SITI BALQIS CHE OTHMAN

DOCTOR OF PHILOSOPHY

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EXPERIMENTAL AND THEORETICAL STUDIES ON THE EFFECTS OF FATTY ACIDS ON THE PERFORMANCE OF ANTIOXIDANTS IN INHIBITING THE OXIDATION OF SELECTED VEGETABLE OILS

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Doctor of Philosophy in School of Fundamental Science Universiti Malaysia Terengganu

Nov 2014

DEDICATION

This thesis is dedicated to Rasimah Binti Daud

I love you.

Abstract of the thesis presented to the Senate of Universiti Malaysia Terengganu
in fulfillment of the requirement for the degree of
Doctor of Philosophy

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SITI BALQIS CHE OTHMAN

November 2014

Main Supervisor : **Associate Professor Ku Halim Ku Bulat, Ph. D**

Co-Supervisor : **Juriffah Ariffin, Ph.D**

School : **School of Fundamental Science**

The aim of this research project was to study the effects of fatty acids on the performance of synthetic antioxidants in inhibiting the autoxidation of selected vegetable oils. In this study, four types of chain-breaking radical scavengers, BHA, BHT, TBHQ and PG, were utilized to test their performances, experimentally and theoretically in the presence or in the absence of fatty acids. Four types of fatty acids, PA, SA, OA and LA were added at several concentrations ranging from 0.25% w/w to 3.0% w/w. The samples of oils either in the presence or in the absence of other intentionally added species such as antioxidants and/or fatty acids were exposed to heat at 60°C in the oven for 15 days. Oil samples at specified days of exposure were then taken for peroxide value (PV), total acid number (TAN) tests, and for infrared analyses. For the theoretical studies, a quantum mechanical software package

of *Gaussian09* at the theoretical level of B3LYP Density Functional Theory 6-31G(d,p) were used for the optimization of the single species structures or the complex structures involving the TAGs (tripalmitic, trioleic, trilinoleic) or the hydroperoxyl radical of TAG COO• with antioxidant and/or fatty acid. Physical parameters such as SCF energy, dipole moment, distance between selected species in question, and the bond length and the bond strength of the O-H of antioxidants were collected and analyzed. Results showed that for these selected vegetable oils, palm olein, canola and safflower, the best antioxidant in reducing the TAG decomposition in the presence of fatty acids was propyl gallate and the highest (largest) negative effect was due to the presence of stearic acid. The unsaturated fatty acids (OA and LA) seem to show larger effects on palm olein in contrast to canola oil. In both oils, palm olein and canola, the performance of propyl gallate was affected very much by the presence of fatty acids. Results also showed that for palm olein, BHA was the least affected by the presence of fatty acids, while BHT and TBHQ were the best antioxidants for canola oil. BHT and TBHQ again were the best antioxidant for safflower oil either in inhibiting the hyroperoxide formation or in reducing the TAG rearrangement to produce free fatty acids. The interaction energy, corrected using the Counterpoise Procedure (CP), between TAGs and antioxidants or between TAGs and fatty acids was the main factor that can be used to determine the effect of fatty acids on the performance of antioxidants under studied. Theoretical results also showed that the interaction energy between antioxidants and TAGs were almost in the same magnitude as of fatty acids: 15 – 39 kJ/mol for $H_{ß,}$ and 10-26 kJ/mol with hydroperoxyl radicals (C_8 OO and C_9 OO). The presence of fatty acid had reduced the interaction between TAG and

antioxidant. The order of percentage reduction in interaction energies is: TAG trioleic $H_{ß}$ (59-98%) > TAG trioleic C_8OO radical (60-77%) > TAG trilinoleic C_9OO radical (7-22%). Almost in all cases, the theoretical findings always support the experimental observations.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KAJIAN EKSPERIMEN DAN TEORI TERHADAP KESAN ASID LEMAK KEATAS PRESTASI ANTIOKSIDA DALAM MENGHALANG PENGOKSIDAAN MINYAK SAYURAN TERPILIH

SITI BALQIS CHE OTHMAN

November 2014

Penyelia Utama : Prof Madya Dr. Ku Halim Ku Bulat, Ph.D

Penyelia Bersama : Dr. Juriffah Ariffin, Ph.D

Pusat Pengajian : Sains Asas

Matlamat utama kajian ini adalah untuk mengkaji kesan asid lemak terhadap keberkesanan antioksida sintetik dalam menghalang dan melambatkan proses autoperoksidaan ke atas minyak sayuran terpilih. Dalam kajian ini, empat antioksida jenis penghapus radikal pemutus rantai iaitu BHA, BHT, TBHQ dan PG telah digunakan untuk melihat keberkesanan dan prestasinya secara experimen dan juga teori sama ada dalam kehadiran atau tanpa kehadiran asid lemak. Empat jenis asid lemak iaiti PA, SA, OA, LA ditambah mengikut kepekatan tertentu iaitu diantara 0.25% b/b sehingga 3.0% b/b. Sampel minyak sama ada dengan kehadiran atau tanpa kehadiran bahan yang ingin ditambah samaada antioksida dan/atau asid lemak didedahkan kepada haba pada 60°C didalam ketuhar pemanas selama 15 hari. Sampel minyak pada hari-hari yang tertentu diambil untuk dianalisis secara kimia iaitu Nilai Peroksida (PV), Jumlah Nilai Asid (TAN) dan juga dianalisis menggunakan kaedah spektroskopi infra merah. Kajian teori pula menggunakan

pakej perisian kuantum mekanikal iaitu Gaussian 09 pada aras teori B3LYP DFT 6-31G(d,p) untuk pengoptimuman struktur spesis tunggal atau dalam keadaan kompleks yang melibatkan rantai TAG (tripalmitik, trioleik, trilinoleik) atau radikal hidroperoksil bagi TAG COO• dengan antioksida dan/atau asid lemak. Parameter fizikal seperti tenaga SCF, momen dwikutub, jarak antara spesis, panjang ikatan dan kekuatan ikatan bagi O-H pada antioksida dikumpul dan dianalisis. Keputusan menunjukkan bahawa bagi minyak masak yang dipilih iaitu minyak sawit, minyak canola dan minyak safflower, antioksida yang paling berkesan dalam mengurangkan penguraian TAG dalam kehadiran asid lemak adalah PG manakala asid lemak yang memberi kesan paling negatif ialah asid stearik. Asid lemak tak tepu (OA and LA) menunjukkan kesan yang lebih besar terhadap minyak sawit berbanding minyak canola. Dalam kedua-dua kes minyak sawit dan canola, propil galat amat terkesan dengan kehadiran mana-mana asid lemak. Keputusan bagi minyak sawit juga menunjukkan BHA merupakan antioksida yang paling kurang terkesan dengan kehadiran asid lemak, manakala BHT dan TBHQ merupakan antioksida terbaik bagi minyak canola. BHT dan TBHQ juga merupakan antioksida terbaik bagi minyak safflower sama ada dalam menghalang pembentukan peroksida mahupun mengurangkan penguraian TAG yang seterusnya akan membentuk asid lemak bebas. Tenaga interaksi, yang telah diperbetulkan dengan Kaedah Penyeimbangan (CP), antara TAG dan antioksida atau antara TAG dengan asid lemak merupakan faktor utama yang boleh digunakan untuk menentukan kesan asid lemak terhadap prestasi antioksida yang dikaji. Hasil pengiraan secara teori menunjukkan tenaga interaksi antara antioksida dengan TAG adalah dalam magnitud yang sama dengan asid lemak: 15-39 kJ/mol bagi $H_{ß,}$ and 10-26 kJ/mol

dengan hydroperoksil radicals (C_8OO and C_9OO). Kehadiran asid lemak telah melemahkan interaksi antara TAG dengan antioksida. Urutan peratus penurunan tenaga interaksi dalam tertib menurun adalah: TAG trioleik $H_ß$ (59-98%) > TAG trioleik radikal C_8OO (60-77%) > TAG trilinoleik radikal C_9OO (7-22%). Dapatan hasil pengiraan secara teori sentiasa menyokong hampir semua keputusan eksperimen.

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APPROVAL

I certify that an Examination Committee has met on 23th November 2014 to conduct the final examination of Siti Balqis Binti Che Othman on her Ph.D. thesis entitled "Experimental and Theoretical Studies on The Effects of Fatty Acids on The Performance of Antioxidants in Inhibiting The Oxidation of Selected Vegetable Oils"in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Hamdan Suhaimi, Ph. D
Professor
Universiti Malaysia Terengganu
(Chairperson)

Ku Halim Ku Bulat, Ph.D.
Associate Professor
Universiti Malaysia Terengganu
(Member)

Juriffah Ariffin, Ph.D.
Universiti Malaysia Terengganu
(Member)

Mohd. Sukeri Mohd Yusof, Ph.D.
Associate Professor
Universiti Malaysia Terengganu
(Internal Examiner)

Mohd. Ambar Yarmo, Ph.D.
Professor
Universiti Kebangsaan Malaysia
(External Examiner)

_____
NAKISAH MAT AMIN, Ph.D.
Professor/Dean of
School of Fundamental Science
Universiti Malaysia Terengganu

Date:

This thesis has been accepted by the Senate of Universiti Malaysia Terengganu as fulfillment of the requirements for the degree of Doctor of Philosophy.

NAKISAH MAT AMIN, Ph.D.
Professor/Dean of
School of Fundamental Science
Universiti Malaysia Terengganu

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Terengganu or other institutions.

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SITI BALQIS CHE OTHMAN
Date: 11 March 2015

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AO	-	Antioxidant
AOCS	-	American Oil Chemists' Society
AOM	-	Active oxygen method
B3LYP	-	Becke 3-Parameter, Lee, Yang and Parr
BDE	-	Bond Dissociation Energy
BHA	-	Butylated Hydroxyanisole
BHT	-	Butylated Hydroxytoulene
BSSE	-	Basis Set Superposition Error
C	-	Carbon
CLA	-	Conjugated linoleic acid
cm^{-1}	-	Wavenumber
°C	-	Degree Celcius
CO	-	Canola oil
cp	-	Counterpoise
CPOME	-	Crude Palm Oil Methyl Ester
DSC	-	Differential Scanning Calorimeter
DHA	-	Docosahexanoic acid
DFT	-	Density Functional Theory
EPA	-	Eicosapentaenoic acid
FA	-	Fatty Acid
FDA	-	Food and Drug Administration

FFA	-	Free Fatty Acid
FTIR	-	Fourier-transform infrared
g	-	Weight in grams
GTO	-	Gaussian Type Orbital
GRAS	-	Generally recognized as safe
H	-	Hydrogen
$H_ß$	-	Hydrogen Beta
HF	-	Hartree-Fock
hrs	-	Hours
Ir	-	Increase ratio
It	-	Induction time
kg	-	Weight in kilograms
KI	-	Potassium Iodide
KOH	-	Potassium hydroxide
kPa	-	Kilopascal
LA	-	Linoleic Acid
LEAR	-	Low erucic rapeseed
$N_2S_2O_3.5H_2O$	-	Sodium thiosulphate
mg	-	Milligrams
ml	-	Millilitres
MPA	-	Mullikan Population Analyses
MUFA	-	Monounsaturated fatty acid
NBO	-	Natural Bond Orbital
O	-	Oxygen

OA	-	Oleic Acid
OH	-	Hydroxy
PA	-	Palmitic Acid
PC	-	Phosphatidylcholine
PE	-	phosphatidylethanolamine
PG	-	Propyl Gallate
PO	-	Palm olein
ppm	-	parts per million
PUFA	-	Polyunsaturated fatty acid
PV	-	Peroxide Value
R_f	-	Retention factor
RBD	-	Refine bleach deodorized
RHF	-	Restricted Hartree-Fock
RIP	-	Rancimat Induction Period
SA	-	Stearic Acid
SAW	-	Surface acoustic wave
SCF	-	Self-Consistent Field
SD-DFT	-	Slater Determinant- Density Functional Theory
SO	-	Safflower oil
STO	-	Slater Type Orbitals
TAG	-	Triacylglyceride
TAN	-	Total Acid Number
TBARS	-	2-thiobarbituric acid reactive substances
TBHQ	-	*tert*-butylhydroquinone

TDDFT	-	Time-Dependent Density Functional Theory
W	-	Weight
%	-	Percentage
º	-	Degree

CHAPTER 1

INTRODUCTION

1.1 Scope of the study

The degradation process of vegetable oils not only involves oxidation but also the *cis-trans* isomerization and free fatty acids (FFA) production. The degradation of the oil had cause major quality losses in food industry. The oil degradation could take place during preparation, processing and storage of food products. The oxidation changes appearance, taste, as well as odor (Zhang et al., 2010; Lee et al., 2007). These changes degrade functional and nutritional compounds of food, damage essential fatty acids and produce oxidized polymers which could raise safety concerns, carcinogenic, mutagenesis, cytotoxicity and atherosclerosis and also reduces shelf life of food (Ai-li and Chang-hai, 2006). Hence, susceptibility of food towards lipid oxidation affects quality and consumer acceptability and limits its applications.

