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Antioxidative effect of wild lettuce (*Launaea taraxaciafolia*) on stability of refined soybean oil

Arawande, Jacob Olalekan[1*], Komolafe, Eniayo Ayodeji[2] and Ijitona, Olugbenga Olufemi[3]

[1]Department of Science Laboratory Technology, Rufus Giwa Polytechnic, P.M.B. 1019, Owo, Ondo-State, Nigeria, [2]Department of Food Science and Technology, Rufus Giwa Polytechnic, P.M.B. 1019, Owo, Ondo-State, Nigeria, [3]Department of Chemical Sciences, Osun State University, Osogbo, Osun- State, Nigeria

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Wild Lettuce extracts as a natural source of antioxidant was evaluated during twelve months storage of Refined Soybean Oil (RSBO) in white transparent plastic bottles at room temperature (27^0C-33^0C). Extracts of Wild Lettuce were prepared by separately dissolving dried, ground and sieved Wild Lettuce into acetone, chloroform, ethylacetate, methanol and water in ratio 1:10 for 72hours. Maximum yield of extracts were obtained with methanol (12.40±0.14%) and water (10.92±0.22%). The Methanol Wild Lettuce Extract (MWLE) and Water Wild Lettuce Extract (WWLE) were separately added at varying concentration (200ppm to 1000ppm) to RSBO. Another set of RSBO which contained no additive (0ppm (control)) and 200ppmBHT 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 RSBO samples were monitored monthly using standard methods for a period of twelve months. The colour of RSBO containing MWLE (12.0-16.0units) was higher than RSBO containing WWLE (10.0-12.0units) while colour of RSBO sample containing no additive (0ppm) was 8.0units and 10.0units for RSBO containing 200ppm BHT. The refractive index of RSBO containing MWLE and WWLE were of the same value (1.472) with that of RSBO containing 200ppmBHT. The FFA, AV and PV of RSBO containing MWLE and WWLE were lower than RSBO containing 200ppm BHT. The MWLE is more effective in stabilizing RSBO hydrolytically and oxidatively than WWLE.

Key words: Wild Lettuce Extract, BHT, Refined Soybean Oil, Quality Assurance Tests, Stability

[*] Corresponding author: Arawande, Jacob Olalekan; e-mail: joawande1@yahoo.com

Introduction

Refined Soybean Oil is obtained from crude oil extracted from soybean seeds (*Glycine max*). The crude soybean oil passes through different stages of processing such as degumming, neutralization, bleaching and deodourization before becoming refined vegetable oil. It is well known for its good organoleptic qualities and low level of cholesterol hence it is widely consumed in United State and some other part of the world where the seeds thrive well (Fradin and Day, 2002). In the growing season of 2002-2003, 30.6millon metric tons of soybean oil was produced worldwide, constituting about half of the worldwide edible vegetable oil production (USDA, 2004).

In Nigeria, refined soybean oil serves as one of the commonest and widely consumed vegetable oil. It has an increasing demand which has invariably led to its scarcity. The oil is bought in larger quantity during the last quarter of the year when it is always available and cheaper by oil merchants. In most cases, the oil is hoarded and sold during the second and third quarters of the following year when the price would have been skyrocketed. Over the period of storage, the oil would have been deteriorating in its physical and chemical qualities. Therefore there is need to prevent or reduce the extent of oil deterioration by adding antioxidant that will impede the oil rancidity. Although there are a quite number of known antioxidants (Butylatedhydroxytoluene (BHT), Butylatedhydroxyanisole (BHA), Propylgallate (PG), Citric acid etc) that have been used (Arawande and Abitogun, 2009; Khanahmadi and Janfeshan, 2006; Amir *et al*., 2005; Ullah *et al*., 2003). It has now been established that these known synthetic antioxidants are toxic and carcinogenic therefore their use as food additives are being discouraged in international market (Enrol *et al*., 2004; Carrasquero *et al*., 1998; Tian and White, 1994).This necessitates the need to seek for safer food additives that are of plant origin which will have comparatively the same or higher efficiency in combating oil rancidity. The uses of different plant extracts to stabilize sunflower oil have been documented (Rehab, 2010; El Anany, 2007; Shaker, 2006).

