Effects of Antioxidants on the Oxidative Stability of Vegetable Oil at Elevated Temperature

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Abstract

Unstable nature of vegetable oil posed serious health risk to humans. The oxidative stability of vegetable oils (Palm Olein, Soyabean Oil and Linseed Oil) with direct incorporation of antioxidants (tertiary butyl hydroxoquinone (TBHQ), butylated hydroxyl toluene (BHT) and mixed (TBHQ and BHT) at room temperature and 70^0C for 168 hours was aimed to be investigated. Peroxide value was determined and the oxidative stability was evaluated. TBHQ had significant effect on the oxidative stability of palm olein at 70^0C while (TBHQ and BHT) had synergetic effect on stability of Soya bean oil at room temperature and Linseed oil at 70^0C.

Key words: Vegetable oil, peroxide value, oxidative Stability, antioxidants)

1.0 Introduction

Production of vegetable oils that meet the global standard of consumption of the cosmetics and specialty food industries is a multi-step process which involves procurement of raw materials, extraction, refining and packaging. The inability of the oil to remain stable due to the presence of acyl-lipids such as monomeric, dimeric and oligomeric triacyl glecerols, and sterols like holesterols (animal sterols) and phytosterols (plant sterols) which can oxidize through the exposure to air or presence of moisture at high temperature to form lipid oxidation products with initial reaction products known as hydroperoxide and later form compounds such as aldehydes, ketones, alcohols carboxylic acids which has great consequences (Rossell, 2001a; 2001b; Tabee, 2008; Dobarganes and Marquez-Ruiz, 2003; 2005).

Lipid oxidation causes food spoilage, deterioration of important qualities in vegetable oil, rancidity of the vegetable oil and reduces the organleptic characteristics of the fried food which have negative effects on the taste, colour and odour of vegetable oil (Rossell, 2001a; Tabee, 2008; Ellen, 2008; Dutta, 2002). Cholesterols oxidation products (COPs) have received much attention due to their biological effects such as cytotoxicity, atherogenicity, sterol metabolism interference, mutagenicity, carcinogenicity and absorption of compounds which poses health risk to human if consumed in large quantities (Dobarganes and Marquez-Ruiz, 2003; Dutta et al, 2002; Hovenkamp et al, 2008; Osada, 2002; Garcia-Cruset et al, 2002; Ryan et al, 2005; Abramsson-Zetterberg et al, 2007; Zembron-Lacny et al, 2009; Said et al, 2007; Amaral et al, 2003).

Though, phytosterols oxidation products have not show any evidence of genotoxic effect (Dutta et al, 2006; Ryan et al, 2005; Abramsson-Zetterberg et al, 2007) but unstable due to the presence of unsaturated fatty acid content which may combine with oxygen on exposure to air (Abramsson-Zetterberg et al, 2007; Dunn, 2005; Gertz et al, 2000; Aluyor and Ori-Jesu, 2008; Rudnik et al, 2001; Dostálová et al, 2005) and it reduces cancers (colon, breast and prostate cancers) if properly treated (Amaral et al, 2003; Awad and Fink, 2000; Awad et al, 2001). Methods of improving stability of vegetable oils against oxidation include hydrolysis (Rossell, 2001a; 2001b; Erickson, 2006; Aluyor and Ori-Jesu, 2008), hydrogenation process in the presence of nickel catalyst(Rossell, 2001a; Aluyor and Ori-Jesu, 2008; Orthoefer and G. R. List, 2006), genetic modification (Aluyor and Ori-Jesu, 2008) and indirect addition of antioxidant (Rossell, 2001a; 2001b; Aluyor and Ori-Jesu, 2008; Zembron-Lacny et al, 2009) reduces the degree of unsaturation and promote the quality of vegetable oil with storage period (Dostálová et al, 2005; Ullah et al, 2003; Yoshida et al, 2002). The choice of antioxidants used based on the solubility and ability to combine with other antioxidants (ECC, 2006; Amaral et al, 2003).