Due to the differences in their fatty acid content of the oils, palm olein, high in saturated of palmitic 16:0, 46% (Rossi et al, 2007), canola oil, high in

monounsaturated of oleic 18:1, 65% (Huang et al, 2007), and safflower oil, high in polyunsaturated of linoleic 18:2, 70% (Bozan & Temelli, 2008) are selected to be evaluated in this work. Four different fatty acids were employed in this study which is palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic acid (18:2). Samples were prepared by adding different amount of fatty acid with and without antioxidant, to the selected oil. Four types of antioxidants were used for this study which is Propyl gallate (PG), Butylhydroxyanisole (BHA), Butylhydroxytoulene (BHT) and Tert-butylhydroquinone (TBHQ). The fatty acids were added at several concentrations ranging from 0.25% w/w to 3.0% w/w. The samples of oils either in the presence or in the absence of other intentionally added species such as antioxidants and/or fatty acids were exposed to heat at 60°C in the oven for 15 days. Oil samples at specified days of exposure were then taken for peroxide value (PV), total acid number (TAN) tests, and for infrared analyses.

Quantum mechanical software package of *Gaussian09* at the theoretical level of B3LYP Density Functional Theory 6-31G(d,p) were employed for the optimization of the single species structures or the transition state structures involving the TAGs (tripalmitic, trioleic, trilinoleic) or the hydroperoxyl radical of TAG COO• with antioxidant and/or fatty acid. Physical parameters such as SCF energy, dipole moment, distance between selected species in question, and the bond length and the bond strength of the O-H of antioxidants were collected and analyzed. Concentration of lipid free radicals and the quantity of inherent

antioxidants in lipids are important factors for predicting the stability of edible oils against oxidative stress (Lee et al, 2007; Choe & Min, 2005).

Results of these experimental and theoretical studies on the performance of antioxidant in the presence of free fatty acids during processing and storage are expected to shine new light on understanding the chemical and physical interaction between these important species, and therefore to further improve the world food security. This research will contribute most to the vegetable oil industries as well as in the development of specific antioxidant for specific vegetable oil hence the theoretical concern in oleochemical food security R&D. The use of proper or ideal antioxidant would combine effectiveness in low concentrations with minimal toxicity and also can help to extend the shelf life of vegetable oil without any expensive genetic modification which can save a lot of government money. Since this research involved the free radicals species and the oxidation (antioxidant), this finding should also be benefited in the pathogenesis studies of chronic diseases, such as cardiovascular disease, cancer, atherosclerosis, and age-related macular degeneration.

1.2 Vegetable Oils

Vegetable oil can be classed as edible and inedible vegetable oils. The inedible vegetable oils such as linseed oil, castor oil, and tung oil, usually used in

lubricants, paints, cosmetics, pharmaceuticals, and other industrial purpose. Their production and use is based on a wide range and supporting science such as physics, chemistry, biochemistry, agriculture, seed breeding, food science, nutrition and medicine among others. The main constituents of vegetable oils are triacylglycerides. Triacylglycerides have lower densities then water, which make it as hydrophobic substance and at normal room temperature, it may be solid or liquid (Formo *et al*, 1979). When solid, they are called fats or butters and when liquid they are called oils. Triacylglycerol, are a chemical compound formed from one molecule of glycerol and three fatty acids (Knothe and Dunn, 2001). The basic structure of triacylglycerol is shown in Figure 1.1. Besides triacylglycerol, other component of vegetable oils are monoglycerol, diacylglycerol, free fatty acids, phospholipids, free and esterified sterols, triterpene alcohols, tocopherols, tocotrienols, carotenes, chlorophylls and other coloring matters, and hydrocarbons as well as traces of metals, oxidation products, undesirable flavors and many more.

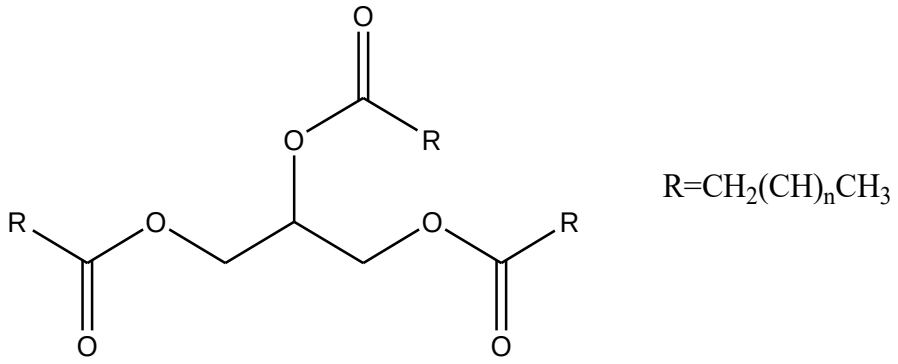


Figure 1.1: Structure of triacylglycerol

1.2.1 Palm Olein

The palm tree belongs to a family of plants known as *Palmae* or *Palmaceae*. Palm oil is derived from the flesh of the fruit from oil palm species *Elaeis guineensis*. It is originated in West Africa and now has spread to most parts of the tropical and subtropical zone of the world but particularly Malaysia and Indonesia. Malaysia is now the world second largest producer and exporter of palm oil.

Palm oil is one of the most important sources of revenue for Malaysia. Palm oil has now gained worldwide acceptance due to its unique properties and versatile applications as well as the competitive traded price over other vegetable oils (Choo *et al.*, 2007). Statistic retrieved from Malaysia Palm Oil Board (MPOB) states that Malaysia is currently the second major producer of palm oil right behind Indonesia where Malaysia produced 10.7 mill tons of palm oil from January till July this year. As one of the leading countries in the palm oil business, attempts should be taken to ensure that palm oil is of good quality.

The oil palm tree has the appearance of a date palm with a large head of pinnate feathery fronds growing from sturdy trunk. The fruit growing in bunches weighing 22 to 110 pounds and each containing 800 to 2000 individual fruits. The fruits consist of the outer pulp which is the source of crude palm oil, an inner shell which is used for fuel and another locate in the middle is kernel which are the source

of palm kernel oil. The physical and physicochemical properties of palm (*E. guineensis*) fruit pulp oil are presented in Table 1.1.

Table 1.1: Physicochemical properties of palm (*E. guineensis*) fruit pulp oil

Characteristics	**Physicochemical properties**
Refractive index (30 ^{o}C)	1.4612
Specific gravity (g/cm^3) at 25 ^{o}C	0.918
Acid number (mg KOH/g)	2.36
Peroxide value (meq/kg)	2.96
Iodine value (g/100 g)	45.6
Saponification value (mg KOH/g)	183.8
Cetane number	42.0
Caloric value (MJ/kg)	39.5
Cloud point (^{o}C)	31.0
Flash point (^{o}C)	267
Kinematic viscosity (cSt at 38 ^{o}C)	39.6

Source: Bora *et al*, 2003

Palm oil is composed of fatty acids, esterified with glycerol just like any ordinary fat. It contains a balance of polyunsaturated, monounsaturated and saturated fatty acids. In biological systems, single double bond is always in CIS configuration. According to Sambanthamurthi (2000), PO are highly suitable for deep frying because of low content of polyunsaturated fatty acid and higher level of saturated fatty acids. The oil palm gives its name to the 16-carbon saturated fatty acids, palmitic acid found in palm oil; monounsaturated oleic acid is also a major

constituent of palm. The detailed fatty acids composition of palm oil are presented in Table 1.2.

Table 1.2: Fatty acid composition of palm oil

Fatty acid	Formula	IUPAC Name	Structure	Weight (%)
Lauric	$C_{12}H_{24}O_2$	Dodecanoate	$C_{12:0}$	0.35
Myristic	$C_{14}H_{28}O_2$	Tetradecanoic	$C_{14:0}$	1.08
Palmitic	$C_{16}H_{32}O_2$	Hexadecanoic	$C_{16:0}$	43.79
Palmitolic	$C_{16}H_{30}O_2$	Cis-9-hexadecenoic	$C_{16:1}$	0.15
Stearic	$C_{18}H_{36}O_2$	Octadecanoic	$C_{18:0}$	4.42
Oleic	$C_{18}H_{34}O_2$	Cis-9-Octadecenoic	$C_{18:1}$	39.9
Linoleic	$C_{18}H_{32}O_2$	cis-9,cis-12-Octadecedianoic	$C_{18:2}$	9.59
Linolenic	$C_{18}H_{30}O_2$	cis-6,cis-9,cis-12-Octadecatrienoic	$C_{18:3}$	0.17
Arachidic	$C_{20}H_{40}O_2$	Eicosanoic	$C_{20:0}$	0.38

Source: Darnako and Cheryan, 2000

Palm oil is the largest natural source of tocopherols, carotinoids and tocotrienol which is part of vitamin E family that acts as natural antioxidants against damaging free-radicals. Palm oil also contains high in vitamin K and dietary magnesium. Splitting oils and fats produces the fatty acids. Glycerin is produced as by-product. The split fatty acids are a mixture of fatty acids ranging from C6 to C18 depending on the type of oil/fat. The pure fatty acid is used as an important raw material in the manufacture of soaps, washing powder and other personal care

products. According to Kuntum and Hamirin (2000), about 10 % of palm oil is used for non-food product such as oleochemicals, cosmetics and biofuels (Chuah *et al*., 2006).

