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Article

Antioxidative Potential of Sweet Orange Peel Extracts on the Stability of Refined Soybean Oil

Jacob Olalekan Arawande [1, 2, *], Isiaka Adekunle Amoo [2], Labunmi Lajide [2]

[1] *Department of Science Laboratory Technology, Rufus Giwa Polytechnic, P.M.B. 1019 Owo, Ondo-State, Nigeria*

[2] *Department of Chemistry, Federal University of Technology, P.M.B. 704 Akure, Ondo-State, Nigeria*

* Author to whom correspondence should be addressed; E-Mail: joawande1@yahoo.com; Tel: +2348034391608.

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Abstract: Sweet orange peel 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 - 33^{o}C). Extracts of sweet orange peel were prepared by separately dissolving dried, ground and sieved orange peel into acetone, chloroform, ethyl acetate, methanol and water in ratio 1:10 for 72 h. Maximum yield of extracts were obtained with water (17.60 ± 0.75%) and methanol (12.82 ± 0.76%). The methanol orange peel extract (MOPE) and water orange peel extract (WOPE) were separately added at varying concentrations (200 - 1000 ppm) to RSBO. Another set of RSBO 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 RSBO samples were monitored monthly using standard methods for a period of twelve months. The colour of RSBO containing MOPE (10.0 - 12.0 units) was higher than RSBO containing WOPE (9.0 - 10.0 units) while colour of RSBO sample containing no additive (0 ppm) was 8.0 units and 9.0 units for RSBO containing 200 ppm BHT. There was no remarkable difference in refractive index of RSBO containing MOPE and WOPE (1.470 - 1.471) with that of RSBO containing 200 ppm BHT (1.470). There was significant difference at $P < 0.05$ in FFA, AV and PV of RSBO containing MOPE and WOPE with RSBO containing no additive. The MOPE and WOPE at all concentrations considered are more effective in stabilizing RSBO hydrolytically and oxidatively than 200 ppm BHT.

Keywords: refined soybean oil; stability; free fatty acid; acid value; peroxide value; sweet orange peel; antioxidant.

1. Introduction

The search for chemical substance that will stabilize edible oils hydrolytically and oxidatively has been on increase because a lot of cardiovascular diseases such as hypertension, stroke, arteriosclerosis and thrombosis are associated to the indiscriminate consumption of fats, oils and their products [1-4]. There have been earlier discovery of synthetic chemicals (antioxidants) such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), tertiary butylated hydroxy quinone (TBHQ) and propyl gallate (PG) that are found effective in stabilizing edible oils and fats against hydrolytic and oxidative rancidity [5-7]. However, the unsafe nature of these synthetic chemicals due to their toxicity, carcinogenicity and mutagenicity have been noticed and reported; this has led to restriction on the usage of these additives to foods and food products in the international markets [7-15]. Consequently, there is growing interest in finding suitable and safer natural alternatives to synthetic antioxidants currently in use to prevent lipid peroxidation [12, 16-21].

Refined soybean oil is obtained from soybean seeds (*Glycine max*). The seeds are dried, cracked, adjusted for moisture content (bean-conditioning), rolled into flakes and extracted with commercial food grade hexane to obtain crude soybean oil. The crude soybean oil is then subjected to different processing stages such as degumming, neutralization, bleaching and deodourization before becoming refined vegetable oil [22-24]. There has been increasing demand for refined soybean oil in United State of America and other countries in the world because of its good organoleptic characteristics and very low cholesterol level which makes it more fit and safer for consumption. In 2004, United State Department of Agriculture (USDA) reported that about 31 million metric tons of soybean oil was produced worldwide in 2002-2003, constituting about half of the world edible vegetable oil production [25]. In Nigeria, a lot of money is made from soybean oil business. Oil merchants purchase the oil when it is cheap and store it for about six months or more and later sell it when the price has gone up without minding the deterioration that might have set in during the period of storage. At times for economic reasons, some house wives also buy it in larger quantities when it is cheaper and store it for months in various containers and keep it inside cupboards or on the cooking table or kitchen shelves for further domestic and ceremonial uses.