Antioxidants are substances that when introduced into substrate at low concentration significantly inhibit oxidation of the substrate (Aluyor and Ori-Jesu, 2008; Zubr and Matthäus, 2002). Antioxidants are of two types: Primary or chain breaking type involves the addition of trace amount to inhibit oxidation of the substrate. This is further classified into natural antioxidants such as panda leaf, tocophenol, carotene, flavonols, rosemary from labiates family etc (Abramovic and Abram, 2006; Ullah 2003; Robards et al, 2002) and synthetic antioxidants such as TBHQ, BHT,TBH, BHA, BNB, TBA, propyl gallates, Irgafos 168, Irganox 1010 and 1330, Chimassorb 944, tocophenol etc (Azizkhani and Zandi, 2009; Müller et al, 2009; Zubr and Matthäus, 2002; Yoshida et al, 2002). The Secondary type or preventive antioxidant retards the rate of oxidation through the removal of the substrate or singlet oxygen quenching (Müller et al, 2009). Though, several methods are available to promote the quality of vegetable oil which may not be satisfactory or economically feasible in many instances (Tabee, 2008).

Researchers have established that antioxidant retards the development of rancidity due to oxidation in vegetable oils and thereby, used to control the quality of vegetable oils as well as assessment of the suitability of the products with respect to oxidation resistance (Müller et al, 2009; Zembron-Lacny et al, 2009). The initiative of this study was based on direct supplementation of antioxidants used on vegetable oil before refining and storage at elevated temperature so as to reduce the negative effect posed by vegetable oil. Moreover, whether phytosterols has any biological effect as cholesterols do or not, it undergoes oxidation which causes deterioration of the vegetable oils. The objective of this work is to establish the effectiveness of synthetic antioxidant supplementation: Butylated hydroxyl toluene (BHT), tertiary butylhydroquinone (TBHQ) and mixed antioxidants with equivalent proportion of the antioxidants at elevated temperature on the oxidative stability of the palm olein, soybean oil and linseed oil.

2.0 Materials and Methods

2.1 Preparation of the Vegetable Oil

Palm olein (non-drying oil), soya bean oil (semi-drying oil) and linseed oil (drying oil) were extracted by Ellen (2008) method from their respective seeds using solvent extraction after being crushed and steam cooked. Hexane was used as a solvent and later separated from the oils by evaporation. The crude oils were purified through degumming, neutralization and deodorization processes. Degumming was done to precipitate the phosphatides, gums, impurities and other water soluble impurities by heating the mixture of one litre of the crude oil sample and 100millilitre of phosphoric acid to a temperature of 70^0C in a three litre beaker. One litre of deionized water was added and stirred for about 10minutes to obtain an even distribution of the colloids; oil mixture was separated by the use of separating funnel and washed with deionized water. Neutralization process was carried out by addition of 25ml of benzene to 5ml of degummed vegetable oil followed by 25ml of ethanol and a few drops of phenolphthalein indicator were added.

The mixture was shaken and titrated simultaneously against aqueous solution of 0.1N NaOH. The titre value for neutralization was recorded. The degummed vegetable oils and 0.1N NaOH were set at a temperature of 70^0C. The required amount of sodium hydroxide solution used for titration was added to the degummed oil sample and stirred continuously on the heater for 5minutes. The mixture was washed with water and salt, and then drained off soap stock after settling in the separating funnel. This was repeated until all soap stocks were completely removed.

Adequate proportion of activated charcoal was added to the neutralized oil, stirred thoroughly for removal of pigment and oxidized component, and then filtered. The drained oil was dried in the oven to removed volatile materials. The twelve samples (A to L) were formulated as stated in the table 1 and composed of refined vegetable oil blended with adequate proportion of antioxidants by direct method and even distribution and homogeneity was ensured.

The method of Maisuthisakul and Charuchongkolwongs (2007) was employed to determined peroxide value of vegetable oil in which 5g each of samples A to L were weighed in a conical flask and 30ml of acetic acid-chloroform solution was added and then stirred until the sample dissolved. 0.5ml of saturated potassium iodide solution was added, swirled for one minute and 30ml of distilled water was then added followed by few drops of starch solution. The vegetable oil samples were titrated with 0.01M Sodium thiosulphate solution for every storage period of 24hours in 168 hours at room temperature and at 70^0C with vigorous shaking until the blue colour disappeared, a blank test was also performed and the titre values were recorded.

