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The Antioxidative Effect of Chaya Leaf Extract on Refined Soybean Oil

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Abstract: The antioxidative effect of methanol and water chaya leaf extracts on Refined Soybean Oil (RSBO) stored for twelve months at room temperature (27^{0}C–33^{0}C) was determined by monitoring the colour, refractive index, free fatty acid (FFA), acid value (AV), and peroxide value (PV). Extracts of chaya leaf were prepared by separately dissolving dried, ground, and sieved orange peel into acetone, chloroform, ethylacetate, methanol, and water in ratio 1:10 for seventy-two hours. The methanol chaya leaf extract (MCLE) and water chaya leaf extract (WCLE) were separately added at varying concentrations (200 ppm to 1000 ppm) to RSBO. The oil was also dosed with 200 ppm Butylated Hydroxy Toluene (BHT). Maximum yield of extracts was obtained with water (14.33±1.10%) and methanol (12.56±0.98%). The colour and refractive index of RSBO containing the extracts ranged between 10.0–18.5 units and 1.470–1.472 respectively whereas the colour and refractive index of RSBO with no additive (0 ppm) and 200 ppm BHT were 8.0–10.0 units and 1.470 accordingly. The FFA and AV of RSBO containing methanol (expect 200 ppm) and water chaya leaf extracts were lower than RSBO containing no additive and 200 ppm BHT. The PV of RSBO containing chaya leaf extracts was lower than PV of RSBO that contained no additive and 200 ppm BHT. The methanol and water chaya leaf extracts are more effective than Butylated Hydroxy Toluene in combating both hydrolytic and oxidative rancidity of RSBO.

Keywords: Chaya Leaf Extracts, BHT, Refined Soybean Oil, Antioxidant, Quality Characteristics

Introduction

The health problems associated with the indiscriminate consumption of lipids or lipid containing foods in recent decades have been wearisome to concerned food scientists. These health hazards are not unconnected with the degradation processes that are inherent in lipids and lipid containing foods known as rancidity. The rancidity of oils which reduces the shelf life has been prevented by the use of synthetic chemicals called synthetic antioxidants. Examples of such chemicals are Butylated Hydroxy Toluene (BHT), Butylated Hydroxy Anisole (BHA), Tertiary Butylated Hydroxy Quinone (TBHQ) and Propyl Gallate (PG) (Amir et al. 2005; Ullah et al. 2003; Malecka 2002). These chemicals have been proven effective even at very small concentration but it has been equally found out that they pose more serious health challenge owing to their carcinogenicity, toxicity and mutagenicity at the small proportion of their usage. As a result of this, their usage as food additives is not encouraged in international markets (Erol et al. 2004; Murkovic 2003; Malecka 2002; Carrasquerro et al. 1998; Frankel 1996; Mahdavi and Salunke 1995; Lehman et al. 1995; Naimiki 1990 and Ito et al. 1986).

Consequently, there have been provoking interests in searching for safer and cheaper antioxidants from natural sources. Identification and usage of some solvent extracts from plants as antioxidants have been reported in very recent times (Rehab 2010; Arawande and Komolafe 2010; Arawande and Abitogun 2009; Kanaze et al. 2008; Akinmoladun et al. 2007 Kroyer 2004; Kuti and Konumi 2004, Abdalla and Roozen 1999; Marinova and Yanishlieva 1997).

Chaya leaf is botanically known as *Cnidoscolus acontifolus* and it is an ever green leafy vegetable that is available throughout the year. It is a fast growing perennial shrub, native to the Yucatan Peninsula of Mexico and Central American (Sarmiento et al. 2003). The English names of the plant are tree spinach, treadsifly and cabbage star while its Spanish names are chaya, copapayo and papayeulo and its French names is manioc batard. Jatropha aconitifolia is its Latin

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name (Oboh 2005; Burkilli 1995). The leaves of the plant have been reported that it was very rich in total phenolic compound and flavonoids (kaempferol-3-O-glycosides and quercetin-3-O-glycosides) (Joseph and Hima 2004)

Therefore the focus of this research is to obtain extracts from chaya leaf using different solvents; investigating the antioxidative potential of two highest solvent yield extracts at varying concentrations (200ppm–1000ppm) on refined soybean oil; determining the effect of the extracts on colour and refractive index of the oil as well as comparing the antioxidant activities of the extracts with that of Butylated Hydroxy Toluene (200ppm BHT) by monthly monitoring their free fatty acid (FFA), acid value (AV) and peroxide value (PV) for twelve months.