1.2.2 Canola Oil

Canola is the registered trademark of the canola council of Canada for the genetically modified seed, oil and meal derived from rapeseed cultivars *Brassica napus* and *Brassica campestris.* Rapeseed is one of the oldest vegetable oils known but its edible use has been limited because of high levels of erucic fatty acids (C-22:1) and glucosinolates. Oils which contain high in erucic fatty acids have been shown to cause heart muscle lesions followed by other cardiac problems and the presence of glucosinolates in meals reduce its nutritive value as an animal feed. The world first low erucic acid, low glucosinolate rapeseed cultivar was released in 1974. In response to a petition from Canada, the United Stated affirmed low erucic rapeseed oil (LEAR oil) as food substance generally recognized as safe (GRAS) in 1985. In 1988, the US Food and Drug Administration (FDA) agreed that LEAR oil (2.0% maximum) could be identified as canola. The genetically modified oilseed plant has become the world third leading source of vegetable oil and meal in less than 30 years. Commodity canola oil, with its low level of saturated fatty acids and containing both n-6 and n-3 essential fatty acids, is perceived as a healthy oil (Gunstone, 2001). The characteristic of canola oil are shown in Table 1.3 while the fatty acids composition are shown in Table 1.4

Table 1.3: Typical characteristic for canola oil

Characteristics	**Physicochemical properties**
Refractive index (30 °C)	1.470 to 1.474
Specific gravity (g/cm^3) at 25 °C	0.914 to 0.920
Acid number (mg KOH/g)	-
Peroxide value (meq/kg)	-
Iodine value (g/100 g)	109.5
Saponification value (mg KOH/g)	182 to 193
Cetane number	-
Caloric value (MJ/kg)	
Cloud point (°C)	-5.0
Melting point (°C)	-9.0
Kinematic viscosity (cSt at 38 °C)	-

Source: Vaisey-Gensor, 1987

Canola seed is flaked and cooked to inactivate the enzyme *myrosinase* to prevent hydrolysis of glucosinolates into undesirable breakdown products. The oil is extract from the cooked flake by pressing and solvent extraction procedures. Usually the crude canola oil is degummed to remove the water-hydrated gums to phosphorus levels of approximately 240 ppm with water degumming or approximately 50 ppm with acid degumming procedures (Bagge, 1993; List *et al*., 1996; Ackman, 2001).

Table 1.4: Fatty acids composition of genetically modified Canola oil

Fatty Acid (%)	Canola	Low-Linolenic Canola	High Oleic Canola	Lauric Canola
Lauric (C-12:0)	0	0	0	37.0
Myristic(C-14:0)	0.1	0.1	0.1	4.4
Palmitic(C-16:0)	4.2	3.8	3.0	3.2
Palmitoleic(C-16:1)	0.3	0.3	0.3	0.3
Stearic (C-18:0)	2.3	2.4	2.0	1.3
Oleic(C-18:1)	62.5	64.1	73.7	31.5
Linoleic(C-18:2)	19.2	23.8	14.4	13.1
Linolenic(C-18:3)	7.9	2.1	2.9	6.7
Arachidic(C-20:0)	0.7	0.7	0.7	0.5
Gadoleic(C-12:1)	1.3	1.2	1.4	1.0
Eicosadienoic(C-20:2)	0.1	0.1	0.1	0.1
Behenic(C-22:0)	0.3	0.3	0.3	0.3
Erucic(C-22:1)	0.3	0.3	0.1	0.2
Lignoceric(C-24:0)	0	0	0.2	0
Nervonic(C-24:1)	0.2	0.2	0.2	0.1

Source: Vaisey-Genser, 1987

Canola oil is low in saturated and high in monounsaturated and contains a high level of oleic, characteristic to those similar to olive, high oleic sunflower and high oleic safflower oils. Canola oils are the lowest in saturated and its mono unsaturated level is exceeded only by high oleic sunflower and safflower oils. Distribution of the two fatty acids importance for flavor stability, linoleic and linolenic have been found primarily in the sn-2 position of the triglyceride similar to high-erucic rapeseed oil. This differ from other oils, which usually have a random distribution for linoleic and linolenic fatty acids and the somewhat lower total unsaturation indicates better resistance to oxidation than oil with similar linoleic and linolenic fatty acid contents.

1.2.3 Safflower Oil

Safflower, *Carthamus tinctorius*, is among the oldest crop known. The spesies is believed to be indigenous to Southeastern Asia but has long been cultivated in China, the Near East, and the Northern Africa. Until recent years, the history of safflower has been concerned almost entirely with the use of its florests, from which carthamine, a dye, was extracted. Later, the introduction of other more stable dyes replaced this use for safflower plant. Safflower was relatively insignificant oilseed crop until the early 1950s, when higher yielding oil-bearing varieties were developed and it was established as a source of oil for surface coatings. The composition of safflower oil is largely made up of linoleic fatty acid with a very low level of linolenic acid, which results in very nearly an ideal drying oil. Interest in the ability of the unsaturated liquid oil to lower serum cholesterol levels catalyzed the development of an edible grade of safflower oil.

Safflower oil is obtained by pressing the seed or by solvent extraction. Safflower oil occupies a unique position in that it has a higher level of linoleic fatty acid available commercially. The characteristic and compositions of safflower oil are shown in Table 1.5 and Table 1.6.

Table 1.5: Typical characteristic for safflower oil

Characteristics	**Physicochemical properties**
Refractive index (30 °C)	1.473 to 1.476
Specific gravity (g/cm^3) at 25 °C	0.919 to 0.924
Acid number (mg KOH/g)	-
Peroxide value (meq/kg)	-
Iodine value (g/100 g)	141 to 147
Saponification value (mg KOH/g)	186 to 194
Unsaponifiable number	0.3 to 0.6
Caloric value (MJ/kg)	
Cloud point (°C)	-
Melting point (°C)	-18 to -16
Kinematic viscosity (cSt at 38 °C)	31.3

Source: Vaisey, 1987

Table 1.6: Fatty Acid composition of safflower oil

Fatty Acid (%)	Weight (%)
Myristic(C-14:0)	0.1
Palmitic(C-16:0)	6.8
Palmitoleic(C-16:1)	0.1
Stearic (C-18:0)	2.3
Oleic(C-18:1)	12.0
Linoleic(C-18:2)	77.7
Linolenic(C-18:3)	0.4
Arachidic(C-20:0)	0.3
Gadoleic(C-12:1)	0.1
Behenic(C-22:0)	0.2

Source: Bozan & Temelli, 2008

Safflower oil has appeal to health conscious consumers. Linoleic, its principle fatty acid, is an essential fatty acid that cannot be synthesized by the human body. It is required for ensuring the integrity of plasma membrane s for growth, reproduction, skin maintenance, and general body functioning. The health benefits of conjugated linoleic fatty acids are also attracting interest. The potential therapeutic properties are that it is anti-carcinogenic, anti-atherosclerotic, growth promoting and lean body mass enhancing. Safflower oil is the natural raw material for the production of conjugated linoleic fatty acid (Terrones, 2001)

The oxidative stability of crude safflower oil precludes storage for indefinite periods before processing. Generally, standard active oxygen method (AOM) stabilities of crude safflower oil without added antioxidants range from 4 to 8 hours shortly after crushing, which reduces 1 to 3 hours after 2 to 4 month of normal storage. Thus, it is obvious that safflower oil with high linoleic fatty acid content and low level of natural antioxidant is not particularly stable.

Safflower oil has been used in food products where high polyunsaturated fatty acid content is desired. It has been utilized in mayonnaise, salad dressing and liquid margarine and was the original source oil for the first soft tub margarine. Flavor stability has been a constant problem with products containing appreciable quantities of safflower oil due to the high linoleic fatty acid content. Safflower oil is readily hydrogenated in conventional processing equipment. Hydrogenation improves oxidative stability and the products can be used in margarine or shortening products to replace the usual β-crystal–habit base-stocks. However, the oxidative

stability of hydrogenated safflower oil is significantly less than similar products produced with soybean oil or corn oil hardened to the same degree (Blum, 1966).

1.3 Oxidation of Vegetable Oils

Oxidative stability is one of the most important indicators for maintaining the quality is oxidative stability (Gapinski *et al*., 1994; Becker and Knorr, 1996). It is known as the resistance to oxidation under defined conditions and is expressed as the period of time required to reach an end point, which can be selected according to different criteria (e.g. development of rancidity), but usually corresponds to a sudden increase in oxidation rate (Velasco et al., 2004). Lipid oxidation products are responsible for the development of rancidity by production of low molecular weight fission compounds that impart undesirable flavors. Oxidation of lipids not only produces rancid odors and flavor but also can decrease the nutritional quality and safety by the formation of secondary products in food after cooking and processing (Frankel, 1996). The reaction of oxygen with unsaturated lipid produces wide range of compounds that have attracted considerable interest and research. Oxidation mechanisms can be divided into two which are autoxidation and photo-oxidation. Autoxidation involves the formation of free radicals, highly unstable species that initiate a chain reaction while photo-oxidation involves direct addition of the highly reactive singlet oxygen to lipid (Azaredo et al., 2004).

The oxidative stability of fats and oils is related to their TAGs composition and antioxidants present (Hrncirik and Fritsche, 2005; Matoes et al., 2005) and iron content (Coscione and Artz, 2005). Vegetable oils contain natural antoxidants such as tocopherols (Hrncirik and Fritsche, 2005) so even without added antioxidant they exhibit some resistance to oxidation. Unsaturated carbon–carbon bonds function as active sites for many reactions for oxidation and many other reactions. A majority of triglyceride-based vegetable oils contain unsaturated fatty acids and are susceptible to oxidation. This is primarily due to the presence of bisallylic protons which is highly susceptible to radical attack (Adhvaryu *et al*, 2005). The greater the level of unsaturation, the more susceptible the oil becomes to oxidation (Sherwin, 1978).

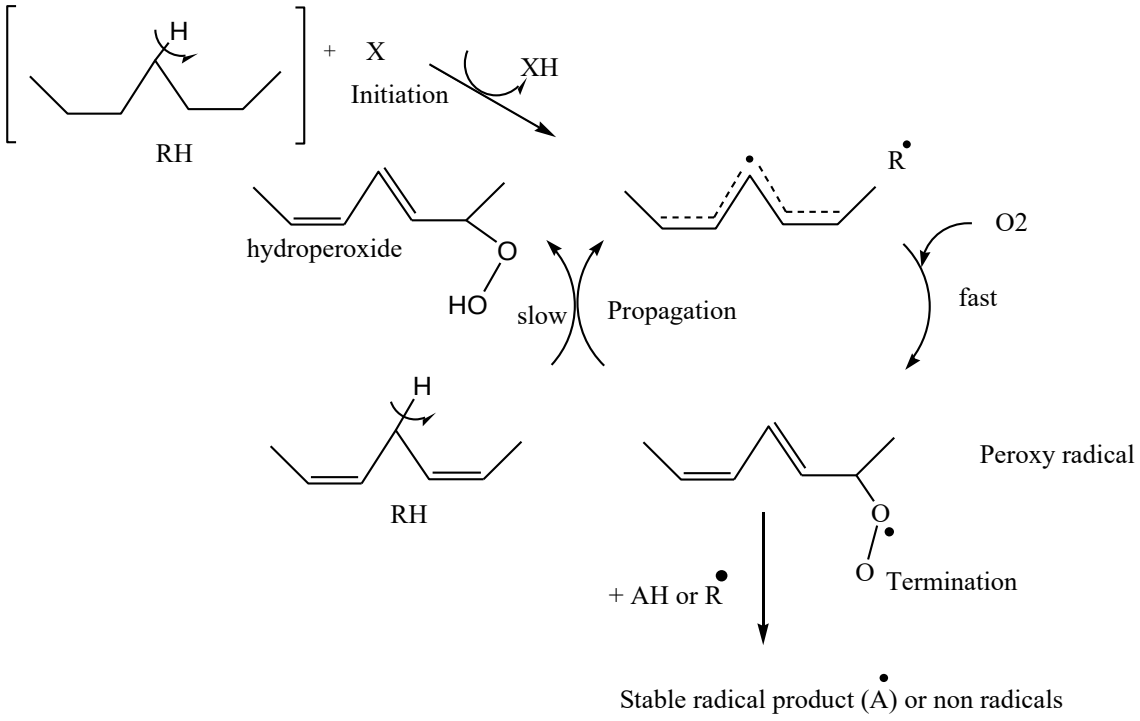
Rancidity is another factor that makes edible products unacceptable to consumers. Oxidative rancidity in vegetable oils occurs when heat, metals or other catalysts cause unsaturated oil molecules to convert to free radicals. These free radicals are easily oxidized to yield hydroperoxides and organic compounds such as aldehydes, ketones or acids (Das et al., 2009; Vlachos et al., 2006), which give rise to the undesirable odors and flavors characteristics of rancid fats (Naz et al., 2004). This process proceeds rapidly in vegetable oils as a chain reaction. Hydroperoxides are primary products formed by oxidation mechanism is quite unstable and their decomposition products are responsible for oxidative rancidity (Azaredo et al., 2004). It rates can be decreased by primary antioxidants, which compete with lipids, breaking off free radical chain. The role of peroxides is

exploited in monitoring oxidative deterioration by measuring peroxide values (PV) (Mochida *et al*., 2006).