Sweet orange fruit (*Citrus sinesis*) is an important fruit of economic and health values. Their consumption appears to be associated with lower risk of colorectal, esophageal, gastric and stomach cancers and stroke. It is a rich source of vitamin C, folate, dietary fibre, minerals as well as phytochemicals [26]. Sweet orange peel also has a lot of health importance, and it is very rich in antioxidants (vitamins A & C, flavonoids) that helps to fight illness, and helps in reducing cholesterol, promotes healthy skin, aids digestion, good source of vitamin-packed flavoring [27]. Orange peel is an

agricultural waste, hardly can one find any one in Nigeria eating the orange peel but its health properties suggest that they are rich in phytochemicals and antioxidants.

Therefore this research work aims at determining the extractive value of sweet orange peel using different solvents; investigating the antioxidative potential of two highest solvent yield extracts at varying concentrations (200 - 1000 ppm) 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 butylatedhydroxytoluene (200 ppm BHT) by monthly testing for chemical quality parameters of the oil for twelve months.

2. Materials and Methods

2.1. Sources of Materials

Sweet Orange fruits were purchased from a local farmer at Utelu farm camp 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.

2.2. Preparation and Extraction of Sweet Orange Peel

The ripen peels were removed by knife and cut into smaller pieces for easy drying. The dried peels were ground using electric blending machine and it was sieved with 40 mm mesh size. The powdery samples were packed into a black polyethene bags labeled appropriately prior to extraction.

Twenty gram of the powdery sample was weighed into five cleaned and dried reagent bottles, and 200 mL of each solvent (methanol, ethyl acetate, acetone, water and chloroform) 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^{o}C by a rotary evaporator. Weight of extract obtained was used to calculate the percent yield of extract in each solvent [5, 17].

2.3. Addition of Additives to Refined Soybean Oil

Methanol and water extracts of orange peel at concentration of 200 ppm (0.02 g per 100 mL oil) to 1000 ppm (0.10 g per 100 mL 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 200 ppm BHT (0.02 g per 100 mL oil) and that contained no additive (0 ppm, control) was also set-up. Each container was appropriately labeled and stored in an open place at room temperature ranging from 27 to 33^{o}C.

2.4. Physical and Chemical Analyses

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^{o}C [28]. 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 [28] for a period of twelve months.

2.5. 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) [29].

3. Results and Discussion

3.1. Extractive Values of Orange Peel in Different Solvents

The extractive values (% yield) of acetone, chloroform, ethyl acetate, methanol and water extracts of orange peel are shown in Table 1. The extractive value obtained using water as solvent was the highest (17.60 ± 0.75%) while that of chloroform was the lowest (2.83 ± 0.09%). The yield of methanol extract (12.82 ± 0.76%) was next to water. The extractive values using acetone and ethyl acetate were 3.98 ± 0.00% and 3.27 ± 0.13%, respectively. There was significantly different at $P < 0.05$ at the extractive values obtained when chloroform, methanol and water were used as solvents while the yields in acetone and ethyl acetate were not significantly different at $P < 0.05$. 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 [5]. Chloroform, acetone and ethyl acetate extract yields were about 22 - 31% and 16 - 23% of methanol and water extract yields, respectively.

Table 1: Extractive value of orange peel

Solvent	Extractive Value (% yield) *
Acetone	3.98 ± 0.00 ^{b}
Chloroform	2.83 ± 0.09 ^{a}
Ethyl acetate	3.27 ± 0.13 ^{b}
Methanol	12.82 ± 0.76 ^{c}
Water	17.60 ± 0.75 ^{d}

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.

3.2. Impact of Additives on Colour and Refractive Index of Refined Soybean Oil

Table 2 reveals changes in colour and refractive index of refined soybean oil stored with varying concentration of methanol and water orange peel extracts and 200 ppm BHT. Colour of edible oils is an important physical quality factor that influences consumer decision of acceptance or otherwise. It is a psychological interpretation of a physiological response by the eye and brain to the physical stimulus of light radiation at different wavelength [30]. The most acceptable colour of edible oils 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 1 inch cell.