Materials and Methods

Sources of Materials

Chaya leaf (stems and leaves) was obtained from open land near an ancient building in Iyere Owo, Ondo-State, Nigeria. The refined soybean oil was obtained before being fortified with vitamin A at JOF Ideal Family Farms Limited, Owo, Ondo-State, Nigeria.

Preparation and Extraction of Chaya Leaf

The stems and leaves of Chaya leaf were rinsed in water, cut into smaller pieces for easy sun drying. The dried plant parts were ground using electric blending machine and sieved with 40mm mesh size. The powdery sample was packed into a black polyethene bags prior to extraction.

Ten grams of the powder sample were weighed into five cleaned and dried reagent bottles; and 100ml of each solvent (methanol, ethyl acetate, acetone, water, and chloroform) was separately added to each bottle and left for seventy-two hours during which it was intermittently shaken on a shaking orbit machine. The mixture was filtered through a 0.45µm Nylon membrane filter. The extracts were evaporated to dryness under reduced pressure at 40^0C by a rotary evaporator. Weight of extract obtained was used to calculate the percent yield of extract in each solvent (Arawande and Komolafe 2010; Amir et al. 2005).

Addition of Additives to Refined Soybean Oil

Methanol and Water extracts of Chaya leaf at concentration of 200ppm (0.02g per 100mL oil) to 1000ppm (0.10g per 100mL oil) were separately added to Refined Soybean Oil (RSBO) contained in white transparent plastic bottles of equal capacity and they were thoroughly shaken for proper mixing. RSBO containing 200ppm BHT (0.02g per 100mL oil) and that which contained no additive (0ppm=control) were also set up. Each container was appropriately labeled and stored in an open place at room temperature ranging from 27^0C to 33^0C.

Physical and Chemical Analysis

As soon as the setup is done, the colour of the oil sample was determined as described by AOCS 2004 method using Lovibond Tintometer (Model 520). The refractive index was also determined using Abbe's Refractometer at 40^0C (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

Table1: Extractive Value (% Yield) of Chaya Leaf

Solvent	*Extractive Value (% Yield)
Acetone	6.17^{b} ±0.21
Chloroform	4.98^{a} ±0.13
Ethylacetate	6.54^{b} ±0.18
Methanol	12.56^{c} ±0.98
Water	14.33^{c} ±1.10

Note: Within 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 triplicate determination ± Standard Deviation.

The extractive values (% yield) of acetone, chloroform, ethyl acetate, methanol and water extracts of chaya leaf are shown in Table 1. The extractive value obtained using water as solvent was the highest (14.33 ± 1.10%) while that of chloroform was the lowest (4.98 ± 0.13%). The yield of methanol extract (12.56 ± 0.98%) was next to water. The extractive values using acetone and ethylacetate were 6.17 ± 0.21% and 6.54 ± 0.18%, respectively. There was no significant difference at $P < 0.05$ at the extractive values obtained for methanol and water as well as for acetone and ethylacetate. The amount of extracts obtained increase as the polarity of the solvent increases. According to the rule of Thumb, natural antioxidants are polar compounds (polyphenolics) and they are best extracted using polar solvents (Amir et al. 2005). Chloroform, acetone and ethyl acetate extract yields were about 40–52 % and 34–46% of methanol and water extract yields, respectively.

Table2: Changes in Colour and Refractive Index of Refined Soybean Oil stored with varying concentration of Methanol and Water Chaya Leaf Extract and 200ppm BHT

Concentration of Additive	Colour (Units) in 1 inch cell	Refractive Index at $40^{0}C$
0ppm(No additive)	1R+3Y=8.0	1.470
200ppm MCLE	1R+7Y=12.0	1.470
400ppm MCLE	1R+7Y=12.0	1.471
600ppm MCLE	1.2R+9Y=15.0	1.471
800ppm MCLE	1.5R+10Y=17.5	1.472
1000ppm MCLE	1.5R+11Y=18.5	1.472
200ppm WCLE	1R+5Y=10.0	1.470
400ppm WCLE	1R+6Y=11.0	1.470
600ppm WCLE	1R+6Y=11.0	1.470
800ppm WCLE	1R+6Y=11.0	1.470
1000ppm WCLE	1R+7Y=12.0	1.471
200ppm BHT	1R+5Y=10.0	1.470