The autoxidation of the vegetable oils produced by chain reaction where the molecule of oxygen is added to carbon atom adjacent to a double bond to form hydroperoxide, that have a double intact link (Mannekote et al., 2012). The autoxidation mechanism essentially a free radical chain reaction consists of initiation, propagation and termination. Propagation reactions are primarily responsible for the autocatalytic nature of autoxidation.

Figure 1.2 and Table 1.7 shows the mechanism and phases of the oil autoxidation. As mention earlier, oxidation is initiated by formation of free radicals. Free radicals can easily be formed from the removal of a hydrogen atom from the methylene group next to a double bond. Free radicals rapidly react with oxygen to form a peroxy radical. The peroxy radical can then attack another lipid molecule to remove a hydrogen atom to form a hydroperoxide and another free radical, propagating the oxidation process. The abstraction of a hydrogen atom by the peroxy radical to generate a hydroperoxide is the rate-limiting step of vegetable oil autoxidation (Porter *et al*., 1995). The rate constant for the rate-limiting step depends primarily on the strength of the carbon–hydrogen bond being destroyed. The strength of a carbon–hydrogen bond next to a carbon–carbon double bond is lowered and the hydrogen can be removed easily, thus those oils containing double bonds are more susceptible to autoxidation. As the number of double bonds

increases there become more sites susceptible to the abstraction of a hydrogen atom and the autoxidation process can occur at a faster rate. Vegetable oils containing a high percentage of monounsaturated fatty acids will typically autoxidise only at high temperatures, whereas those oils containing polyunsaturated, such as linoleic and linolenic acid, readily autoxidise at room temperatures.



where; RH = allylic of oleic or linoleic
R• = alkyl radical

Figure 1.2: Phases of the autoxidation process (Rizwanul *et al*., 2014)

Table 1.7: Classical representation of oil autoxidation mechanism (Fox and Stachowiak, 2007).

Initiation	$RH \rightarrow R\cdot + H\cdot$
Propagation	$R\cdot + O_2 \rightarrow ROO\cdot$ $ROO\cdot + RH \rightarrow ROOH + R\cdot$
Branching	$ROOH \rightarrow RO\cdot + OH\cdot$ $RO\cdot + RH + O_2 \rightarrow H_2O + ROO\cdot$
Termination	$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$ $ROO\cdot + R\cdot \rightarrow ROOR$ $R\cdot + R\cdot \rightarrow R\text{-}R$
Peroxide decomposition	ROOH → various lower molecular weight compound
Polymerization	ROOH → various higher molecular weight compound

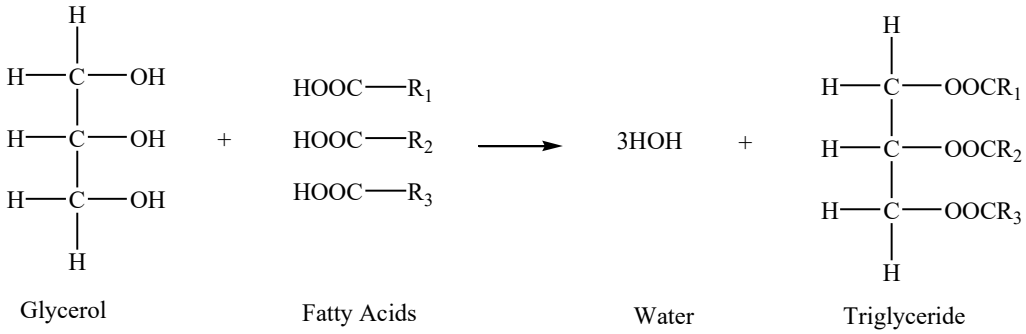
where; RH = allylic of oleic or linoleic
R• = alkyl radical
RO• = alkoxy radical
ROO• = peroxy radical
ROOH = fatty acid hydroperoxide

Oxidation causes a decrease in the relative percentages of the unsaturated fatty acids and an increase in the relative percentages of the saturated fatty acids (Liu and White, 1992). Therefore, Linoleic acid and palmitic acid are usually used as indicators of the extent of oil deterioration because linoleic acid is more susceptible to oxidation, whereas palmitic acid is more stable toward oxidation.

1.4 Fatty Acids

Fatty acids are included amongst compounds naturally present in low amounts in vegetable oils. The physical and chemical characteristic of the oils are greatly influenced by the kind and proportion of the fatty acids on the triacylglycerol (Senanayake and Shahidi, 2002).

Structurally, a triglyceride is the reaction product of one molecule of glycerol with three fatty acid molecules to yield three molecules of water and one molecule of triglyceride (Goering *et al.,* 1982). As illustrated in Figure 1.2, up to three fatty acids are linked to a glycerol molecule with ester linkages. The fatty acids vary in their carbon chain length and in the number of unsaturated bonds.



$R_1, R_2, R_3 = CH_2 (CH_2)_n CH_3$

Figure 1.3: Formation of triglycerides

There are three main types of fatty acids that can be present in a triacylglycerides which can be classified in classes as saturated (Cn:0), monounsaturated (Cn:1), MUFA and polyunsaturated with two or three double bonds (Cn:2,3), PUFA. Fatty acids are almost entirely straight chain aliphatic carboxylic acids. The broadest definition includes all chain lengths, but most natural fatty acids are C4 to C22, with C18 most common. Table 1.8 Shows type of fatty acids in commodity oils and fats. Ideally the vegetable oil should have low saturation and low polyunsaturation i.e is high in monounsaturated fatty acid (Gunstone, 2004).

The most reactive sites of fatty acids are the carboxyl group and double bonds. The methylene groups that are adjacent to them are activated, increasing their activity. Only rarely do saturated chains show reactivity. Carboxyl groups and unsaturated centers usually react independently, but when in close proximity, both may react through neighboring group participation. In enzymatic reactions, the reactivity of carboxyl group can be influence but the presence of a nearby double bond.

Fatty acids in vegetable oil appear during the degradation of vegetable oils (Serri *et al.*, 2008). Fatty acids are primarily released from the TAG by β- hydrogen elimination and hydrolysis. Both β-hydrogen elimination and hydrolysis are not oxidation reactions but are likely to occur coincidentally with oxidation as another degradation process (Fox and Stachowiak, 2007). In TAG, there is a single

hydrogen atom on the second, or β, carbon. This β-hydrogen is readily susceptible to elimination. If the β-hydrogen is removed, the middle carbon-oxygen bond grows weak, and a free fatty acid will form (Goyan *et al*., 1998; Fox and Stachowiak, 2007).

Table 1.8: Fatty Acids in commodity oils and fats

Fatty acid	Trivial Name	Formula	Chain length
4:0	Butyric	$CH_3(CH_2)_2CO_2H$	short
6:0	Caproic	$CH_3(CH_2)_4CO_2H$	short
8:0	Caprylic	$CH_3(CH_2)_6CO_2H$	short
10:0	Capric	$CH_3(CH_2)_8CO_2H$	medium
12:0	Lauric	$CH_3(CH_2)_{10}CO_2H$	medium
14:0	Myristic	$CH_3(CH_2)_{12}CO_2H$	medium
16:0	Palmitic	$CH_3(CH_2)_{14}CO_2H$	medium
18:0	Stearic	$CH_3(CH_2)_{16}CO_2H$	long
18:1	Oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$	long
18:2	Linoleic	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6CO_2H$	long
18:3	Linolenic	$CH_3CH_2(CH{=}CH{=}CH_2)_3(CH_2)_6CO2H$	long
22:1	Erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}CO_2H$	long
20:5	EPA	$CH_3CH_2(CH{=}CHCH_2)_5(CH_2)_2CO_2H$	long
22:6	DHA	$CH_3CH_2(CH{=}CHCH_2)_6CH_2CO_2H$	long

Being a product of hydrolytic degradation of triglycerides, their amount is considered an important quality index for oils, so that threshold values of fatty acids content in vegetable oils are provided by regulations, and vary according to the oil type and the commercial class

1.5 Antioxidants

Antioxidants are chemicals compounds that provide greater oxidative stability and longer shelf life for edible fats and oils by delaying the onset of oxidative rancidity (Lundberg, 1962). Oxidation occurs in a series of steps often referred as free radical oxidation because the initial step is the formation of a free radical on the fatty acid portion of the fat molecule. The free radical is highly reactive and forms peroxides and hydroperoxides by reaction with oxygen. These free radicals also initiate further oxidation by propagating other free radicals. Finally, the hydroperoxides split into smaller compounds such as aldehydes, ketones, alcohols, and acids (Fox and Stachowiak, 2007; Wasowicz *et al*., 2004). These compounds provide the offensive odor and flavor characteristic of oxidized oil. Antioxidants function by inhibiting or interrupting the free radical mechanism of glyceride autoxidation. Their ability to do this is based on their phenolic structure or the phenolic configuration within their molecular structure. Antioxidants or phenolic substances function as a free radical acceptor, thereby terminating oxidation at initial step. Fortunately, the antioxidant free radical that forms is stable and does not split into other compounds that provide off flavors and odors nor does it propagate further oxidation of the glyceride.

There are two major classes of antioxidant: chain breaking radical scavengers and peroxide decomposers. Chain breaking antioxidants react with radicals to form stable compounds and prevent propagation of the oxidation

reaction. The most effective of the type quench the initial peroxy and hydroperoxy radicals as well as the alkoxy and hydroxyl radicals formed during the branching stages. Some less effective radical scavengers only quench the alkoxy and hydroxyl radicals formed during the branching stages (Fox and Stachowiak, 2007). Commonly utilized chain-breaking antioxidants include butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), mono-tert-butylhydroquinone (TBHQ), propyl gallate (PG), and naturally occurring tocopherols.

1.5.1 Butylated Hydroxyanisole

Butylated hydroxyanisole (BHA) is an antioxidant which is widely used in food industry as preservatives (Shyamala *et al*., 2005). BHA is an aromatic compound with the IUPAC name of 2-tert-Butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole (mixture). Other name for BHA are 2-and 3-tert-butyl-4-methoxyphenol, tert-Butyl-4-hydroxyanisole (Yang Hua *et al*., 2002), (1,1-Dimethylethyl)-4-methoxyphenol and Antioxyne B. In its pure form, BHA is a waxy white or pale yellow solid with a melting point of 48-55˚C and a boiling point of 264-270˚C. It is normally insoluble in water. Several studies on BHA was done such as oxidative stability of biodiesel (Dunn, 2005; Maia *et al*., 2011), on medication (Gabriel, 1988; Gaunt *et al*. 1965) and many more. Figure 1.3 shows structure of BHA.