Table 2: Changes in colour and refractive index of refined soybean oil stored with varying concentration of methanol and water orange peel extracts and 200 ppm BHT

Concentration of Additive	Colour (Units) in 1 inch cell	Refractive Index at 40 ^{o}C
0 ppm (No additive)	1R + 3Y = 8.0	1.470
200 ppm MOPE	1R + 5Y = 10.0	1.471
400 ppm MOPE	1R + 5Y = 10.0	1.470
600 ppm MOPE	1R + 5Y = 10.0	1.471
800 ppm MOPE	1R + 7Y = 12.0	1.470
1000 ppm MOPE	1R + 7Y = 12.0	1.471
200 ppm WOPE	1R + 4Y = 9.0	1.471
400 ppm WOPE	1R + 5Y = 10.0	1.470
600 ppm WOPE	1R + 5Y = 10.0	1.471
800 ppm WOPE	1R + 5Y = 10.0	1.470
1000 ppm WOPE	1R + 5Y = 10.0	1.470
200 ppm BHT	1R + 4Y = 9.0	1.470

Note: MOPE = methanol orange peel extract; WOPE = water orange peel extract, BHT = butylated hydroxytoluene; R = red slide; Y = yellow slide**.**

It is observed that the addition of additives (methanol orange peel extract (MOPE), water orange peel extract (WOPE) and BHT) increased the colour units of refined soybean oil (RSBO) at varying degrees. The colour units slightly increased as the concentration of the extracts increased. RSBO containing 200 ppm to 1000 ppm MOPE had colour unit ranged from 10.0 to 12.0 units while it was between 9.0 to 10.0 units for RSBO containing 200 ppm to 1000 ppm WOPE. RSBO containing 200 ppm BHT had colour of 9.0 units. It can be seen that WOPE and BHT competed favorably well with each other and gave better colour unit in oil than MOPE. Refractive index of RSBO containing additives was measured at 40 ^{o}C. The water and methanol extract of orange peel did not change the refractive index of RSBO. The oil which contained no additive had refractive index of 1.470 while

RSBO which contained both natural extract and BHT had refractive index of 1.470 and 1.471. Refractive index of edible oil is a measure of the extent of oil adulteration or purity [31], hence the addition of WOPE and MOPE to RSBO did not reflect that the oil was adulterated and it had the same refractive index with RSBO containing 200 ppm BHT.

3.3. Effects of Varying Concentrations of Additives on Free Fatty Acid of Refined Soybean Oil

Fig. 1 depicts free fatty acid (FFA) of RSBO stored with MOP extract and BHT for twelve months. It was observed that RSBO containing 200 ppm to 1000 ppm MOP extract had lower FFA values than oil sample containing 200 ppm BHT after the first three months of induction period. The FFA of oil containing MOP extract was lower than that of oil containing no additive at all (0 ppm, control).

Fig. 2 shows free fatty acid (FFA) of RSBO stored with WOP extract and BHT for twelve months. The FFA of oil sample which contained no additive was higher than oil sample that contained WOP extract. The FFA of oil containing WOP extract was lower than the FFA of oil containing 200 ppm BHT and this became apparent in the last six to seven months of storage.