Note: MCLE= Methanol Chaya Leaf Extract; WCLE= Water Chaya Leaf Extract, BHT= Butylated Hydroxyl Toluene, R = Red Slide; Y = Yellow Slide

The changes in colour and refractive index of refined soybean oil stored with varying concentration of methanol and water chaya leaf extract and 200ppm BHT is presented in Table 2. Colour of edible oils is an important physical quality factor that influences consumer decision of acceptance or otherwise (Ihekoronye and Ngoddy 1985). The lower the colour unit, the more acceptable and attractive the oil becomes. The colour unit is measured as red and yellow slides by using Lovibond Tintometer in 1 inch cell. It is observed that the addition of methanol and water chaya leaf extract as well as BHT did increase the colour units of refined soybean oil. The oil that contained no additive had a colour unit of 8.0 units. The colour unit of refined soybean oil stored with Methanol Chaya Leaf Extract (MCLE) and Water Chaya Leaf Extract (WCLE) ranged between 12.0 to 18.5 units and 10.0 to 12.0 units respectively; and the oil colour that contained BHT was 10.0 units. In terms of colour, methanol extract of chaya leaf was inferior to water extract because RSBO containing methanol extract had a higher colour unit than that of RSBO containing the water extract. The colour units of RSBO control and that of 200ppm BHT was better than that of RSBO containing water chaya leaf extract. However, it was only addition of 800ppm and 1000ppm MCLE to RSBO that makes its colour above maximum of 15.0units recommended by Standards Organisation of Nigeria (SON 2000). The water and methanol extract of chaya leaf slightly increased the refractive index of RSBO by 0.001 and 0.002. The oil which contained 200ppm BHT as well as the oil which contained no additive had refractive index of 1.470 while RSBO which contained 200ppm–1000ppm MCLE had refractive index of between 1.470 and 1.472; and RSBO containing 200ppm–1000ppm WCLE had refractive index of between 1.470 and 1.471. The value of refractive index of RSBO containing additives was approximately within 1.470 recommended by Standards Organisation of Nigeria (SON 2000).

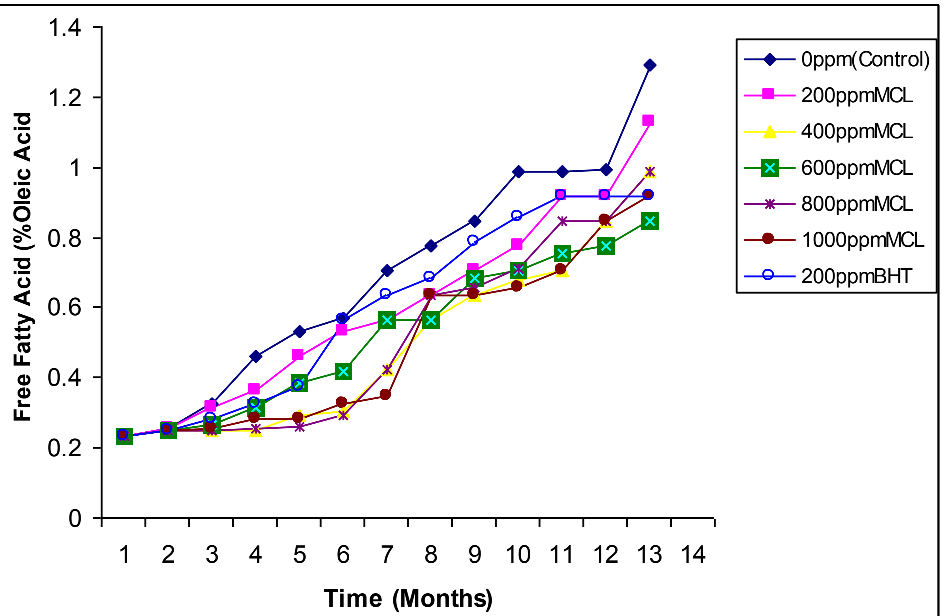

Figure1: Free Fatty Acid of Refined Soybean Oil stored with Methanol Chaya Leaf Extract (MCLE) Extract and BHT for twelve months