Table 1: Composition of Experimental Oil Samples

SAMPLE	COMPOSITION
A	450g of refined Palm Olein + 0.45g TBHQ
B	450g of refined Soyabean Oil + 0.45g TBHQ
C	150g of refined linseed Oil + 0.15g TBHQ
D	450g of refined Palm Olein + 0.45g BHT
E	450g of refined Soyabean Oil + 0.45g BHT
F	150g of refined Linseed Oil + 0.15g BHT
G	450g of refined Palm Olein + 0.45g of equal amount of TBHQ + BHT
H	450g of refined Soyabean Oil + 0.45g of equal amount of TBHQ + BHT
I	150g of refined Linseed Oil + 0.15g of equal amount of TBHQ + BHT
J	450g of refined Palm Olein + no additive
K	450 of refined Soyabean Oil + no additive
L	150g of refined Linseed Oil + no additive

2.2 Models

Peroxide Values (PV) were calculated for all vegetable oil samples using equation given by Maisuthisakul and Charuchongkolwongs (2007):

$$PV = \frac{(V_s - V_b)NF}{W} x\ 100 \qquad (1)$$

PV is peroxide value of vegetable oil sample measured in milliequivalent of peroxide per kg of oil sample, V_s is Volume of sodium thiosulphate solution (ml) used for neutralization, V_b is Volume of sodium thiosulphate solution used for neutralization for blank test determined as 2.8ml, $W\ is$ weight of vegetable oil sample measured (g), F is the factor from standardization with Potassium Iodide and N is normality of sodium thiosulphate solution (0.01 M). Oxidative stability (S) was evaluated from peroxide values as thus:

$$S = \frac{PV_i - PV_j}{P_{Vj}} x100 \qquad (2)$$

S is the oxidative stability measured in percent, PV_i is the peroxide value of vegetable oil with antioxidant, PV_j is the peroxide value of sample without antioxidants.

2.3 Statistical Analysis

The data were analyzed by SPSS software program (Version 17.0) with bivariate correlations. The Pearson's correlation coefficient test was conducted for determination of significance with one tail of p-value less than 0.01 considered statistically significant between vegetable oils at room temperature and at 70^0C in the presence of the antioxidants, and vegetable oil samples with different antioxidant.

3.0 Results and Discussion

Table 2 shows the evaluated peroxide value of vegetable oils in the presence of the antioxidants at room temperature and at elevated temperature of 70^0C using equation 1.

It was be observed that the peroxide value for palm olein (A_n) remains unchanged throughout storage period of 168 hours at room temperature using TBHQ antioxidants but peroxide value increased by 10 percent at elevated temperature of 70^0C throughout of storage period of 168 hours of sample A_W. It can also be deduced from the Table 2 that the peroxide value of all samples of vegetable oil decreased with increasing storage time in the presence of antioxidants irrespective of temperature effect but with significant variation at elevated temperature.

Table1: Evaluated Peroxide Value of Vegetable Oils in the Presence of Antioxidants