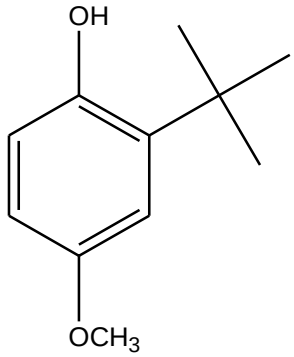


Figure 1.4: Molecular structure of Butylated Hydoxyanisole

1.5.2 Butylated hydoxytoulene

Butylated hydroxytoluene (BHT) is an antioxidant which is almost similar as BHA and also used as preservatives in food containing fats (Tuner and Korkmaz, 2007; Grochowska and Buta, 1985). BHT is an aromatic compound with it IUPAC names of 2,6-bis(1,1-dimethylethyl)-4-methylphenol. Other chemical name for BHT are 2,6-*ditert*-butyl-4-methylphenol, 2,6-*di-tert*-butyl-*p*-cresol, 3,5-di-tert-butyl-4-hydroxytoluene and Additin RC 2110. Its physical appearances are in form of white granular crystals with a melting point of 68-69 ˚C and boiling point of 265 ˚C. Several studies on BHT was done especially on controlling oxidation in biodiesel (Das *et al*., 2009; Dunn, 2005) and during vegetable oil or food storage (Ansorena and Astiasaran, 2004 ; Suja *et al*., 2004). Figure 1.4 is the structure of BHT.

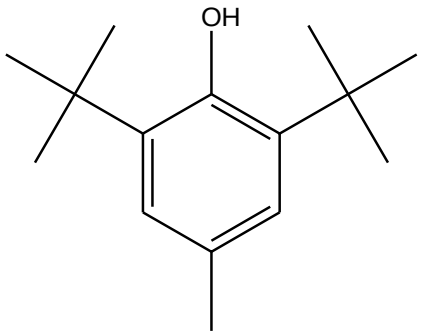


Figure 1.5: Molecular structure of Butylated Hydoxytoulene

1.5.3 *Tert*-Butylhydroquinone

Tert-butylhydroquinone (TBHQ) is another aromatic compound which is act as antioxidant and widely used as food preservative (Yang *et al*., 2002) and in animal processed food (Gharavi and El-Kadi, 2004). IUPAC names for TBHQ is 2-(1,1-Dimethylethyl)-1,4-benzenediol. Physical appearance of TBHQ is in form of tan powder. The melting points of TBHQ are in the range of 127-129 ˚C while its boiling point is around 273 ˚C. Molecular structure of TBHQ is shown in Figure 1.6.

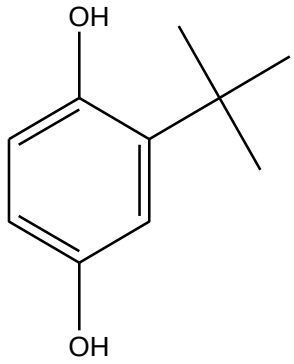


Figure 1.6: Molecular structure of *tert*-butylhydroquinone

1.5.4 Propyl Gallate

Propyl gallate (PG) is a compound which is formed from the condensation of gallic acid and propanol. Propyl gallate has been widely used in food containing oils, cosmetics and food packaging to prevent rancidity and spoilage (Nakagawa *et al*., 1996). IUPAC names for propyl gallate is Propyl 3,4,5-trihydroxybenzoate. Other name for propyl gallate is propyl ester n-Propyl gallate. Physical appearance of PG is in the form of creamy white crystalline. The melting point of PG is 150 ˚C. Molecular structure of PG is shown in Figure 1.7.

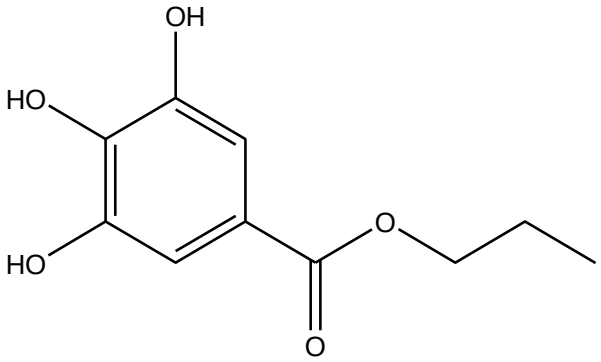


Figure 1.7: Molecular structure of Propyl Gallate

1.6 Ab Initio Quantum Mechanical Studies

Concepts of "Model Chemistry" and "Molecular System" are required for *ab initio* molecular orbital calculation. Model Chemistry refers to all theoretical aspects of calculation, whereas the Molecular System refers to the molecules to be studied (Hehre *et al*., 1986).

A theoretical model chemistry is a complete algorithm for the calculation of the energy of any molecular system. It cannot involve subjective decisions in its application. It must be size consistent so that the energy of every molecular species is uniquely defined.

1.6.1 Theoretical Approach

1.6.1.1 Hartree-Fock Molecular Orbital Theory

The Hartree-Fock (HF) approximation refers to the field approximation where each electron is considered to be independently moving in an average field of nuclei and other electrons. It approximates the N-electron wave function with a product of N one-electron wave functions. To allow for spin and the Pauli Exclusion Principle, Fock anti-symmetrized the product wave function by using a Slater

determinant, leading with external potential to the HF equations (Chien and Yang, 1998).

The HF method is capable of providing good information about geometry of molecules with less than 50 nuclear centers. The energies it predicts are accurate in a relative sense, as long as the number and type of bonds in a molecule remain unchanged. The absolute energies, however, are often not very good approximation to experimental values.

The primary deficiency of HF theory is the inadequate treatment of the correlation between motions of electrons. In particular, single-determinant wavefunctions take no account of correlation between electrons with opposite spin. Correlation of the motions of electrons with the same spin is partially, but not completely, accounted for by virtue of the determinantal form of the wave function. These limitations lead to calculated HF energies are above the exact values. By convention, the difference between the HF and exact (nonrelativistic) energies is the Correlation Energy,

$$E(exact) = E(Hartree\text{-}Fock) + E(correlation)$$

1.6.1.2 Density Functional Theory

In 1964, Hohenberg and Kohn explains that the electron density determines the external potential for an electronic systems, and that the true electron density minimizes the overall energy of the system. Since the external potential determines the wave function, knowing the density is equivalent to knowing the wavefunction, which determines all electronic properties of the system. Further, the true density may be found using a variational approach. Kohn and Sham (1965) followed up with the idea of variationality searching for a density of N non-interacting electrons equal to the true density of the system, leading to Kohn-Sham equations. The exchange-correlation functional is unknown and must be approximated, which has been a central focus of the development of DFT. Von Barth showed that Sleter Determinant - DFT (SD-DFT) in its basic form produces reasonably good results only for states that are the lowest energy of their symmetry, and that can be represented by a single Slater Determinant. Excited states are often not the lowest of their symmetry, and even ground states can fail to be represented by a single determinat. Time-Dependent Density Functional Theory (TDDFT) is a generalization of DFT which starts from the time-dependent Schrodinger equation, instead of the static Schrodinger equation, as in DFT. Runge and Gross proved that, as Hohenberg and Kohn showed in the static case, there is a unique relationship between the time-dependent potential and the time-dependent electron density. As in the static case, since the potential determines the wave function, the density is all we need to know (Casida, 2009).

The last decade has seen significant improvements in both theoretical techniques in the field of quantum chemistry and in computer hardware that allow us to calculate accurate magnetic, as well as other properties of molecules.

The purpose of this research project was to study the interaction of the molecular system of TAG tri oleic, TAG tri linoleic, TAG tri palmitic and it's radical. All calculations were performed using the same theoretical approach of DFT B3LYP/6-31G (d,p).

1.6.2 Basis Sets

A simple model chemistry employs a single theoretical method and basis set while a compound model chemistry combines several theoretical methods and basis sets to achieve higher accuracy at lower cost. Generally, a basis set is a collection of vectors which defines a space in which a problem is solved. In quantum chemistry, the 'basis set' usually refers to the set of (nonorthogonal) one-particle functions used to build molecular orbitals. Sometimes, theorists might also refer to N-electron basis sets, which is something else entirely; sets of Slater Determinants.

Method and basis set deal with the Hamiltonian operator and wavefunction in the Schrodinger equation, respectively (Richard & Horsly, 1970). Various methods and basis sets are available in a commercial *ab initio* molecular orbital

calculation package especially Gaussians softwares. The suitable combination of methods and basis set, as well as the selection of calculation level, is very important for a systematic calculation of the studied system. High model chemistry, means more accurate the results obtain (Forceman and Frisch, 1996). However, the highest model chemistry is to be avoided since the computational cost will increase logarithmically with calculation level. Using the minimal computational resources to achieve accurate enough results is challenge for the *ab initio* molecular orbital calculation (Hehre *et al*., 1986).

1.6.2.1 Minimal Basis Sets STO-3G

STO-3G basis sets are the minimal basis sets where only sufficient orbital are used to contain all electron in the neautral atom Hehre *et al*, 1969). It is also the most widely used basis set for large systems and preliminary geometry determinations. STO-3G were propose by John Pople. In STO-3G, it use three gaussian primitives per basis function (3G). STO stands for Slater Type Orbitals and the STO-3G basis set approximates Slater orbital with gaussian funtions. The example of minimal basis set which contain the minimal number of basis function (i.e: H: 1s ; C: 1s, 2s, $2p_x$,$2p_y$, $2p_z$). Initially Slater-type orbital's (STO's) were used as the basis functions are for calculations on small molecules but the difficulties inherent in the accurate and speedy evaluation of Gaussian Type Orbital's (GTO) is still extremely time consuming for molecules of an appreciable size (Suthers and Linnett, 1974).

The model chemistry of HF/STO-3G which is the minimal basis set and the entry-level method in *ab initio* molecular orbital calculation is a low calculation level which is not sufficient for frequency calculation because there is no scale factor for frequency scaling at this level (Chen and Yang, 1998).

1.6.2.2 Split Valence Basis Sets

One way to increase the size of a basis set is to take more basis functions per atom. Split valence basis sets such as 3-21G and 6-31G basis sets, have two or more sizes of basis function for each valence orbital (i.e.: 3-21G and 6-31G basis have will have, H: 1s, 1s'; C:1s, 2s, 2s', $2p_x$, $2p_y$, $2p_z$, $2p_x$', $2p_y$', $2p_z$'. 6-31G is one of the largest split-valence which defines through the second row of the periodic table. It comprises inner-shell function each written in terms of a linear combination of six-Gaussian, and two valence shells represented by three- and one-Gaussian primitives respectively. 6-31G basis sets for second-row elements are constructed utilizing inner-shell description taken directly from the previously defined 6-21G basis sets, and optimized only with respect to valence-shell parameters (Hehre *et al*, 1986).

1.6.2.3 Polarized basis sets

Split valence basis sets could be improved by adding orbitals with different shapes. Polarized basis sets add orbitals with angular momentums going beyond of

requirement for the proper description of the ground state of each atom at the HF level. For example, polarized basis sets add d-functions to carbon atoms and some of them add p-function to hydrogen atoms. Examples for polarized basis sets are the 6-31G (d) and the 6-31G (d, p) basis sets.

The 6-31G (d) is one of the simplest of polarization basis sets which representations originally proposed by Hariharan and Pople in 1976 for first row atoms and later extended to second-row elements. It is constructed by the addition of a set of description of each heavy (non-hydrogen) atoms. The 6-31G (d) basis contains no provision for polarization of the s orbital on hydrogen and helium atoms. As this feature is desirable for the description of the bonding in many systems, particularly those in which hydrogen is a bridging atom, a second more complete basis set, termed 6-31G (d,p), has been constructed. It is identical to 6-31G (d) except for the addition of a single set of Gaussian p-type function to each hydrogen and helium atom (Hehre *et al*., 1986).

1.6.3 Optimization

Geometry optimization is a name for procedure that attempts to find the configuration of minimum energy of the molecule. The procedure calculates the wave and the energy at starting geometry and then proceeds to search a new geometry of a lower energy. This is repeated until the lowest energy geometry is found. The procedure calculates the force on each atom by evaluating the gradient

(first derivatives) of the energy with respect to the atomic positions. Sophisticated algorithms were then used at each step to select a new geometry, aiming for rapid convergence to geometry of the lowest energy.