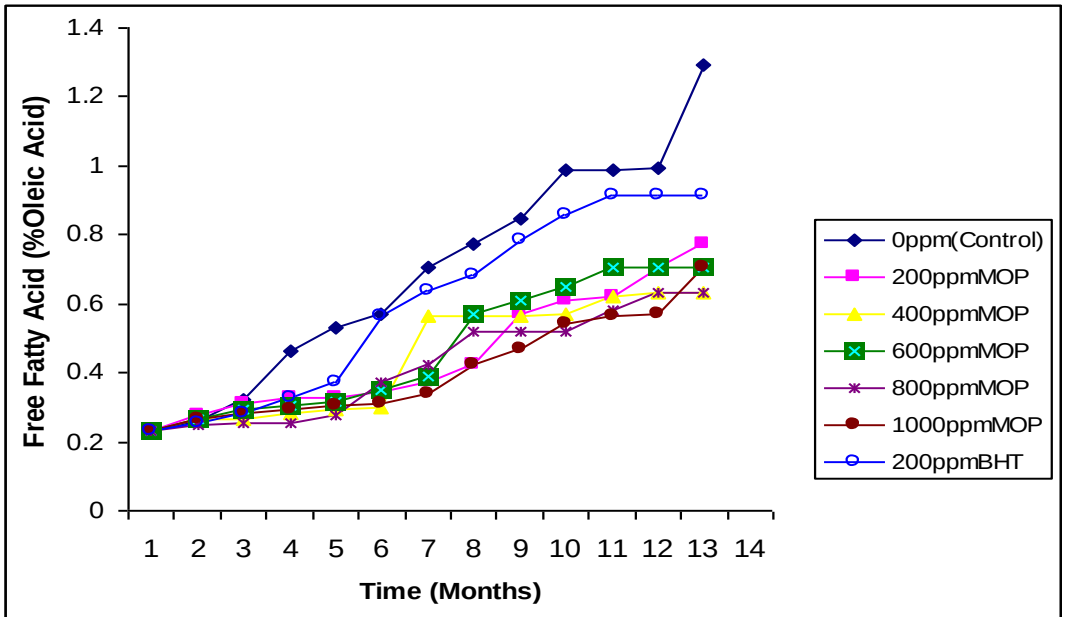

Figure1 : Free Fatty Acid of Refined Soybean Oil stored with Methanol Orange Peel (MOP) Extract and BHT for twelve months

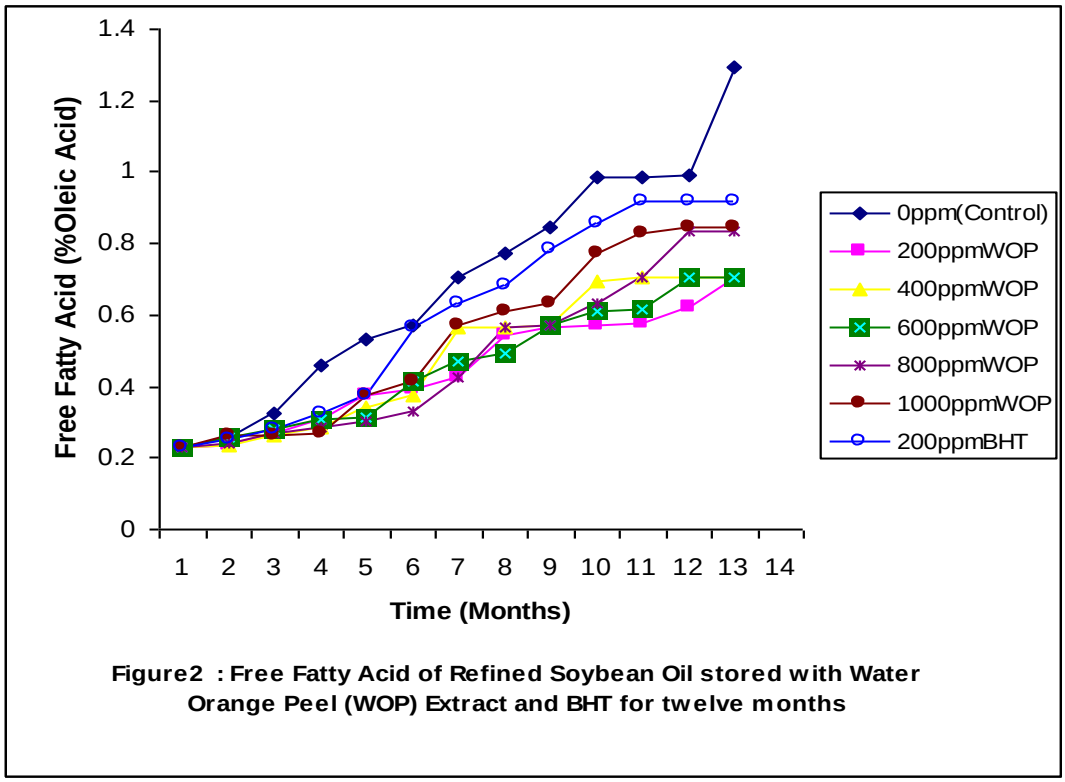

Figure2 : Free Fatty Acid of Refined Soybean Oil stored with Water Orange Peel (WOP) Extract and BHT for twelve months