Time	0	24	48	72	96	120	144	168
A_n	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
A_w	4.0	4.0	4.2	4.2	4.2	4.2	4.4	4.4
B_n	0.8	1.1	1.55	1.8	2.0	2.2	2.6	2.8
B_w	0.8	1.4	1.8	2.3	2.6	3.1	3.4	3.8
C_n	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2
C_w	48.8	50.2	51.6	53.0	54.6	56.0	57.4	58.8
D_n	4.2	4.2	4.2	4.3	4.4	4.4	4.4	4.4
D_w	4.2	4.2	4.4	4.5	4.6	4.6	4.8	4.8
E_n	0.9	1.2	1.7	1.9	2.2	2.4	2.8	3.0
E_w	0.9	1.5	1.9	2.4	2.8	3.3	3.6	4.0
F_n	49.2	50.8	52.4	54	55.6	57.2	58.8	60.4
F_w	49.2	51.4	53.4	55.6	57.6	59.8	61.8	64.0
G_n	3.6	3.6	3.6	3.7	3.8	3.9	3.9	4.0
G_w	3.6	3.7	3.9	4	4.1	4.1	4.3	4.4
H_n	0.8	1.1	1.4	1.6	1.8	1.9	2.1	2.3
H_w	0.8	1.3	1.6	2.0	2.3	2.6	2.8	3.0
I_n	48.4	49.0	49.8	50.4	51.2	51.8	52.6	53.2
I_w	48.4	49.8	51.2	52.6	54.0	55.4	56.8	58.2
J_n	4.4	4.6	4.6	4.8	5.0	5.2	5.2	5.4
J_w	4.4	4.6	5.0	5.2	5.4	5.6	6.0	6.2
K_n	1.0	1.4	2.0	2.4	2.8	3.2	3.8	4.2
K_w	1.0	1.8	2.4	3.2	3.8	4.6	5.2	6.0
L_n	49.8	51.6	53.4	55.2	57.2	59.0	60.8	62.6
L_w	49.8	52.8	55.8	58.8	61.8	64.8	67.8	71.0

Subscript n means at room temperature, w means at elevated temperature of 70^0C.

The peroxide value for 168 hour of storage period for B_n, B_w, C_n, C_w, D_n, D_w, E_n, E_w, F_n, F_w, G_n, G_w, H_n, H_w, I_n, I_w, J_n, J_w, K_n, K_w, L_n and L_w increased by 250%, 375%, 17.21%, 20.49%, 4.76%, 14.29%, 233.33%, 344.44%, 22.76%, 30.08%, 11.11%, 22.22%, 187.5%, 275.00%, 9.92%, 20.33%, 22.73%, 40.91%, 320.0%, 500.00%, 25.70% and 42.57% respectively. It was observed that the peroxide value for 168 hour of storage period for all the vegetable oils reduced with and without temperature effect in the presence of antioxidants (TBHQ, BHT and mixed TBHQ and BHT) compared with the absence of antioxidants at increased percentage of peroxide values of vegetable oils. This indicates that without addition of antioxidants to vegetable oil, there may be rapid deterioration during storage period.

The presence of TBHQ antioxidant gave the lowest percentage change in peroxide value of the palm olein, followed by BHT antioxidant while mixed antioxidants (TBHQ and BHT) gave lower peroxide value when compared with palm olein in the absence of the antioxidant during the storage period. In contrast, mixed antioxidants (TBHQ and BHT) in the soyabean oil gave the lowest peroxide value during the storage period of 168 hours, followed by BHT and TBHQ antioxidant gave lower peroxide value during the storage period of soyabean oil with and without temperature effect. Also, mixed antioxidants (TBHQ and BHT) gave lowest peroxide value with linseed oil during the storage period followed by TBHQ and BHT antioxidant gave lower peroxide value with and without temperature effect. Increased temperature in the presence of antioxidants, increases peroxide value of all samples of vegetable oil as shown in the Table 2. This may be attributed to the reaction kinetics and rheological properties of vegetable oils.