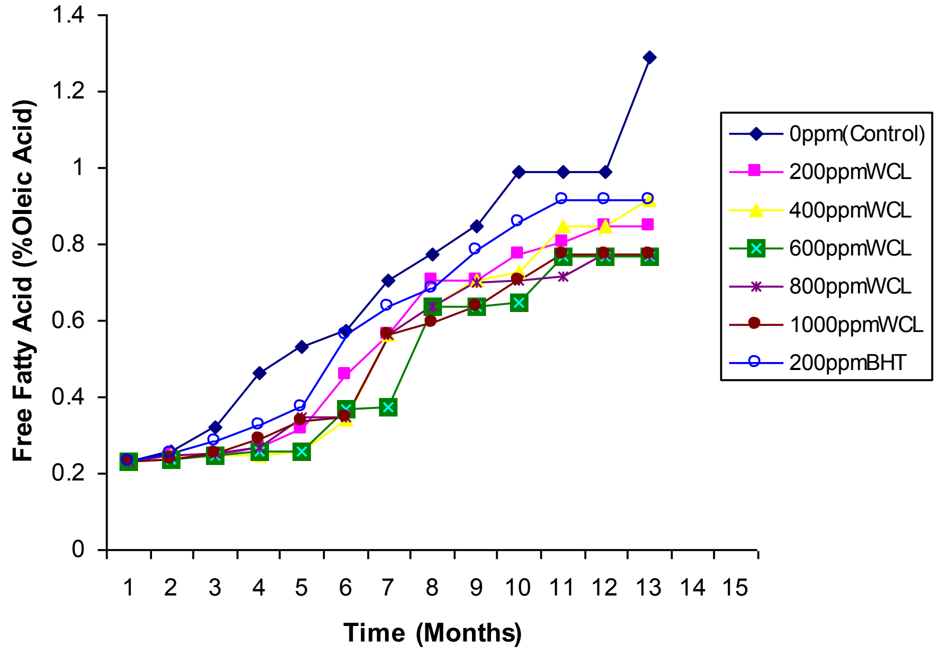

Figure2: Free Fatty Acid of Refined Soybean Oil stored with Water Chaya Leaf Extract (WCLE) Extract and BHT for twelve months

The Free Fatty Acid (FFA) of Refined Soybean Oil (RSBO) stored with Methanol Chaya Leaf Extract (MCLE) and Butylated Hydroxy Toluene (BHT) for twelve months is depicted in Figure 1. It was observed that RSBO containing additives (MCLE and BHT) had lower FFA values than oil sample which contained no additive (control).Within the first ten months of storage, the FFA of RSBO gradually decreases as the concentration of the extract increases. Figure 2 shows Free Fatty Acid (FFA) of RSBO stored with Water Chaya Leaf Extract (WCLE) and Butylated Hydroxy Toluene (BHT) for twelve months. The FFA of oil sample which contained no additive was higher than oil sample that contained WCLE and BHT. Although, the FFA of oil containing WCLE was lower than the FFA of oil containing 200ppm BHT throughout the period of storage.