It is important to recognise that this procedure will not necessarily find the global minimum, for example the geometry with lowest energy. By its nature, a successive search for a minimum finds a local minimum but not necessarily the lowest. In fact the optimization procedure stops when it finds a stationary point where force on an atom is zero, and this may also be a saddle point (i.e a transition structure). This will occur particularly if we restrict the symmetry of the molecule and do not allow the program to search the full space of the molecular configurational degree of freedom.

It is always a good idea to start a procedure of a geometry optimization calculation with a small basis set and relatively poor method (such as HF/STO-3G) before we move to the basis set and method of choice for particular problem.

1.6.4 Counterpoise Correction and Basis Set Superposition Error

The study of intermolecular distances and concomitant artificial strengthening of the intermolecular interaction often encounters an artificial shortening. The problem is called “basis set superposition errors” (BSSEs) and they are more pronounced for smaller basis sets, for instance, as monomer A approaches

monomer B, the dimer can be artificially stabilized as monomer A utilizes the extra basis functions from monomer B to describe its electron distribution, and vice versa. Van Duijneveldt et al. (1994), state that the improvement in the descriptions of monomers A and B through the addition of extra accessible basis functions is not an error in itself; the error arises from the inconsistent treatment of the monomers where they are able to access additional functions from the other monomer at shorter intermolecular distances, but at large intermolecular distances, the other monomer is too far away for its functions to provide stabilization. This inconsistent treatment of the basis set for each monomer as the intermolecular distance is varied as the source of the basis set superposition error. If this inconsistency could be perfectly eliminated, there would remain errors due to the fact that the basis set is incomplete and these would be described as "basis set incompleteness errors" (BSIEs). In the limit of a complete basis set, both the BSSE and the BSIE would be reduced to zero. The Boys and Bernardi counterpoise correction (CP) is a prescription for removing BSSE. The typical, uncorrected interaction energy between monomers A and B would be computed as:

$$\Delta E_{int}(AB) = E_{AB}^{AB}(AB) - E_{A}^{A}(A) - E_{B}^{B}(B) \qquad (1)$$

where the superscripts denote the basis used, the subscripts denote the geometry, and the symbol in parentheses denotes the chemical system considered. Thus, $E_{AB}^{AB}(AB)$ represents the energy of the bimolecular complex AB evaluated in the dimer basis (the union of the basis sets on A and B), computed at the geometry of the dimer. Likewise, monomers A and B are each evaluated at their own geometries in their own basis sets. Note that this can be performed as three separate, standard

computations: one on the dimer, one on monomer A, and one on monomer B. Alternatively, one could obtain the energy of the dissociation limit by a computation of the A+B super molecule at some very large intermolecular separation (where the distance between A and B would be so large that the basis functions of one monomer would not overlap with those of the other); this might be necessary for theoretical methods which are not size-extensive, such as truncated configuration interaction, for which the energy of A+B at infinite separation is not equal to the sum of the energies from separate computations on A and B. Correction of Eq. (1) can be done by estimating the amount by which monomer A is artificially stabilized by the extra basis functions from monomer B (and vice versa). This may be estimated as:

$$E_{BSSE}\,(A) = E_A^{AB}(A) - E_A^A(A) \qquad (2)$$

$$E_{BSSE}\,(B) = E_B^{AB}(B) - E_B^B(B) \qquad (3)$$

where the energy of monomer A in its monomer basis are subtracted from the energy of monomer A in the dimer basis (and likewise for monomer B). Geometries of monomers A and B do not change as they approach each other and form the bimolecular complex. This is often a very good approximation and simplifies the procedure; below. The monomer geometries are considered deformed as they join in the bimolecular complex. The energy of monomer A in the dimer basis must be lower(more stable) than the energy of monomer A in the monomer basis, so $E_A^{AB}(A) < E_A^A(A)$ and thus $E_A^{BSSE} < 0$ as defined above (the error is stabilizing). If this error is subtracted from the interaction energy defined in Eq. 1, the terms $E_A^A(A)$ and $E_B^B(B)$ cancel, yielding:

$$\Delta E_{int}^{CP}(AB) = E_{AB}^{AB} - E_{A}^{AB} - E_{B}^{AB} \quad (4)$$

The energy of monomer A in the dimer basis can be evaluated by placing all the basis functions of monomer B on the atomic centres of monomer B while neglecting the electrons and the nuclear charges of monomer B. The functions on monomer B are thus referred to as "ghost functions," or the atoms of B are referred to as "ghost atoms" in such a computation. The procedure should "overcorrect" for BSSE, because some of the basis functions in monomer B are occupied and hence unavailable to monomer A because of the Pauli Exclusion Principle. Indeed, there do appear to be situations in which the counterpoise correction overcorrects, particularly for smaller basis sets (Dunning, 2000). This appears to happen in particular for hydrogen-bonded complexes (although the counterpoise corrected interaction energies approach the complete basis set more smoothly and are more suitable for extrapolation) (Halkier *et al*, 1999). On the other hand, for dispersion-dominated systems it appears that the counterpoise corrected values are superior (Sherril *et al*, 2009). Van Duijneveldt *et al* (1994) have argued that the counterpoise correction does not overcorrect, 3 and that poorer agreement with experiment or higher-level theory after counterpoise correction is a reflection of BSIE or other errors. Some researchers prefer to use the average of the uncorrected and the counterpoise-corrected values (Kim et al, 2000). This procedure appears to work quite well for hydrogen-bonded complexes among others (Halkier *et al*., 1999).

1.6.5 Counterpoise Correction in Cluster

The trimers and large clusters for counterpoise correction can be define as follows (Van Duijneveldt *et al*,. 1994):

$$\Delta E_{int}^{CP}(ABC) = E_{ABC}^{ABC}(ABC) - E_{A}^{ABC}(A) - E_{B}^{ABC}(B) - E_{C}^{ABC}(C) \quad (4)$$

These trimer interaction energies may be broken down into their two-body and three-body components, according to the definitions of Hankins et al., 1970). The two-body interactions are defined as

$$\Delta^2 E_{int,AB}^{CP} = E_{AB}^{ABC} - E_{A}^{ABC} - E_{B}^{ABC} \quad (5)$$

$$\Delta^2 E_{int,AC}^{CP} = E_{AC}^{ABC} - E_{A}^{ABC} - E_{C}^{ABC} \quad (6)$$

$$\Delta^2 E_{int,BC}^{CP} = E_{BC}^{ABC} - E_{B}^{ABC} - E_{C}^{ABC} \quad (7)$$

The total interaction energy is then written as a sum of these two-body interaction energies plus a three-body interaction energy,

$$\Delta E_{int,ABC}^{CP} = \Delta^2 E_{int,AB}^{CP} + \Delta^2 E_{int,AC}^{CP} + \Delta^2 E_{int,BC}^{CP} + \Delta^3 E_{int,ABC}^{CP} \quad (8)$$

The total interaction energy can be computed according to equation 4 above, so that the three body term is:

$$\Delta^3 E_{int,ABC}^{CP} = \Delta E_{int,ABC}^{CP} - \Delta^2 E_{int,AB}^{CP} - \Delta^2 E_{int,AC}^{CP} - \Delta^2 E_{int,BC}^{CP} \quad (9)$$

1.6.6 Self-Consistent Field Energy

Self-Consistent Field (SCF) calculations are commonly performed with basis sets composed of atom-centred linear combinations of Gaussian orbital's (Lee and Head-Gordon, 2000). It is too complex to be found directly, but can be approximated by simpler wave function. The SCF method involves selecting a Hamiltonian approximation, solving the Schrödinger equation in order to obtain more accurate set of orbital. To calculate a potential energy surface, the electronic Schrödinger equation must be solved, for a system of *n* electrons and *N* nuclei, over a range of nuclear coordinates. This is defined as an *ab intio* method, since it is derived from 'first principles'. In the absence of an applied external magnetic field, the ground state and excited electronic states can be obtained by SCF calculation (De Dios, 1996).

1.6.7 Dipole moment

Dipole moment reflects the molecular charge distribution and given as a vector in three dimensions. It can be used as a parameter to describe the charge movement across the molecule. The direction of the dipole moment vector in a molecule depends on the centres of negative and positive charges. For charged systems, its value depends on the choice of origin and molecular orientation

(Balachandran *et al*., 2012). Dipole moments are always given in units of Debye (Foresman and Frisch, 1993).

1.7 Statement of the problem

The shelf life of vegetable oils in food uses and their applicability in industrial situations is greatly depending on their oxidative stabilities. Vegetable oil mainly composed of triacylglycerols, diacylglycerols, monoacylglycerols and some free acids may go through oxidation which cause food quality deterioration, and it has been a challenge for manufacturers and food scientists alike (Gan *et al*. 2005). Vegetable oils are susceptible to oxidative processes in the presence of catalytic systems (such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives) (Tan *et al*., 2002). Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species (Fox and Stachowiak, 2007).

Several studies have been published on the effects of antioxidants and free fatty acids, separately, on the oxidative stability of vegetable oils during processing and storage. However, the available information is insufficient and most of the previous research studies involved only experimental work. Literature reviews also reveals that up to present date, there still isn't any experimental or theoretical

studies done on the performance of antioxidant in inhibiting the autoxidation of vegetable oils in the presence of different composition of FFA. Therefore, the aim of this research study is to determine the effect of saturated and polyunsaturated fatty acids on performance of selected antioxidants in inhibiting the autoxidation process of vegetable oils.

1.8 Aim and Objectives

The aim of this research is to study the effect and investigate the influence of fatty acids addition towards antioxidants performance in inhibiting TAG degradation process, formation of peroxide group and to seek the physical parameters that can be used to evaluate the performance of antioxidants. Hence, the objectives of the study are:

i) To investigate the effects of fatty acids on TAG hydrolyses and the formation of peroxide group;

ii) To evaluate the performance of antioxidants in inhibiting TAG decompositions and peroxide formation;

iii) To investigate the effects of added fatty acids toward antioxidants performance in inhibiting TAG decomposition and peroxide formation and

iv) To find the physical parameters which responsible in delaying or increased the oxidation of vegetable oil either by hydrolyzing TAG or formation of peroxide group.

CHAPTER 2

LITERATURRE REVIEW

2.1 Oxidation of Palm Olein

Oil obtained from the palm fruits (*Elaies guineensis*) has grown to be one of the most important vegetable oils due to its advantageous properties such as high productivity, low price, high oxidation stability, fatty acid composition and, finally, good plasticity at room temperature (Nor Aini and Miskandar, 2007). Palm oil, with its high smoke point and strong heat-oxidation resistance, is widely used both on its own as well as in various combinations as frying or cooking oil (De Marco *et al*., 2007; Rossel, 2003). Many researches were done previously to studies the oxidative stability of palm olein in various conditions.

In 2010, Bansal *et al* studies the performance of palm olein in repeated deep frying and controlled heating processes. They had discovered that thermo-oxidative reactions induced under the heating conditions were found to be faster as compared to those under the frying conditions. The polymeric triglycerides formed at the end of heating cycles (16.42%) were substantially higher as compared to those formed at the end of frying French fries (9.72%) and chicken nuggets (5.34%).

In 2005, Gan *et al*. had done research on the storage stability of RBD palm olein using surface acoustic wave (SAW) sensor-based electronic nose. They observed that high correlation was seen between electronic nose responses and chemical test data, as well as sensory evaluation score, by using Pearson's correlation. They concluded that the SAW sensor based electronic nose may be utilized as an analytical tool to follow the progress of oxidation and breakdown of vegetable oil.