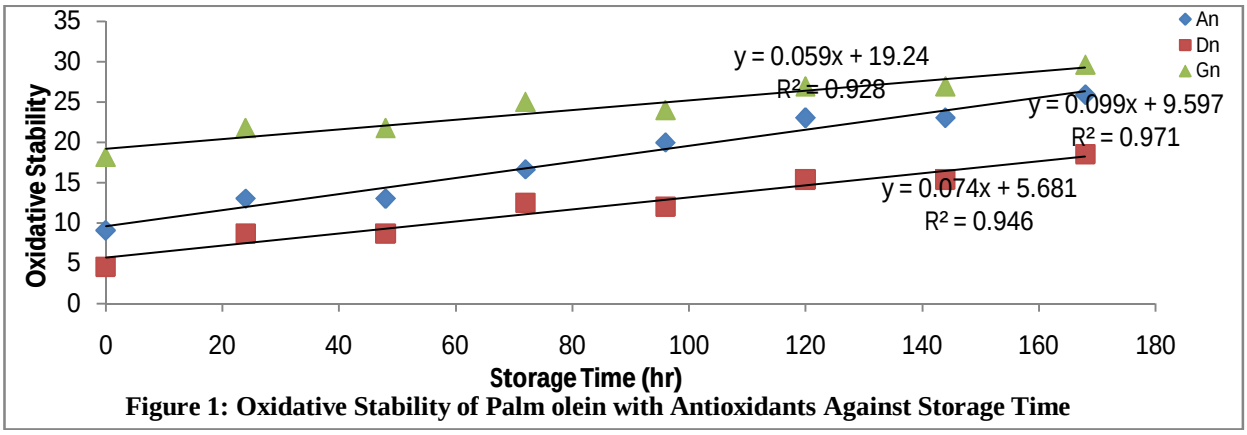

Figure 1: Oxidative Stability of Palm olein with Antioxidants Against Storage Time

Figure 1 shows the rate of oxidative stability of palm olein in the presence of antioxidants at room temperature. It was observed that palm olein with TBHQ antioxidants (0.099) had the highest rate of oxidative stability, followed by BHT antioxidant (0.074) while BHT antioxidant (0.059) had the lowest rate of oxidative stability. This indicates that TBHQ antioxidant was significantly favourable for oxidative resistant of the palm olein oil compared with other antioxidants at room temperature.

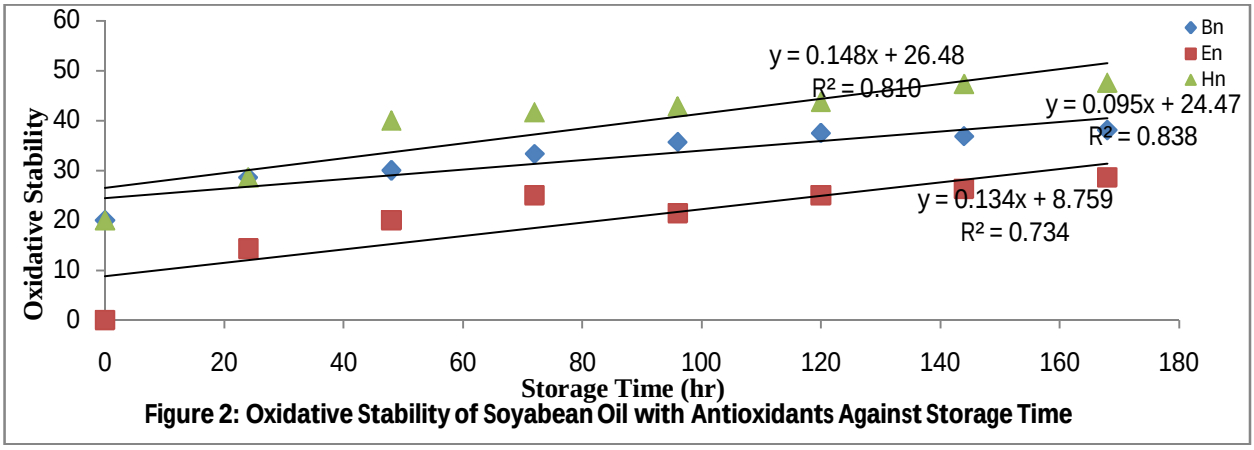

Figure 2: Oxidative Stability of Soyabean Oil with Antioxidants Against Storage Time

Conversely, it was observed that the soyabean oil with mixed (TBHQ and BHT) antioxidants (0.148) had the highest rate of oxidative stability, followed by BHT antioxidant (0.134) while TBHQ antioxidant (0.095) had the lowest rate of oxidative stability as shown in the Figure 2. Also, the linseed oill with mixed (TBHQ and BHT) antioxidants (0.148) had the highest rate of oxidative stability, followed by BHT antioxidant (0.134) while TBHQ antioxidant (0.095) had the lowest rate of oxidative stability as shown in the Figure 3. The highest rate of oxidative stability of soyabean and linseed oils in the presence of mixed (TBHQ and BHT) antioxidants may be attributed to the positive synergistic effect of BHT antioxidant with TBHQ antioxidant in the linseed oil at room temperature. Though, the results obtained for all vegetable oil samples in the presence of antioxidants have high regression correlation coefficients (R^2) which indicates good fitness of the data obtained as shown in the Figure 1, 2 and 3.