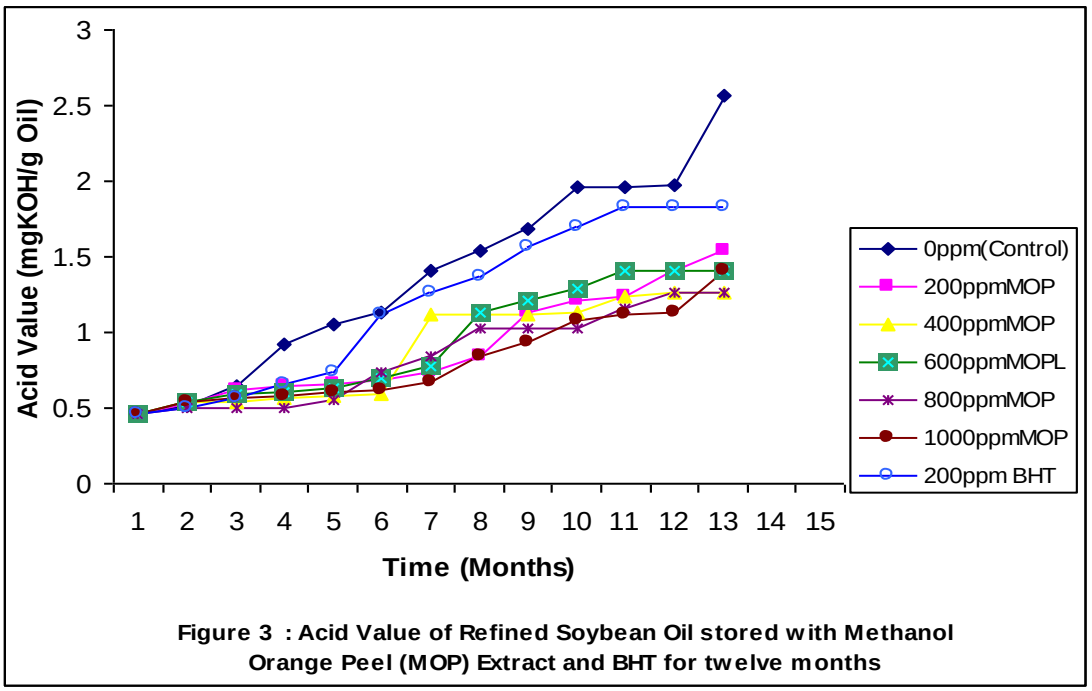

Figure 3 : Acid Value of Refined Soybean Oil stored with Methanol Orange Peel (MOP) Extract and BHT for twelve months

3.4. Effects of Varying Concentrations of Additives on Acid Value of Refined Soybean Oil

The acid value (AV) of refined soybean oil stored with methanol orange peel extract and BHT for twelve months is depicted in Fig. 3. The trend observed resembles that of Fig. 1 above, only that

the acid values obtained were higher than that of free fatty acid. All the varying concentrations of MOP extract were more effective in lowering acid value of refined soybean oil than 200 ppm BHT.

Fig. 4 shows acid value of refined soybean oil stored with water orange peel extracts and BHT for twelve months. The acid value of RSBO that contained no additive was higher than oil samples that contained additives. The 200 ppm BHT was not able to reduce the acid value of RSBO.