Wild Lettuce is a greenish neglected indigenous leafy vegetable among the Yoruba tribe of Southwest, Nigeria. Though it is also locally cultivated in Senegal, Ghana, Dahomey and Sierra Lone (Adebisi, 2000).It is used as medicine (lactation stimulant, yaws, skeletal structure etc) and food (salad, cooked in soup and sauces). The leaves of the plant are used to feed cows-in-milk to increase the milk yield and to feed sheep and goat mixed with natron to produce multiple births (Michael, 2002). The various ways through which the plant is being used suggest that it has phytochemicals of physiological importance. Wild Lettuce is taxonomically known as *Launaea taraxacifolia* or

Lactuca taraxacifolia. The plant is called various names in Nigeria and other West Africa countries. In Nigeria, Hausa tribe call it " Namijin dayii, Nomen barewa and NonanBarya" while Yoruba tribe call it " Yanrin, Efo- Yanrin and Odundun Odo" It is called "Niontoto" in Benin (Dahomey), "Valovalo" in Senegal and " Efo- nyori, Kipo and Bekuhoa-pomboE" in Sierre Lone (Schippers, 2000).

The aim of this research was to determine the extractive values of Wild Lettuce using different solvents, to investigate the antioxidant potential of two highest solvent yield extracts at varying concentration (200ppm-1000ppm) on refined soybean oil, to determine the effect of the extracts on colour and refractive index of the oil as well as to compare the antioxidant activities of the extracts with that of Butylatedhydroxytoluene (200ppmBHT) by monthly monitoring their Free Fatty Acid (FFA), Acid Value (AV) and Peroxide Value (PV) for a period of twelve months.

Materials and methods

Sources of Materials

Wild Lettuce (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 Wild Lettuce

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

Ten gram of the powdery sample was weighed into five cleaned and dried reagent bottles; and 100ml of each solvent (methanol, ethhylacetate, acetone, water and chloroform) was separately added to each bottle and left for 72hours during which it was intermittedly 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 Wild Lettuce 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 set up 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 discussions

The extractive values (%yield) of acetone, chloroform, ethylacetate, methanol and water extracts of Wild Lettuce are shown in Table1. The extractive value of Wild Lettuce using methanol as solvent was highest (12.40±0.14%) while that of chloroform was lowest (3.48±0.12%). The yield of water extract (10.92±0.22%) was next to methanol. The extractive values using acetone and ethylacetate were 4.16±0.17% and 4.43±0.11% respectively. The Wild Lettuce extract yield in chloroform, methanol and water are significantly different at $P<0.05$ while the yields in acetone and ethylacetate were not significantly different at $P<0.05$. The amount of obtained extracts increased as the polarity of the solvent increased. 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 ethylacetate

yield about 28-36% and 32-41% of methanol and water extract yields respectively.

Table 1. Extractive Value (% Yield) of Wild Lettuce

Solvent	*Extractive Value (% Yield)
Acetone	4.16 ± 0.17^{b}
Chloroform	3.48 ± 0.12^{a}
Ethylacetate	4.43 ± 0.11^{b}
Methanol	12.40 ± 0.14^{d}
Water	10.92 ± 0.22^{c}

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.

Table 2. Changes in Colour and Refractive Index of Refined Soybean Oil stored with varying concentration of Methanol and Water Wild Lettuce 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	1.471
200ppm MWLE	1R+7Y=12.0	1.472
400ppm MWLE	1R+7Y=12.0	1.472
600ppm MWLE	1.2+9Y=15.0	1.472
800ppm MWLE	1.2+10Y=16.0	1.472
1000ppm MWLE	1.2R+10Y=16.0	1.472
200ppm WWLE	1R+5Y=10.0	1.472
400ppm WWLE	1R+6Y=11.0	1.471
600ppm WWLE	1R+6Y=11.0	1.472
800ppm WWLE	1R+6Y=11.0	1.472
1000ppm WWLE	1R+7Y=12.0	1.472
200ppm BHT	1R+5Y=10.0	1.472