Jaswir *et al*., (2000) used natural antioxidant in refined palm olein during repeated deep fat frying to study their oxidative stability. The natural antioxidants used were oleoresin rosemary extract, sage extract and citric acid. Results revealed that the use of these natural antioxidants could improve the sensory acceptability of potato chips during a 5-day repeated deep-fat frying.

Sambanthamurti *et al.,* (2000), mention that palm oil is more resistant to oxidation because of its higher level of saturated fatty acids. It is the unsaturated fatty acids in the oil which are susceptible to oxidation. In addition, palm oil contains natural antioxidants such as tocopherols and carotenoids which help to reduce oxidation process.

In 2002, oxidative stability studies by Tan *et al*. mention that microwave power from low to high has been shown to greatly influence the quality of oils where the microwave heating had significant ($P < 0.05$) effect on these quality

parameters. Oil samples heated at high-power setting showed significantly ($P<0.05$) more heat abuse than oil samples heated at medium and low-power settings. Highly significant ($P < 0.05$) increases or decreases for PV, AnV, FFA, IV, C18:2/C16:0, DSC peak temperatures were noticed for oil samples heated at high-power settings. Generally, the experimental results of the anisidine value and free fatty acids showed an increase with heating time.

2.2 Oxidation of Canola Oil

Few researches were also done on oxidative stability on canola oil. In 2011, Moser studied the influence of extended storage on fuel properties of methyl ester prepared from canola and few other selected vegetable oil. Studies revealed that palm oil methyl esters were least affected by oxidative degradation among the methyl esters, as indicated by comparatively high retention factor (R_f) and low increase ratio in viscosity (I_r) values, as well as small changes in total acid number. This was attributed to the relatively high content of saturated and low content of polyunsaturated fatty acid methyl esters identified in palm oil methyl esters. In contrast, canola oil methyl esters were most significantly affected by extended storage, as measured by comparatively low R_f and high I_r values, as well as greater changes in total acid number versus the other methyl esters.

In 1988, Hawrysh and Shand study the storage stability of antioxidant treated canola oils subjected to accelerated storage tests. Evaluations of the efficacy

of additions of varying levels of TBHQ and a combination of BHA and BHT to canola oils were made following exposure to the Schaal oven and fluorescent light accelerated storage tests. They had discovered that TBHQ at 100 or 200 ppm was effective in retarding oxidative rancidity in canola oils subjected to accelerated storage at 65°C for up to 16 days (Schaal oven test). However, the efficacy of TBHQ was not improved by citric acid. Addition of the commonly used mixture of BHA/BHT to canola oils resulted in only a slight decrease in oxidation during the storage test. During accelerated storage in fluorescent light (7500 lux) at 24°C for up to 24 h, none of the antioxidants evaluated (TBHQ and BHA/BHT) was effective in enhancing the storage stability of canola oils.

Lee and Choe (2011) evaluated the effects of phosphatidylcholine (PC) or phosphatidylethanolamine (PE) on the antioxidative activity of α-tocopherol during oxidation of canola oil by singlet oxygen at 10°C for seven hours. Results show that α-tocopherol decreased the singlet oxygen oxidation of canola oil and its antioxidant activity was increased by the presence of PC and PE. During singlet oxygen oxidation of the oil, PC and PE decreased degradation of chlorophyll, but increased degradation of α-tocopherol. They discovered that PC and PE increased chemical quenching of singlet oxygen by alpha tocopherol.

2.3 Oxidation of Safflower oil

In 2004, Lee *et al*. had done oxidation studies safflower oil derivatives using rosemary extract as natural antioxidant. Structured lipid (SL-safflower) showed higher peroxide value (PV), anisidine value (AV), and 2-thiobarbituric acid reactive substances (TBARS) value than safflower oil, indicating that SL-safflower containing conjugated linoleic acid (CLA) which is more susceptible to oxidation than safflower oil. Rosemary extracts with different amount (100, 200 or 300 ppm) could effectively reduce the oxidation, and the most effective concentration is at 300 ppm.

Another study on safflower oil was done by Lee *et al*. (2004). The safflower oil was obtained from safflower seed which was roasted at different temperature (140-180ºC). According to their discovery, the fatty acid compositions of safflower oils did not change with roasting temperature and the major fatty acids is linoleic acid. The content of α-tocopherol in safflower oil gradually increased from 441 to 520 mg/kg as roasting temperature increased from 140 to 180 °C. The oxidative stability showed that, as the roasting temperature increased, the oxidative stability of safflower oil also increased.

2.4 Effect of Antioxidants on the oxidation of vegetable Oil

Antioxidant effectiveness is affected by several factors including the base oil decomposition, environmental conditions and the presence of other additives. The presence of high levels of polyunsaturated fatty acids in the vegetable oil severely reduces the benefit of any added antioxidants. Temperature is a major contributing factor. Some antioxidants decompose at higher temperatures (eg. propyl gallate) or become less effective (tocopherols) (Fox and Stachowiak, 2007). Becker and Knorr (1996) established that protection, or hindering, of the active hydroxyl group was critical for the continued effectiveness of free radical scavengers at high temperatures.

Several researches were carried out to study the oxidative stability of vegetable oil such as Bhatnagar *et al.*, 2009 on coconut oil, Vittadinia *et al.*, 2003 on Olive oil, Durmaz *et al*., 2010 on apricot kernel oil, Polvillo *et al*., 2004 on sunflower oil, Tang *et al*., 2008 and Knothe, 2007 on biodiesel from vegetable oil and many more.

Dunn, 2002 and Canakci *et al.* in 1999 stated that treatment with oxidation inhibitors is a promising approach because it facilitates the use of existing storage tanks and fuel handling systems without requiring upgrades or redesign. Antioxidants such as TBHQ or BHT are known to retard effects of oxidation on IV, TAN and PV of biodiesel (Dunn, 2002; Canakci *et al.,* 1999). Other antioxidants

known to improve resistance to oxidation of vegetable oils include ascorbyl palmitate, tocopherols, BHA and PG (Tan and Che Man, 2002).

Dunn in his research in 2005 increased the resistance of fatty derivatives against autoxidation by treating them with antioxidants. He examines the effectiveness of five such antioxidants, TBHQ, BHA, BHT, PG and α-Tocopherol in mixtures with soybean oil fatty acid methyl esters (SME). Antioxidant activity in terms of increasing oxidation onset temperature (OT) was determined by non-isothermal pressurized differential scanning calorimetry (P-DSC) in static (zero gas flow) and dynamic (positive gas flow) mode under 2000 kPa pressure and 5˚C/rain heating scan rate. Results showed that PG, BHT and BHA were most effective and α-Tocopherol least effective in increasing OT. Increasing antioxidant loading (concentration) showed sharp increases in activity for loadings up to 1000 ppm followed by smaller increases in activity at higher loadings. Phase equilibrium studies were also conducted to test physical compatibility of antioxidants in SME-No. 2 diesel fuel (D2) blends. Overall, this study recommends BHA or TBHQ (loadings up to 3000 ppm) for safeguarding biodiesel from effects of autoxidation during storage. BHT is also suitable at relatively low loadings (210 ppm after blending). PG showed some compatibility problems and may not be readily soluble in blends with larger SME ratios. Although α-Tocopherol showed very good compatibility in blends, it was significantly less effective than other synthetic antioxidants screened in this work.

In 2005, Liang *et al.* used natural and synthetic antioxidants to investigate their effect on the oxidative stability of distilled palm oil methyl esters. It was found that both types of antioxidant showed beneficial effects in inhibiting the oxidation of distilled palm oil methyl esters. Comparatively, the synthetic antioxidants were found to be more effective than the natural antioxidants as lower dosage (17 times less) was needed to achieve the minimum Rancimat induction period (RIP) of 6 h. Crude POME (CPOME) containing not less than 600 ppm of vitamin E were found to exhibit oxidative stability of more than 6 h and thus, conform to the specification of the European standard for biodiesel (EN 14214) while Distilled POME (DPOME) need to be treated with antioxidants in order to make it meet the specification. Synthetic antioxidants, BHT and TBHQ, are found to be more effective than natural antioxidant, α-Tocopherol in terms of their performance to enhance the RIP of DPOME. The RIP of DPOME increases drastically with small increments of the amount of TBHQ used (less than 0.1%). The efficiency of antioxidants investigated in this study was as follows: TBHQ› BHT› α-tocopherol.

Bond dissociation energies (BDE) can be used for the selection and design of chain-breaking antioxidants. BDE influence the effectiveness of hydrogen atom transfer reactions from the antioxidant molecules to the reactive radical intermediates such as hydroxyl, alkoxyl, peroxyl and hydroperoxyl radicals formed during the degradation reactions. For an antioxidant to efficiently quench all destructive radicals its BDE would have to be lower than that of the peroxyl and hydroperoxyl radicals (Zhu *et al*., 1997; Fox and Stachowiak, 2007).

2.5 Effect of Fatty Acids on the oxidation of vegetable Oil

A great deal of attention has been given on the effects of fatty acids on vegetable oil. Several experimental studies have been carried out to explain the interaction of fatty acids with the oxidative phenomena that occur in oils during processing and storage. However, most literature studies shown that results obtained are vary, insufficient and some cases are contradictory. Recently, Paradiso *et al.* (2010) studied the effect of adding increasing amounts of FFA on the oxidative processes occurring in purified olive oil during oxidation at 60^{o}C. They observed that oxidized forms of TAG and polar oligopolymers of TAG increased during oxidation. Low amounts of added FFA caused a further increase of the levels of oxidized TAG and TAG oligopolymers which pointing out a pro-oxidant activities, while higher percentage of added FFA lead to lower amounts of oxidized forms of TAG. It was believed to be due to an increase in peroxide decomposition exerted by FFA when present at higher concentrations.

In 1999, Frega and his group studied the effects of FFA on the oxidative of sunflower oil, grapeseed oil and olive oil. They discovered that FFA added to refined oils shortened their induction time. They also revealed that chain length had no effect on induction time although chain unsaturation made the oil more prone to thermo oxidative degeneration.

In 1986, Miyashita and Takagi studied the oxidative rates of pure FFA, pure fatty acid methyl esters and fatty acid methyl esters added with free fatty acids. They observed that FFA have higher oxidative rate compared to methyl esters. They also mention that the addition of stearic acid has shown to accelerate the rate of autoxidation of methyl linoleate and the decomposition of methyl linoleate hydroperoxide. They suggested that the higher oxidative rate of FFA's than their methyl esters could be due to the catalytic effect of carbonyl groups on the formation of free radicals by the decomposition of hydroperoxides.

Yoshida *et al.* (1992) studied the interaction of FFA with the oxidative processes in purified oils during microwave heating. The studies discovered that the shorter the chain length and the higher the degree of unsaturation of added fatty acids, the higher was the pro-oxidant effect.

In 1990, Handel and Gurrieri investigate oils with different degree of unsaturation in the presence of 1% and 5% of FFA by heating the sample mixture at 200 °C for 24 hours. Addition of FFA revealed to have different effects on different type of oil. A pro-oxidant effect was observed in more saturated oils (IV = 47 and 68). Corn oil (IV = 121) showed extremely varying results.

Colakoglu (2007) has studied the effect of monoolein, stearic acid and iron on soybean oil oxidation kinetics. He discovered that the addition of 1% of stearic

acid to soybean oil during heating at 55 °C up to 24 hours had caused an increment in oxygen consumption and peroxide formation respect to the soybean oil alone.

Few other investigations were performed on evaluating the effect of FFA on the oxidative stability of vegetable oils by using conductivity methods (OSI Rancimat). In 1999, Frega *et al*. found that FFA added to refined oils shortened their induction time (I_t). Different results were obtained for freshly processed virgin olive oils: in unfiltered cloudy oils I_t increased together with the amount of FFA added. Authors hypothesized that lignin from olive nut, primary accountable for cloudy materials, interacted with FFA, liberating the phenolic groups previously bonded and allowing them to exert their antioxidant activity.