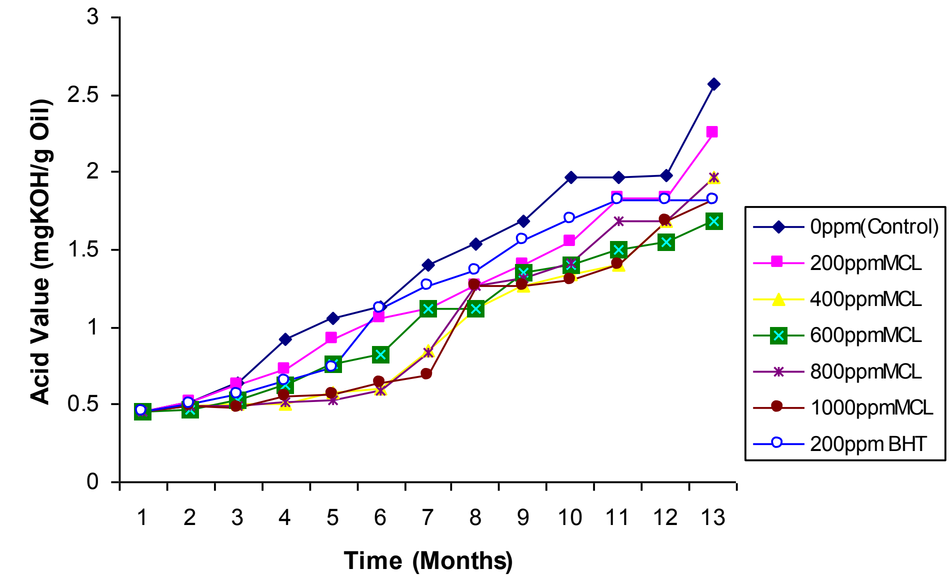

Figure3: Acid Value of Refined Soybean Oil stored with Methanol Chaya Extract (MCLE) Extract and BHT for twelve months

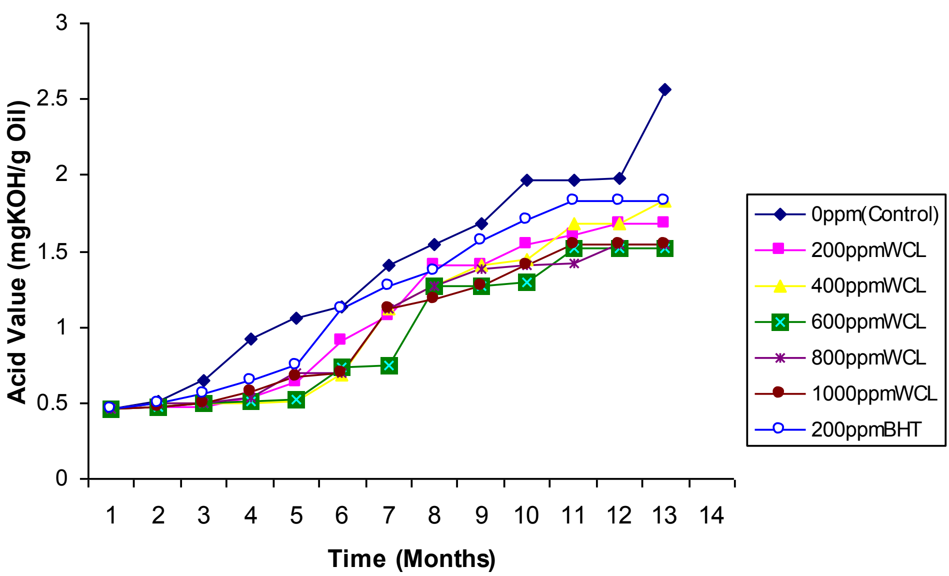

Figure4: Acid Value of Refined Soybean Oil stored with Water Chaya Leaf Extract (WCLE) Extract and BHT for twelve months

The Acid Value (AV) of Refined Soybean Oil stored with Methanol Chaya Leaf Extract (MCLE) and BHT for twelve months is shown in Figure 3. The trend observed is similar to that of Figure1 above, only that the acid values obtained were higher in value than that of free fatty acid. All the varying concentrations of MCLE were effective in lowering the acid value of refined soybean oil than 200ppm BHT. The ability of MCLE to reduce acid value of RSBO 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). The Acid Value of RSBO stored with Water Chaya Leaf Extract (WCLE) and BHT for twelve months is shown in Figure 4. The acid value of RSBO that contained no additive was higher than oil samples that contained additives (WCLE and BHT). As the concentration of WCLE increased in the oil sample, the acid value of the oil decreased remarkably. RSBO containing concentration (200ppm–1000ppm) of WCLE had lower acid value than RSBO containing 200ppm BHT throughout the storage period.