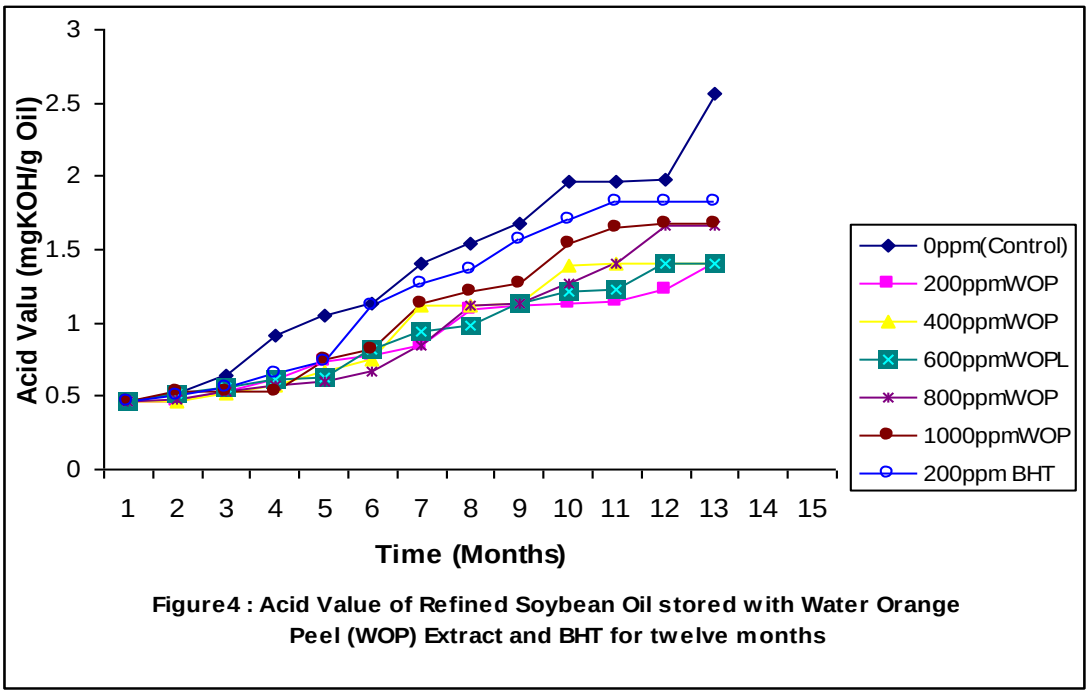

Figure4 : Acid Value of Refined Soybean Oil stored with Water Orange Peel (WOP) Extract and BHT for twelve months

3.5. Effects of Varying Concentrations of Additives on Peroxide Value of Refined Vegetable Oil

Fig. 5 reveals the peroxide value (PV) of refined soybean oil stored with methanol orange peel extract and BHT for twelve months. The trend observed was in agreement with the observation reported by Amir *et al*. [5]. All the additives lowered peroxide value of RSBO. The methanol extract was more effective in combating lipid peroxidation of RSBO than 200 ppm BHT. RSBO containing 800 ppm and 1000 ppm of MOP extract consistently maintained the lowest peroxide value for the period of oil storage.

Peroxide value of refined soybean oil stored with water orange peel extract and BHT for twelve months is depicted in Fig. 6. All the varying concentrations of water orange peel extract were able to inhibit lipid peroxidation in the oil more effectively than 200 ppm BHT and the degree of inhibition increases as the concentration of the extract increases. The 800 ppm and 1000 ppm WOP extract was able to reduce peroxide value most in RSBO. RSBO mixed with WOP extract had lower peroxide value than oil sample mixed with 200 ppm BHT.