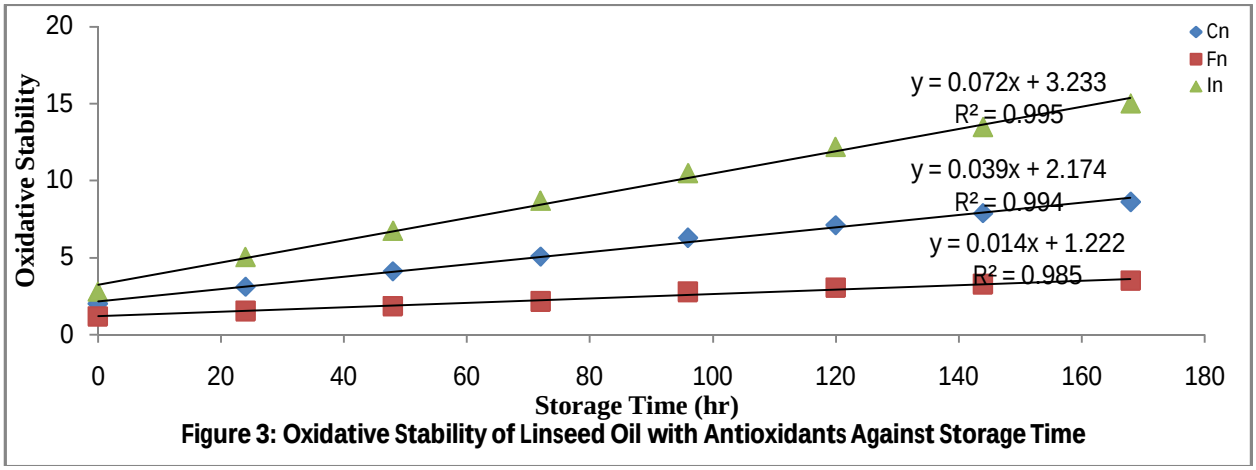

Figure 3: Oxidative Stability of Linseed Oil with Antioxidants Against Storage Time

Figure 4 shows the rate of oxidative stability of palm olein in the presence of antioxidants at elevated temperature of 70^0C. Remarkably, highest rate of oxidative stability was observed for samples of palm olein in the presence of TBHQ antioxidant (0.117) with good correlation coefficient of 0.989 compared with BHT antioxidant (0.099) with correlation coefficient of 0.962. The addition of mixed (TBHQ and BHT) antioxidant (-0.069) with correlation coefficient of 0.645 indicates the negative synergistic effect in the palm olein. The higher value of rate of oxidative stability of palm olein at elevated temperature of 70^0C compared with that at room temperature in the presence of antioxidants with significant value of 0.000 ($p < 0.01$) in the presence of all antioxidants indicated significant effect of heat on palm olein.