MWLE= Methanol Wild Lettuce Extract; WWLE= Water Wild Lettuce 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 Wild Lettuce extract and 200 ppm BHT are shown in Table 2. Colour of vegetable oil is one of the significant physical quality factors of acceptance and it influences consumer decision. It is a psychological interpretation of a physiological response by the eye and brain to the physical stimulus of light radiation at different wavelength (Ihekoronye and Ngoddy, 1985). The most acceptable colour of edible oil is golden yellow and 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 1inch cell. It is observed that the addition of additives (Methanol Wild Lettuce Extract (MWLE), Water Wild Lettuce Extract (WWLE) and BHT) increased the colour units of refined soybean oil (RSBO) at varying degrees. The colour units increased as the concentration of the extracts increased. RSBO containing 200ppm to 1000ppm MWLE had colour unit ranged from12.0 to 16.0units while it was between 10.0 to 12.0units for RSBO containing 200ppm to 1000ppm WWLE.RSBO containing 200ppm BHT had colour of 10.0units. It can be seen that WWLE and BHT competed favorably well with each other and gave better colour unit in oil than MWLE. The superiority of WWLE to MWLE in terms of colour units may be attributed to the lower extractive value of water in comparison with that of methanol. Water extracted less bioactive matter thereby has less impact on the oil colour than methanol. Refractive index of RSBO containing additives was measured at 40^0C. The water and methanol extract of Wild Lettuce did not change the refractive index of RSBO. The oil which contained no additive had refractive index of 1.471 while RSBO which contained both natural extract and BHT had refractive index of 1.471 and 1.472. Refractive index of edible oil is a measure of the extent of oil adulteration or purity (Cocks and Rede, 1966) hence the addition of WWLE and MWLE to RSBO did not reflect that the oil was adulterated and it had the same refractive index with RSBO containing 200ppmBHT.

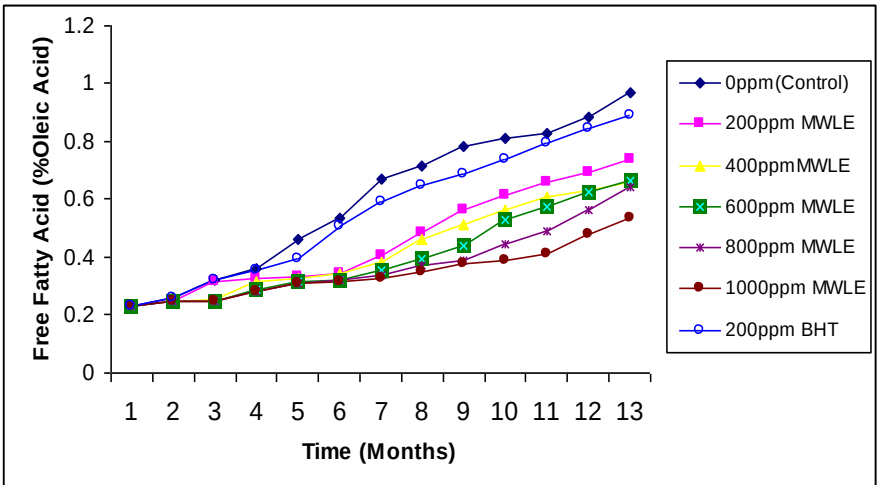

Fig. 1. Free Fatty Acid (FFA) of RSBO stored with MWLE and BHT for twelve months.

Figure 1 depicted Free Fatty Acid (FFA) of RSBO stored with MWLE and BHT for twelve months. It was observed that RSBO containing 200ppm to 1000ppm MWLE had lower FFA values than oil sample containing 200ppmBHT. As the concentration of the extract increased, the FFA of RSBO decreased. The FFA of oil containing MWLE was lower than oil which contained no additive at all (0ppm (control)).

