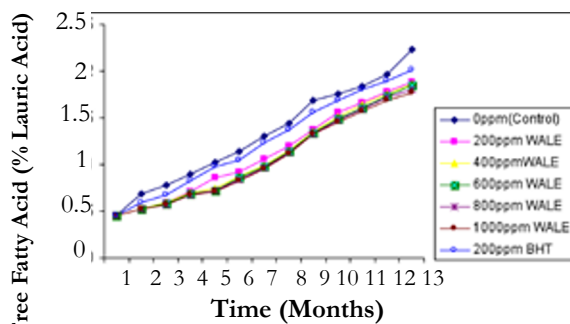


Fig. 2: Free fatty acid of refined palm kernel oil stored with water African lettuce extract (WALE) and BHT for twelve months

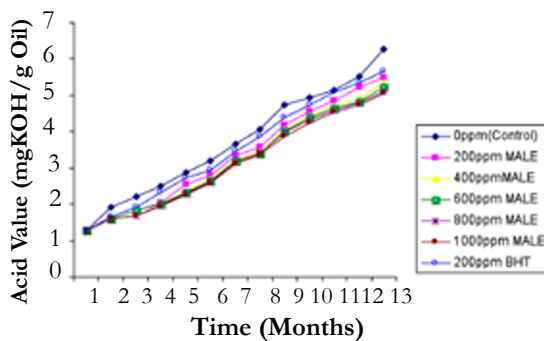


Fig. 3: Acid value of refined palm kernel oil stored with methanol African lettuce extract (MALE) and BHT for twelve months

in lowering the acid value of refined palm kernel oil than 200 ppm BHT. The ability of MALE to reduce the acid value of RPKO increased as the concentration of the extract increased. The acid value of oil is a measure of extent of decomposition of glyceride in oil as caused by lipase enzymes and water (Ihekoronye and Ngoddy, 1985; Cocks and Rede, 1966). Figure 4 depicts Acid Value of Refined Palm Kernel Oil stored with Water African Lettuce Extract (WALE) and BHT for twelve months. The acid value of RPKO that contained no additive was higher than oil samples that contained additives. As the concentration of WALE increased in the oil sample, the acid value of the oil decreased remarkably. At 200 ppm concentration BHT was not able to decrease the acid value of RPKO as African Lettuce extract did.

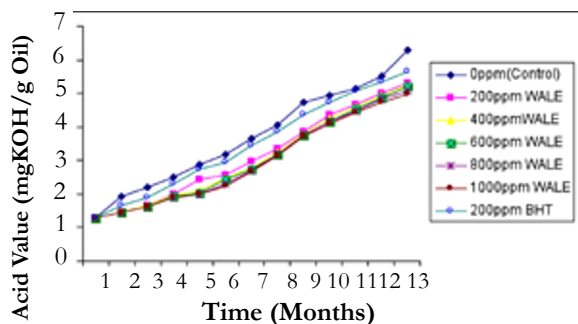


Fig. 4: Acid value of refined palm kernel oil stored with water African lettuce extract (WALE) and BHT for twelve months

Figure 5 reveals the Peroxide Value (PV) of Refined Palm Kernel Oil stored with Methanol African Lettuce Extract (MALE) and Butylatedhydroxytoluene (BHT) for twelve months. The trend observed was in agreement with the observations reported by Amir *et al.* for the plot of peroxide value of soybean oil mixed with pistachio hull extract (Zalejska-Fiolka, 2001), for the plot of peroxide value of oxidation process of

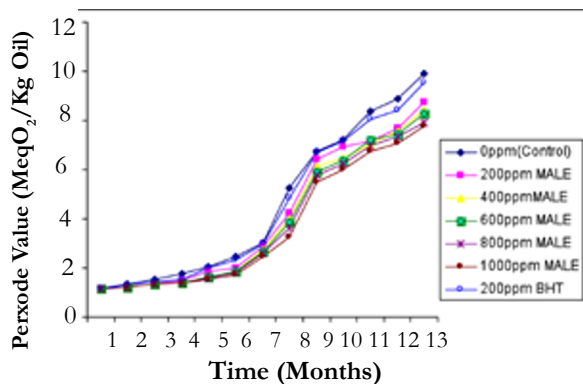


Fig. 5: Peroxide value of refined palm kernel oil stored with methanol African lettuce extract (MALE) and BHT for twelve months

edible oils mixed with garlic extract (Maskan and Karatas, 1998) for the plot of peroxide value of pistachio nut. All the additives lowered peroxide value of RPKO. All the varying concentrations of MALE were more effective than 200 ppm BHT in lowering peroxide value of refined palm kernel oil. RPKO containing 1000 ppm of MALE and WALE consistently maintained the lowest peroxide value for the period of oil storage. Peroxide value (PV) of oil is a measure of primary products of oil oxidation (Rossel, 1994). Peroxide Value of Refined Palm Kernel Oil stored with Water African Lettuce Extract (WALE) and Butylatedhydroxytoluene (BHT) for twelve months is shown in Figure 6. The trend with methanol extract was similar to that of the water extract. The 1000 ppm WALE was able to reduce peroxide value most in RPKO. RPKO mixed with 200 ppm 1000 ppm WALE had lower peroxide value than oil sample mixed with 200 ppm BHT. Generally, the peroxide value of refined palm kernel oil gradually decreased as the concentration of MALE and WALE increased in the oil sample for the twelve months of storage.