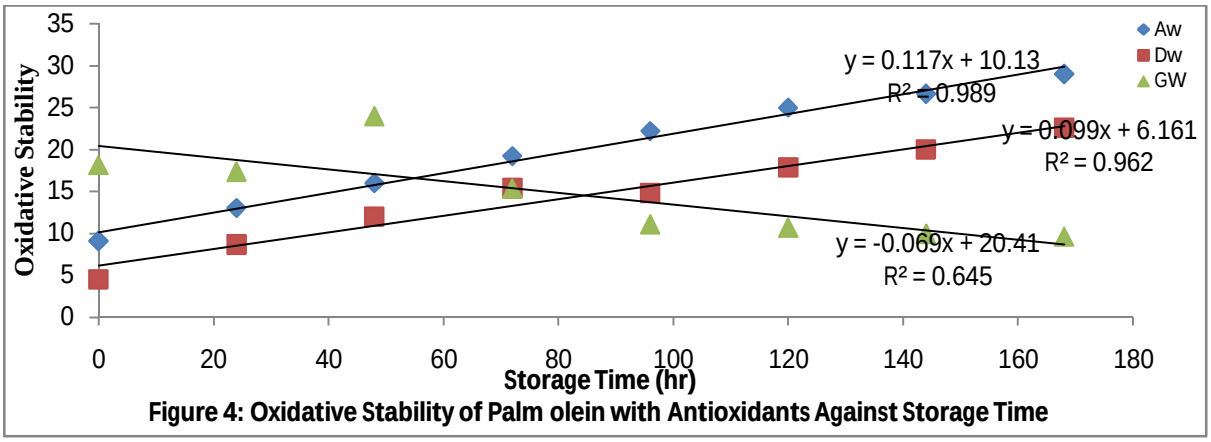

Figure 4: Oxidative Stability of Palm olein with Antioxidants Against Storage Time

The higher rate of oxidative stability was observed for samples of soyabean oil in the presence of mixed (TBHQ and BHT) antioxidants (0.099) with good correlation coefficient of 0.673 compared with BHT antioxidant (0.084) with correlation coefficient of 0.512 as shown in the Figure 5. However, the presence of TBHQ antioxidants in soyabean oil with low rate of oxidative stability (0.035) and correlation coefficient of 0.064 as shown in the Figure 5 indicates the poor effect of TBHQ antioxidant in the soyabean oil. The higher value of rate of oxidative stability of soyabean oil at 70^0C and at room temperature compared with that of elevated temperature in the presence antioxidants with significant value of 0.000 ($p < 0.01$) indicated significant effect of heat on the oxidative stability of soyabean oil.

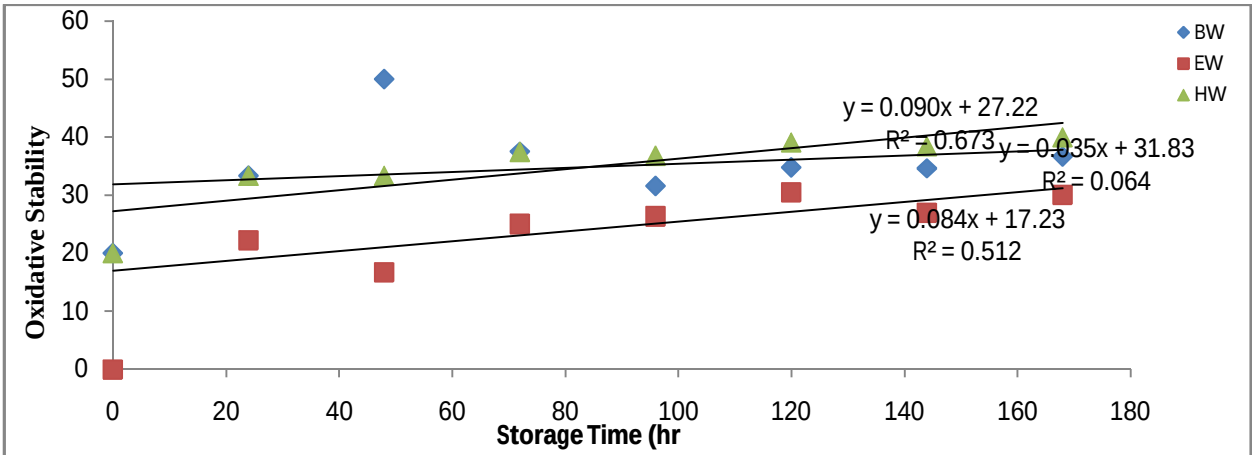

Figure 5: Oxidative Stability of Soyabean Oil with Antioxidants Against Storage Time