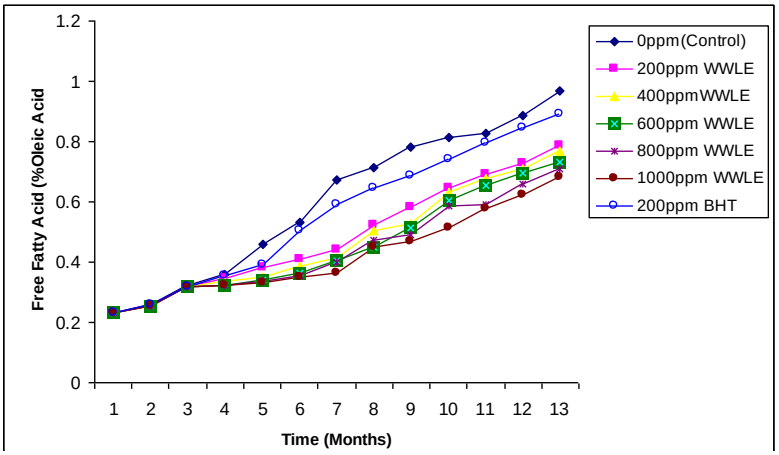

Fig. 2. Free Fatty Acid (FFA) of RSBO stored with WWLE and BHT for twelve months.

Free Fatty Acid (FFA) of RSBO stored with WWLE and BHT for twelve months (Fig.2). The FFA of oil sample which contained no additive was higher than oil sample that contained WWLE. The FFA of oil containing WWLE was lower than the FFA of oil containing 200ppmBHT. The FFA of the oil sample containing WWLE decreased as the concentration of WWLE in the oil increased.

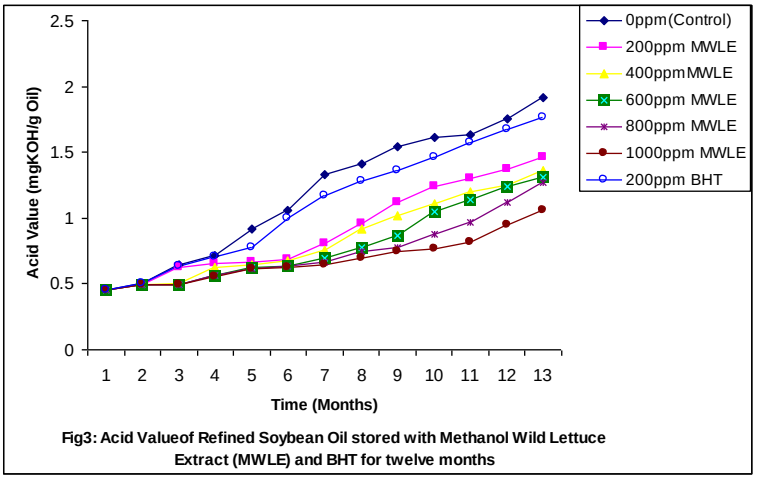

Fig. 3. Acid Value of Refined Soybean Oil Stored with Methanol Wild lettuce Extract (MWLE) and BHT for twelve month.

The Acid Value (AV) of Refined Soybean Oil stored with Methanol Wild Lettuce Extract (MWLE) and BHT for twelve months is shown in Fig 3. The trend observed resemble Fig 1, only the acid values obtained were higher than that of free fatty acid. All the varying concentrations of MWLE were effective in lowering the acid value of refined soybean oil than 200 ppm BHT. The ability of MWLE to reduce acid value of RSBO increased as the concentration of the extract increased.

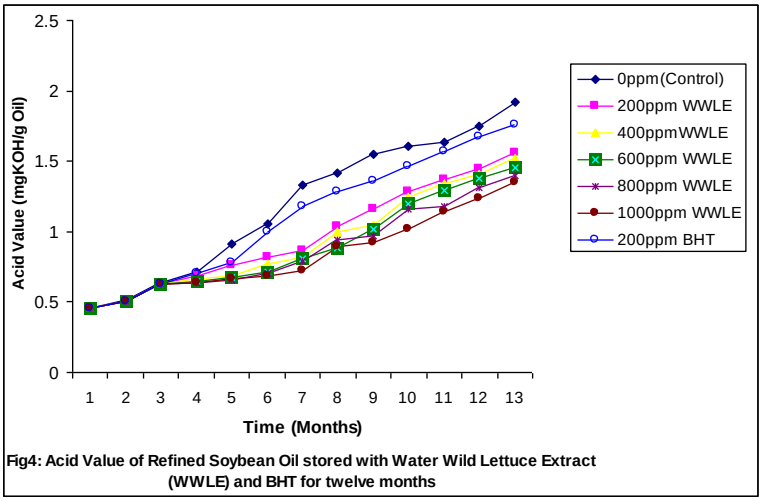

Fig. 4. Acid Value of Refined Soybean Oil stored with Water Wild Lettuce Extract (WWLE) and BHT for twelve months

Acid Value of Refined Soybean Oil stored with Water Wild Lettuce Extract (WWLE) and BHT for twelve months is shown in Fig.4. The acid value of RSBO that contained no additive was higher than oil samples that contained additives. As the concentration of WWLE increased in the oil sample, the acid value of the oil decreased remarkably. 200 ppm BHT was not able to decrease the acid value of RSBO as Wild Lettuce extract did.