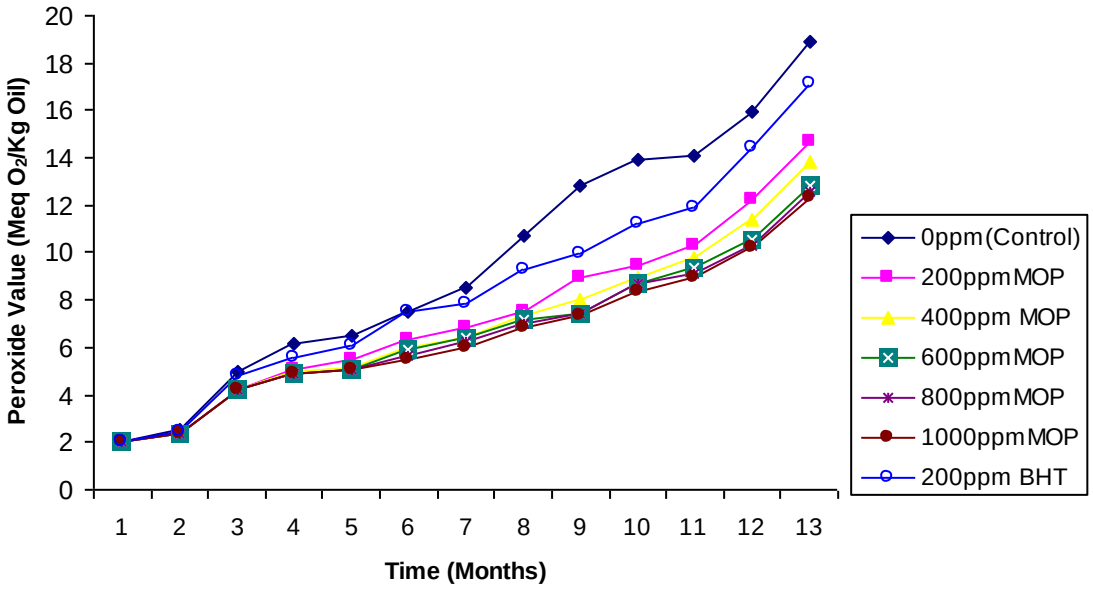

Figure 5 : Peroxide Value of Refined Soybean Oil stored with Methanol Orange Peel (MOP) Extract and BHT for twelve months

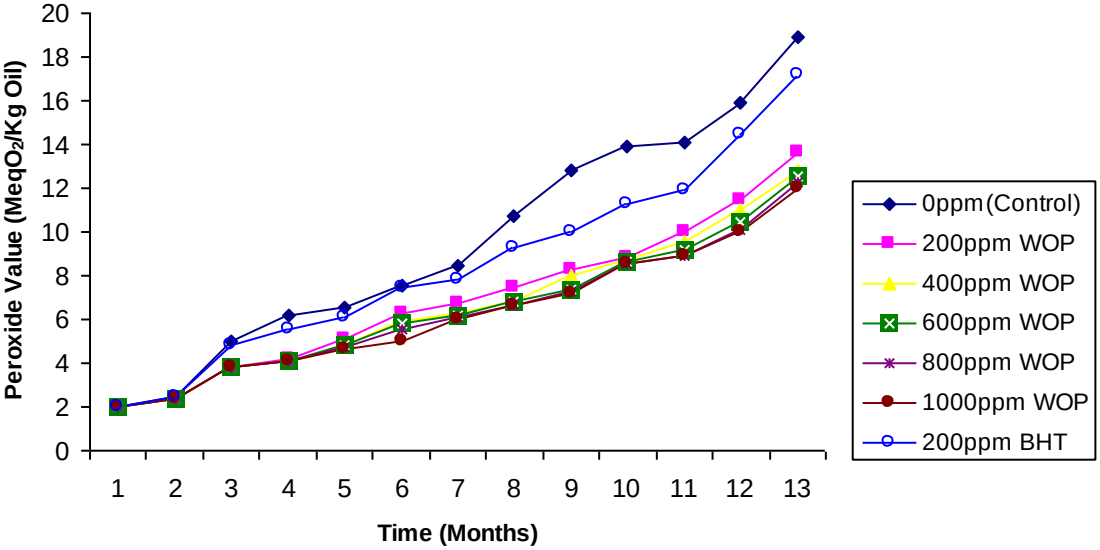

Figure 6 : Peroxide Value of Refined Soybean Oil stored with Water Orange Peel (WOP) Extract and BHT for twelve months