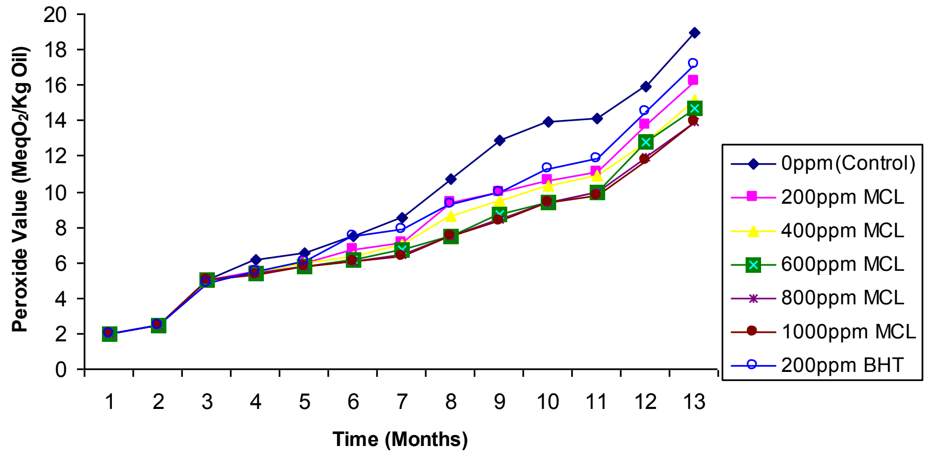

Figure5: Peroxide Value of Refined Soybean Oil stored with Methanol Chaya Leaf Extract (MCLE) Extract and BHT for twelve months

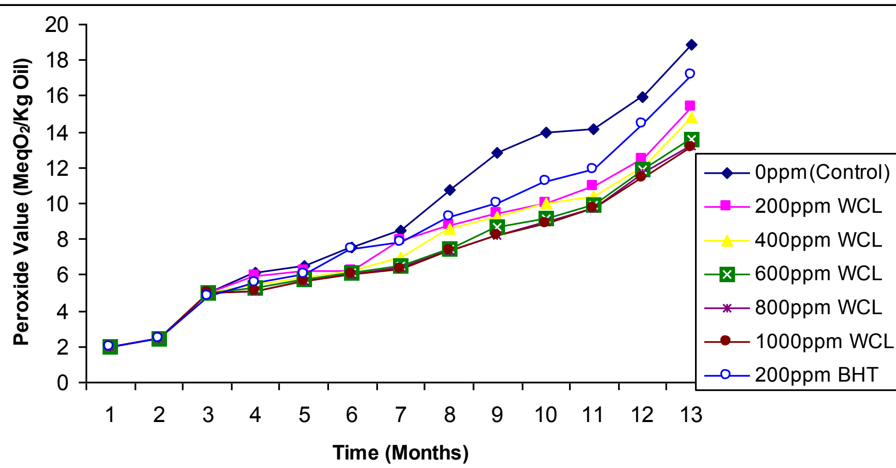

Figure6: Peroxide Value of Refined Soybean Oil stored with Water Chaya Leaf Extract (WCLE) Extract and BHT for twelve months

The Peroxide Value (PV) of Refined Soybean Oil stored with Methanol Chaya Leaf Extract (MCLE) and Butylated Hydroxy Toluene (BHT) for twelve months is presented in Figure 5. The trend observed was in agreement with the observations reported by Amir et al. 2005 for the plot of peroxide value of soybean oil mixed with pistachio hull extract; Zalejiska-Fiolka 2001 for the plot of peroxide value of oxidation process of edible oils mixed with garlic extract and Maskan and Karatas 1998 for the plot of peroxide value of pistachio nut. All the additives lowered peroxide value of RSBO. All the varying concentrations of MCLE were more effective than 200ppm BHT in reducing peroxide value of refined soybean oil. Peroxide value (PV) of oil is a measure of primary products of oil oxidation (Rossel 1994). Peroxide Value of Refined Soybean Oil stored with Water Chaya Leaf Extract (WCLE) and Butylated Hydroxy Toluene (BHT) for twelve months is shown in Figure 6. The trend with methanol extract was similar to that of the water extract. The 1000ppm WCLE was able to reduce peroxide value most in RSBO. RSBO mixed with 200ppm–1000ppm WCLE had lower peroxide value than oil sample mixed with 200ppm BHT. Generally, the peroxide value of refined soybean oil gradually decreased as the concentration of MCLE and WCLE increased in the oil sample for the twelve months of storage.