More so, the highest rate of oxidative stability was observed for samples of linseed oil in the presence of mixed (TBHQ and BHT) antioxidants (0.089) with good correlation coefficient of 0.991, followed by the presence of TBHQ antioxidant (0.088) with correlation coefficient of 0.990 as shown in the Figure 6. Though, low value of rate oxidative stability of linseed oil in the presence of BHT antioxidants (0.051) with correlation coefficient of 0.992 indicates the poor effect of BHT antioxidant in linseed oil as shown in the Figure 6. The higher value of rate of oxidative stability of linseed oil at elevated temperature of 70^0C compared with that at room temperature in the presence antioxidants with significant value of 0.000 ($p < 0.01$) for both mixed antioxidants and BHT antioxidant, and 0.001 ($p < 0.01$) for TBHQ indicates significant effect of elevated temperature on linseed oil. This indicated that oxidative stability of vegetable oil in the presence of antioxidants influenced at elevated temperature of 70^0C.

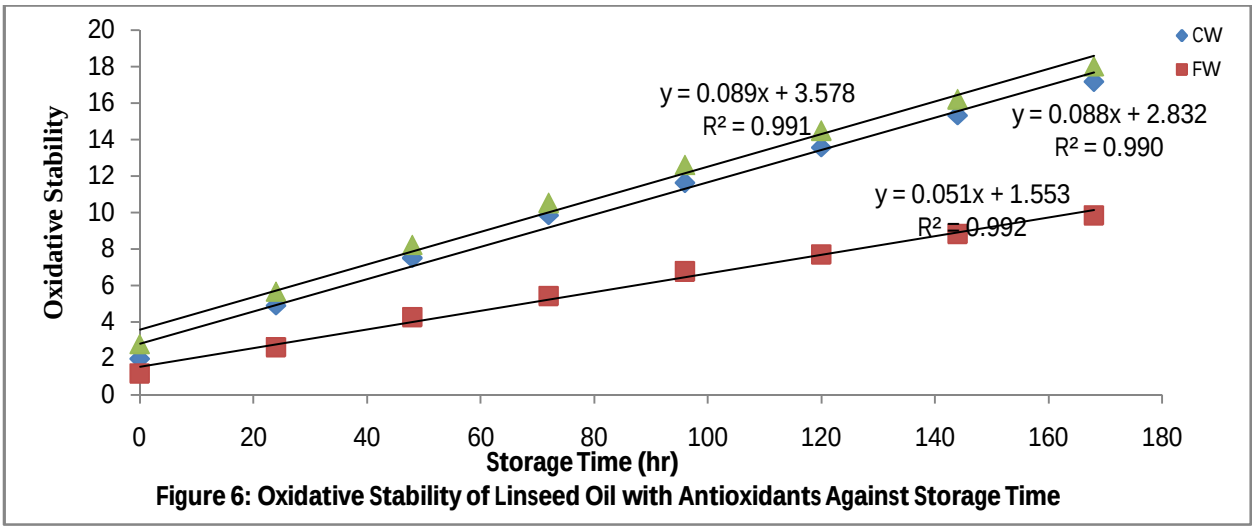

Figure 6: Oxidative Stability of Linseed Oil with Antioxidants Against Storage Time

4.0 Conclusions

Based on the result obtained, all vegetable oils (Palm olein, Soyabeen Oil and Linseed Oil) are prone to oxidative deterioration during storage period. Addition of antioxidants is effective in ensuring oxidative stability of refined vegetable oil. It can be deduced that TBHQ was superior to BHT and mixed (TBHQ and BHT) antioxidants for ensuring oxidative stability of palm olein while mixed antioxidant was significantly superior for the oxidative stability of soyabean at room temperature and linseed oil at elevated temperature. This may be attributed to the synergy effect of BHT antioxidant with TBHQ antioxidant in the linseed oil at elevated temperature during the storage period. Temperature at 70^0C significantly influenced the oxidative stability of vegetable oils used except soyabean oil in the presence of antioxidants which may be attributed to the reaction kinetics and rheological properties of the vegetable oils. More so, introduction of commercial antioxidants (TBHQ, BHT or equal blend of both) to vegetable oil increased oxidative stability. This may be used to improve the oxidative stability of crude vegetable oil during refining.

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