The overall averages of free fatty acid, acid value and peroxide value for twelve months give a better explanation of the antioxidative performance of the additive in an understandable manner. Table 3 shows the mean value of FFA, AV and PV of refined soybean oil stored with varying concentrations of methanol and water orange peel extracts and 200 ppm BHT for a period of twelve months. The addition of methanol and water extracts of orange peel to RSBO resulted in reducing FFA, AV and PV

of oil sample than 200 ppm BHT. However, methanol extract (expect 600 ppm) gave lower values of FFA and AV in oil sample than water extract. Free fatty acid and acid value of any lipid are measure of hydrolytic rancidity [16, 30, 32, 33]. The higher the values of FFA and AV of any lipid, the higher the degree of hydrolytic rancidity that set-in [18]. The FFA and AV of RSBO containing orange peel extracts (expects 1000 ppm MOP extract) were not significantly different at $P < 0.05$. The FFA and AV of RSBO stored with 1000 ppm MOP extract and 200 ppm BHT were not also significantly different at $P < 0.05$ but there was significant difference in FFA and AV of RSBO containing additives and the control which contained no additive (0 ppm).

The peroxide values of RSBO containing methanol and water orange peel extracts at varying concentration were lower than RSBO that contained 200 ppm BHT and there was significant difference 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 [5, 30]. Methanol orange peel extract is less effective in combating oxidative rancidity of RSBO than water orange peel extract.

Table 3: Mean values of some selected quality properties of refined soybean oil stored with varying concentration of methanol and water orange peel extracts and 200 ppm BHT over a period of twelve months

Concentration of Additive	Free Fatty Acid (% Oleic acid) *	Acid Value (mg KOH/g oil) *	Peroxide Value (meq O_2/Kg oil) *
0 ppm (No additive)	0.689 ± 0.327 ^{b}	1.371 ± 0.651 ^{a}	9.597 ± 5.275 ^{e}
200 ppm MOPE	0.454 ± 0.179 ^{a}	0.901 ± 0.358 ^{a}	7.345 ± 3.726 ^{c}
400 ppm MOPE	0.446 ± 0.168 ^{a}	0.888 ± 0.335 ^{a}	6.958 ± 3.437 ^{b}
600 ppm MOPE	0.470 ± 0.189 ^{a}	0.935 ± 0.375 ^{a}	6.685 ± 3.122 ^{a}
800 ppm MOPE	0.420 ± 0.155 ^{a}	0.836 ± 0.309 ^{a}	6.583 ± 3.048 ^{a}
1000 ppm MOPE	0.408 ± 0.149 ^{a}	0.812 ± 0.300 ^{a}	6.472 ± 2.974 ^{a}
200 ppm WOPE	0.447 ± 0.159 ^{a}	0.890 ± 0.317 ^{a}	6.927 ± 3.505 ^{b}
400 ppm WOPE	0.480 ± 0.195 ^{a}	0.956 ± 0.389 ^{a}	6.627 ± 2.291 ^{a}
600 ppm WOPE	0.460 ± 0.172 ^{d}	0.916 ± 0.341 ^{a}	6.472 ± 3.157 ^{a}
800 ppm WOPE	0.480 ± 0.224 ^{a}	0.955 ± 0.446 ^{a}	6.345 ± 3.043 ^{a}
1000 ppm WOPE	0.533 ± 0.242 ^{ab}	1.060 ± 0.482 ^{ab}	6.244 ± 3.003 ^{ca}
200 ppm BHT	0.596 ± 0.273 ^{ab}	1.185 ± 0.543 ^{ab}	8.477 ± 4.476 ^{d}

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. MOPE = methanol orange peel extract; WOPE = water orange peel extract; BHT = butylated hydroxytoluene.

4. Conclusions

Methanol and water gave the highest yield of orange peel 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. Water extract was superior to methanol extract in inhibiting oxidative rancidity of refined soybean oil. The antioxidant activity of both extracts in refined soybean oil was higher than that of 200 ppm BHT. However, further investigation can be carried out on other oils using these extracts or other solvent extracts of this fruit peel or other fruit peels.

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