Table 3: Mean Value of Some Selected Quality Properties of Refined Soybean Oil stored with varying concentration of Methanol and Water Chaya Leaf Extract and 200ppm BHT over a period of twelve months

Concentration of Additive	*Free Fatty Acid (FFA) (% Oleic acid)	*Acid Value (AV) (mgKOH/gOil)	*Peroxide Value (PV) ($meqO_2$/KgOil)
0ppm(No additive)	0.689^{c} ±0.327	1.372^{c} ±0.653	9.6597^{c} ±5.275
200ppm MCLE	0.600^{b} ±0.800	1.195^{b} ±0.557	8.138^{b} ±4.173
400ppm MCLE	0.493^{a} ±0.258	0.982^{a} ±0.514	7.808^{a} ±3.869
600ppm MCLE	0.517^{ab} ±0.222	1.032^{ab} ±0.441	7.443^{a} ±3.684
800ppm MCLE	0.511^{ab} ±0.278	1.01^{ab} ±0.553	7.265^{a} ±2.452
1000ppm MCLE	0.489^{a} ±0.248	0.972^{a} ±0.496	7.205^{a} ±3.422
200ppm WCLE	0.540^{ab} ±0.252	1.067^{ab} ±0.506	7.924^{a} ±3.826
400ppm WCLE	0.525^{ab} ±0.268	1.044^{ab} ±0.534	7.618^{a} ±3.669
600ppm WCLE	0.476^{a} ±0.227	0.948^{a} ±0.452	7.209^{a} ±3.380
800ppm WCLE	0.505^{a} ±0.223	1.005^{a} ±0.444	7.073^{a} ±3..278
1000ppm WCLE	0.502^{a} ±0.224	0.998^{a} ±0.445	7.011^{a} ±3.225
200ppm BHT	0.596^{b}±0.273	1.185^{b} ±0.543	8.477^{bc}±4.476

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. MCLE= Methanol Chaya Leaf Extract; WCLE= Water Chaya Leaf Extract, BHT= Butylated Hydroxy Toluene

Table 3 depicted the mean values of FFA, AV and PV of Refined Soybean Oil stored with varying concentration of methanol and water chaya leaf extract and 200ppm BHT over a period of twelve months. The addition of additives (MCLE, WCLE and BHT) to RSBO lowered the FFA, AV and PV of Refined Soybean Oil during the twelve months of storage. RSBO containing MCLE and WCLE except 200ppm MCLE gave lower values of FFA and AV than RSBO containing 200ppm BHT. 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; Shaker 2006; Carelli et al. 2005; Tian and White 1994). The oil samples containing additives were significantly different at P<0.05 in FFA and AV compared to oil sample which contained no additive (control). There was also significantly difference at P<0.05 in FFA and AV of oil samples containing chaya leaf extract (except 200ppm MCLE) and BHT. However, there was no significant difference at P<0.05 in FFA and AV containing 400ppm MCLE 1000ppm MCLE, 600ppm to 1000ppm WCLE. The peroxide value (PV) of RSBO containing MCLE, WCLE and BHT were lower than the control and significantly different at P<0.05 during the twelve month storage. The PV of RSBO decreased progressively as the concentration of MCLE and WCLE increased from 200ppm to 1000ppm. WCLE gave lower values of PV than MCLE in all the sample treatments. And there was no significant difference at P<0.05 in PV of MCLE and WCLE (except in 200ppm MCLE). Peroxide Value being a measure of oxidative rancidity of oil and the lower the PV value the better is the oil quality (Shaker 2006; Carelli, et al. 2005; Amir et al. 2005; Ruger et al. 2002; Ihekoronye and Ngoddy 1985). It was also noticed that the chaya leaf extract was superior to BHT in combating oxidative rancidity of RSBO.

Conclusion

The use of methanol and water extracts of chaya leaf as additives in refined soybean oil improved the shelf life of the oil. The plant extracts exhibited antioxidant activity against both hydrolytic and oxidative rancidity of refined soybean oil stored in white transparent plastic bottles. The antioxidant activity of both extracts in refined soybean oil was higher than that of 200ppm BHT, although that of water chaya leaf extract proved superior to methanol chaya leaf extract in improving oxidative stability of refined soybean oil stored in plastic bottles. Further research work can be conducted using higher concentrations of these extracts on soybean oil and other oils store in plastic, glass and tin containers.

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