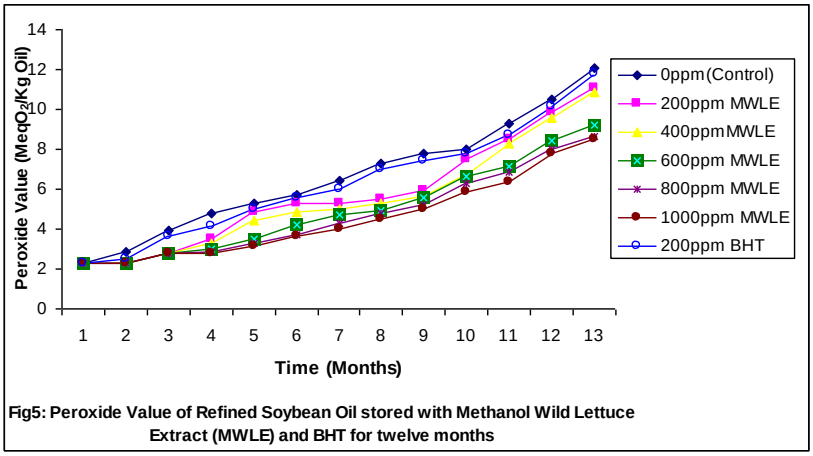

Fig. 5. Peroxide Value of Refined Soybean Oil stored with Methanol Wild Lettuce Extract (MWLE) and BHT for twelve months

The Peroxide Value of Refined Soybean Oil stored with Methanol Wild Lettuce Extract (MWLE) and BHT for twelve months is shown in Fig.5. The trend observation was in agreement with Amir *et al*., 2005. All the additives lowered peroxide value of RSBO. Although, 200 ppm BHT was not affected in lowering peroxide value as MWLE. RSBO containing 1000 ppm consistently maintained the lowest peroxide value for the period of oil storage.

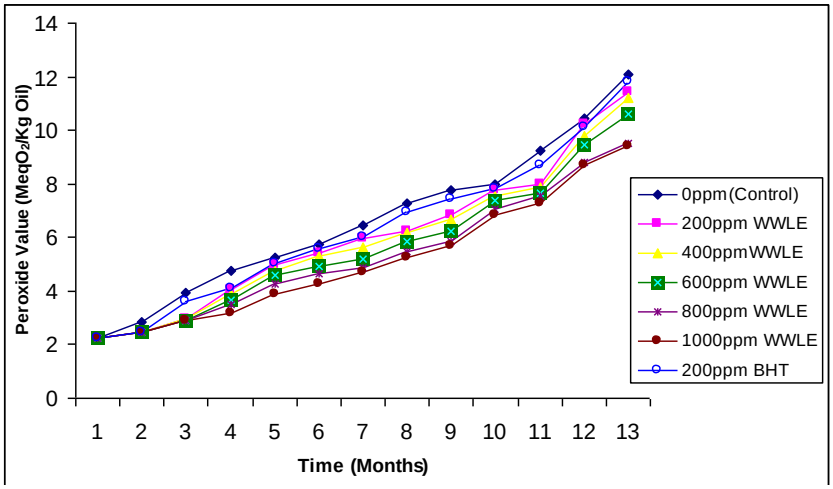

Fig. 6. Peroxide Value of Refined Soybean Oil stored with Water Wild Lettuce Extract (WWLE) and BHT for twelve months

Peroxide Value of Refined Soybean Oil stored with Water Wild Lettuce Extract (WWLE) and BHT for twelve months is depicted in Fig 6. The same trend with methanol extract was observed with water extract. 1000 pppm WWLE was able to reduce peroxide value most in RSBO. RSBO mixed with WWLE had lower peroxide value than oil sample mixed with 200 ppm BHT.