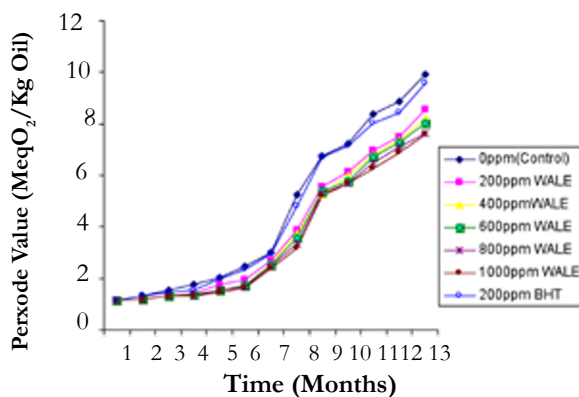


Fig. 6: Peroxide value of refined palm kernel oil stored with water African lettuce extract (WALE) and BHT for twelve months

Table 2 shows the mean values of FFA, AV and PV of refined palm kernel oil stored with varying concentrations of methanol and water African Lettuce extract and 200 ppm BHT for a period of twelve months. The addition of methanol and water extracts of African Lettuce to RPKO resulted in lower FFA, AV and PV of oil samples compared to those with 200 ppm BHT. However, water extract African Lettuce gave lower values of FFA, AV and PV in oil sample than its methanol extract. Free Fatty Acid and Acid Value of lipids are used to measure the hydrolytic rancidity (Rehab, 2010; Farag *et al.*, 2006; Farag *et al.*, 2003; Ihekoronye and Ngoddy, 1985). The higher the values of FFA and AV of any lipid, the higher the degree of hydrolytic rancidity that set in (Arawande and Amoo, 2009). The FFA and AV of RPKO containing different

concentrations of both MALE and WALE (expect 600 ppm and 800 ppm MALE) were significantly different at $P < 0.05$. The Peroxide Values of RPKO containing methanol and water African Lettuce extracts at varying concentrations were lower than RPKO that contained 200 ppm BHT. The PV of RPKO containing 800 ppm MALE and 400 ppm WALE as well as 800 ppm and 1000 ppm WALE were not significantly different at $P < 0.05$. The peroxide value of oil samples decreased progressively as the concentration of additives increased. Peroxide Value being a measure of oxidative rancidity of oil and the lower the PV value the better is the oil quality (Amir *et al.*, 2005; Ihekoronye and Ngoddy, 1985). Water African Lettuce Extract is more effective in combating oxidative rancidity of RPKO than Methanol African Lettuce Extract.

Table 2: Mean value of some selected quality properties of refined palm kernel oil stored with varying concentration of methanol and water african lettuce extract and 200 ppm 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)	1.323 ± 0.548 ^b	3.717 ± 1.541 ^b	4.592 ± 3.243 ^s
200 ppm MALE	1.185 ± 0.512 ^f	3.329 ± 1.440 ^s	4.085 ± 2.870 ^e
400 ppm MALE	1.147 ± 0.488 ^c	3.223 ± 1.372 ^f	3.927 ± 2.788 ^{ed}
600 ppm MALE	1.132 ± 0.477 ^{cd}	3.182 ± 1.342 ^{ed}	3.873 ± 2.757 ^d
800 ppm MALE	1.125 ± 0.477 ^{cd}	3.138 ± 1.330 ^{ed}	3.761 ± 2.659 ^e
1000 ppm MALE	1.101 ± 0.464 ^d	3.093 ± 1.303 ^d	3.626 ± 2.567 ^{ab}
200 ppm WALE	1.121 ± 0.496 ^c	3.150 ± 1.393 ^e	3.858 ± 2.722 ^d
400 ppm WALE	1.087 ± 0.492 ^c	3.054 ± 1.381 ^c	3.715 ± 2.645 ^e
600 ppm WALE	1.074 ± 0.485 ^b	3.015 ± 1.359 ^b	3.654 ± 2.600 ^b
800 ppm WALE	1.061 ± 0.476 ^{ab}	2.975 ± 1.342 ^b	3.561 ± 2.506 ^a
1000 ppm WALE	1.050 ± 0.466 ^a	2.943 ± 1.315 ^a	3.487 ± 2.463 ^a
200 ppm BHT	1.240 ± 0.524 ^s	3.484 ± 1.472 ^s	4.421 ± 3.134 ^f

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.

MALE = Methanol African Lettuce Extract; WALE = Water African Lettuce Extract, BHT = Butylated hydroxyl-toluene

Conclusion

Both methanol and water extracts of African Lettuce exhibited antioxidant activity against hydrolytic and oxidative rancidity of refined palm kernel oil stored in white transparent plastic bottles. The antioxidant activity of both extracts in refined palm kernel oil was higher than that of 200 ppm BHT but Water African Lettuce Extract proved superior in improving both hydrolytic and oxidative stability of refined palm kernel oil stored in plastic bottles than methanol extract.

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