Table 3. Mean Value of Some Selected Quality Properties of Refined Soybean Oil stored with varying concentration of Methanol and Water Wild Lettuce 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)	0.602 ± 0.254^{g}	1.192 ± 0.504^{g}	6.622 ± 2.916^{h}
200ppm MWLE	0.458 ± 0.177^{d}	0.909 ± 0.351^{d}	5.729 ± 2.822^{e}
400ppm MWLE	0.426 ± 0.155^{cd}	0.847 ± 0.313^{cd}	5.482 ± 2.734^{cd}
600ppm MWLE	0.402 ± 0.151^{c}	0.797 ± 0.299^{c}	4.972 ± 2.304^{b}
800ppm MWLE	0.376 ± 0.128^{b}	0.745 ± 0.253^{b}	4.714 ± 2.165^{ab}
1000ppm MWLE	0.347 ± 0.092^{a}	0.686 ± 0.181^{a}	4.530 ± 2.060^{a}
200ppm WWLE	0.488 ± 0.186^{e}	0.966 ± 0.368^{e}	6.037 ± 2.832^{f}
400ppm WWLE	0.470 ± 0.180^{ed}	0.930 ± 0.357^{ed}	5.880 ± 2.737^{e}
600ppm WWLE	0.452 ± 0.171^{d}	0.896 ± 0.339^{d}	5.634 ± 2.625^{d}
800ppm WWLE	0.440 ± 0.157^{cd}	0.872 ± 0.312^{cd}	5.324 ± 2.514^{cd}
1000ppm WWLE	0.422 ± 0.143^{c}	0.835 ± 0.284^{c}	5.132 ± 2.456^{c}
200ppm BHT	0.558 ± 0.230^{f}	1.105 ± 0.460^{f}	6.306 ± 2.889^{g}

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.
MWLE= Methanol Wild Lettuce Extract; WWLE= Water Wild Lettuce Extract, BHT= Butylated hydroxyl toluene

The overall averages of Free Fatty Acid (FFA), Acid Value (AV) and Peroxide Value (PV) for twelve months explain the antioxidative performance of the additive in a comparative manner. The mean value of FFA, AV and PV of refined soybean oil stored with varying concentrations of methanol and water wild lettuce extract and 20 ppm BHT for a period of twelve months are shown in Table 3. The addition of methanol and water extracts of wild lettuce to RSBO resulted in lowering FFA, AV and PV of oil sample than 200 ppm BHT. However, methanol extract gave lower values of FFA, AV and PV in oil sample than water extract. Free Fatty Acid and Acid Value of any lipid are measure of hydrolytic rancidity (Rehab, 2010; Farag *et al*., 2003; Ihekoronye and Ngoddy, 1985). The higher values of FFA and AV of any lipid, the higher degree of hydrolytic rancidity that set-in (Arawande and Amoo, 2009). The FFA and AV of RSBO containing 200ppm MWLE and 600 ppm WWLE were not significantly different at $P<0.05$. The FFA and AV of RSBO stored with 400 ppm MWLE and 800 ppm WWLE were not also significantly different at $P<0.05$.There was no significance difference in FFA and AV of RSBO mixed with 600 ppm MWLE and 1000 ppm WWLE. The Peroxide Values of RSBO containing methanol and wild lettuce extracts at varying concentration were lower than RSBO that contained 200 ppm BHT. The PV of all the oil containing additive (apart from 400 ppm MWLE and 800 ppm WWLE) were significantly different at $P<0.05$. The peroxide value of oil samples decreased progressively as the concentration of additives increased. Peroxide Value is 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). Methanol Wild Lettuce Extract is more effective in combating oxidative rancidity of RSBO than Water Wild Lettuce Extract.

Conclusion

Methanol and water gave the highest yield of wild lettuce extract. Both methanol and water extracts had pronounced antioxidant activity against 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 200 ppm BHT. Further investigation can be conducted by using glass and tin containers as well as using higher concentrations (>1000 ppm) of the plant extract on other edible oils.

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