Aubourg (2001) used fluorescence assessment to appraise the pro-oxidant effect of FFA on marine lipids. Increasing fluorescence development was observed as higher amounts of FFA were employed (0.01–1.00%). Results approved that, at 30°C, the shorter the chain length and the higher the unsaturation degree, the stronger was the pro-oxidant activity. The author also stated that, a short chain length should exert less positive inductive effect on the carboxyl group and steric hindrance; a high degree of unsaturation should lead to higher ability of the fatty acid for free radical formation as a result of the delocalization of the radical formed through the double-bond system.

In 1968, Rao and Achaya had discovered that oleic acid should act as synergist for phenolic antioxidants in oxidation of safflower oil. They observed that both the carboxyl function and the presence of unsaturation are important factor for such synergism. They suggest that oleic acid probably functions by antioxidant regeneration.

Consequently, it appears that the role of fatty acid in oxidation of fats and oils is far to be ascertained. In this study, the aim is to see the effect of fatty acids on palm olein, canola and safflower oil and to see the effect of fatty acid on the performance of antioxidant used during heat treatment of the selected vegetable oil respectively.

2.6 Ab Initio Calculation Studies

The key to theoretical chemistry is molecular quantum mechanics. It is the science relating molecular properties to the motion and interaction of electron and nuclei. Concepts of "Model Chemistry" and "Molecular System" are required for *ab initio* molecular orbital calculation. Model Chemistry refers to all theoretical aspects of calculation, while the Molecular System refers to the molecules to be studied (Hehre *et al*., 1986).

The accuracy of the computational methods is primarily depends upon the combination of basis set and the use of electron correlation The suitable combination of methods and basis set as well as the selection of calculation level are very important for a systematic calculation of the studied system. The higher the model chemistry used, the more accurate the results (Chien and Yang, 1998). Basis set is a mathematical representation of the molecular orbital within a molecule. The basis set can be interpreted as a restricting each electron to a particular region of space. Larger basis sets impose fewer constraints on electron and more accurately approximate exact molecular orbital (Foresman and Frisch, 1996).

Simple molecular orbital theory can be extended to open-shell systems in two possible ways. First, is described as spin-restricted Hartree-Fock (RHF), theory where a single set of molecular orbital is used. Disadvantages are that is computationally cumbersome and that it is somewhat unsatisfactory as a starting point for a perturbation treatment of electron correlation. The second type of molecular orbital theory is spin-unrestricted Hartree-Fock (UHF) theory. In this approach, different spatial orbitals are assigned to α- and β- electrons. It is better than RHF because it generally gives a lower energy than the corresponding RHF treatment, capable of providing a qualitatively correct description of bond dissociation and computationally more efficient than the corresponding RHF procedure (Hehre et al., 1986).

Density Functional Theory (DFT) based method ultimately derived from quantum mechanics research from the 1920's, especially the Thomas-Fermi-Dirac model, and from Slater's fundamental work in quantum chemistry in the 1950's. The DFT approach is based upon a strategy of modeling electron correlation *via* general functional of the electron density (Foresman and Frisch, 1996).

Hohenberg and Kohn, in 1964, showed the electron density determines the external potential for an electronic system, and that the true electron density minimizes the overall energy of the system. Since the external potential determines the wave function, knowing the density is equivalent to knowing the wave function, which determines all electronic properties of the system. Further, the true density maybe found using a variational approach

CHAPTER 3

METHODOLOGY

3.1 Summary of Experimental

Vegetable oil consisting of ester mixtures derived from glycerol with chain of fatty acids. The physical and chemical characteristics of vegetable oils are greatly influenced by the kind and proportion of the fatty acids on the triacylglycerol. Due to the differences in their fatty acid content of the oils, palm olein, high in saturated of palmitic 16:0, 46% (Rossi *et al*., 2007), canola oil, high in monounsaturated of oleic 18:1, 65% (Huang *et al*., 2007), and safflower oil, high in polyunsaturated of linoleic 18:2, 70% (Bozan and Temelli, 2008) were selected to be evaluated in this work. Their percentage of fatty acids and initial characteristic of the oils are presented in Table 2.1 & Table 2.2. Four different fatty acids that were employed in this study are palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic acid (18:2).

Samples were prepared by adding different amount of fatty acid (0.25%, 0.5%, 1% and 3%), with and without antioxidant, to the selected oil. Four types of antioxidants that were used in this study are BHA, BHT, TBHQ and PG. Mixtures were then heated in a forced air oven at 60^{o}C for 15 days (Paradiso *et al.*, 2010). The heat treated samples were taken at 1, 3, 6, 10 and 15 days for routine analyses.

Table 3.1: Fatty acid composition of palm olein (PO), canola(CO) and safflower oils (SO)

Fatty acid (FA)			Percentage		
IUPAC name	Trade name		PO[a]	CO[b]	SO[c]
Saturated FA					
Dodecanoic	Lauric	C12:0	0.43	-	-
Tetradecanoc	Myristic	C14:0	1.19	0.00	0.16
Hexadecanoic	Palmitic	C16:0	46.3	2.77	7.59
Octadecanoic	Stearic	C18:0	3.99	1.04	2.42
Eicosanoic	Arachidic	C20:0	0.30	0.37	0.36
Monounsaturated FA					
cis-9-Hexadecenoic	Palmitolic	C16:1	Tr	0.11	0.28
cis-9-Octadecenoic	Oleic	C18:1	38.2	64.6	11.04
Polyunsaturated FA					
cis-9,cis-12-Octadecadienoic	Linoleic	C18:2	8.16	21.1	70.46
cis-6,cis-9,cis-12-Octadecatrienoic	Linolenic	C18:3	0.19	7.60	-

Sources: a – Rossi *et al*. (2007); b- Huang *et al*. (2007); c- Bozan and Temelli (2008)

Table 3.2: Initial characteristic of palm olein, canola and safflower oils

Characteristics	Palm Olein	Canola	Safflower
Free fatty acid (%)	0.10	0.04	0.09
Peroxide value (meq/kg)	0.82	6.68	5.07
Iodine value (g/100 g)	56.6	108	145
Anisidine value	1.33	1.15	6.41

Source: Tan *et al*., 2002

3.1.1 Preparation of oil and chemicals

Palm olein (Buruh brand) and Canola oil (Natural brand) were purchased from the local grocery store while safflower oil was supplied by Sigma Aldrich Company. Fatty Acids; palmitic, stearic, oleic and linoleic acid were supplied by Merck and used without any further purification. All antioxidants: BHA, BHT, TBHQ, PG and other chemical for analyses were also supplied by Merck.

3.1.2 Experimental Procedures

Oven test was conducted to evaluate the oxidation during the accelerated storage of oil. The test was carried out on three different vegetable oils, palm olein, canola oil and safflower oil.

The samples were group into 4 different categories. The first category is to study the oxidation of the oil itself. The second category is to study the oxidation of oils in the presence of different antioxidant. The third category is to study the effect of fatty acid on the oxidative stability of the selected oil and the fourth category is to study the performance of antioxidant in the presence of fatty acids.

There are two major classes of antioxidant available nowadays which is chain breaking radical scavenger and peroxide decomposer. The antioxidants those were tested in this study are synthetic antioxidants: BHA, BHT, TBHQ and PG which were also known as chain breaking radical scavenger. The proportion of antioxidant used in this study was 5000 ppm or 0.5% antioxidant/vegetable oil (w/w).

Approximately 250 grams of vegetable oils were weighted in 500 mL conical flask. The oils were stored uncovered in the dark at 60ºC for a definite period in an oven. According to Frankel (1993), 60ºC is a suitable temperature to be used as a storage temperature since the temperature is recognized as having the fewest limitation and results correlate well with evaluation of actual shelf life. Labuza (1971) state that at higher temperature, there is the risk that autoxidation proceeds by different mechanism, yielding products which are not representative of a shelf-deterioration. Evan *et al*. (1971) mention that 1 day storage at 60ºC is equivalent to 1 month of storage at room temperature. In this study, the samples were heat treated at 60ºC for 15 days which is equivalent to 15 months of storage

at room temperature. Most vegetable oil reached its threshold of shelf life at 12 month of storage at ambient temperatures (Vaisey-Gensor *et al*., 1987). Vegetable oils that were heat treated without antioxidant or fatty acids or both antioxidant and fatty acids will be set as control. Samples were taken for analyses after 1, 3, 6, 10 and 15 days. Analyses were also performed on the samples before the samples mixture were heat treated and will be set as day zero or control. The chemical analyses used to evaluate the oxidation and the performance of antioxidant was peroxide value (PV) and total acid number (AV). All experiments were conducted with duplicate sets and analyses of samples were run in triplicate and average.

3.1.2.1 Total acid number (AOCS method Cd 3A-63)

Free fatty acids often expressed as total acid number (TAN), defined as the amount of KOH (mg) required in neutralizing 1g of oil or fat (Barthet *et al*., 2008). Thus, the total acid number indicates the degree to which the triacylglycerides in the oil have broken down to release free fatty acids. The reaction of the total acid number is shown in Figure 2.1.

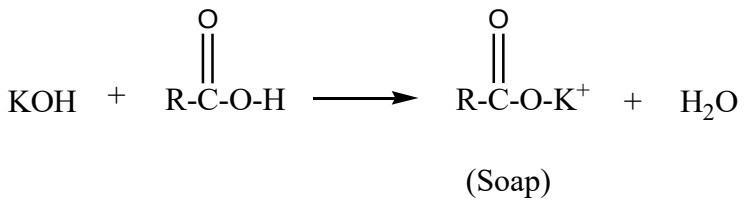


Figure 3.1: Reaction for total acid number determination

Preparation of solutions

Phenolphthalein solution was prepared by mixing 1% (v/v) in 95% ethanol using standard volumetric flask while Potassium Hydroxide (KOH) with 0.05M solution was prepared in standard volumetric flask and standardized before used.

Procedure

About 3 g of oil sample was accurately weighted in a 250 mL Erlenmeyer flask. The solvent which is the combination of 25mL of diethyl ether and 25 mL ethanol (95%) and the phenolphthalein indicator were then added to the oil sample. The mixture was shaken gently for 10 minutes to ensure the oil was entirely dissolved in the solvent. The mixture was titrated with 0.05 N KOH. The solution was swirled vigorously until the end point of titration in order to avoid carbon dioxide dissolved into the solution. At the end point, the solution changed into pink colour and maintained for 15 seconds. Blank determination (without sample) was carried out using the above mentioned procedure. The process was repeated for three times for all samples to avoid the errors that may occurred during titration.

Expression of results

The total acid number was calculated as below:

$$\text{Total acid number} = \left[\frac{(V_s - V_b) \times N \times 56.1}{W} \right] \text{mg KOH/g}$$

Where;

V_s is the volume in millilitres, of the potassium hydroxide solution of normality N, used for determination;

V_b is the volume in millilitres of potassium hydroxide solution used for blank test;

W is weight in grams of test portion;

N is the molarity of potassium hydroxide solution;

56.1 is a molecular weight of KOH.

3.1.2.2 Peroxide Value (AOCS Cd 8-53 (1989) ISO 3960 (1977))

The peroxide value is a measure of oxidation degree in a lipid sample and not measurement of lipid stability. It is express in terms of miliequivalents of active oxygen per kilograms fats and oils which oxidize potassium iodide, KI under the

conditions of the test. It is also a measure of formation of peroxide and hydroperoxide groups that are the initial products of the lipid oxidation.

The peroxide value was determined by measuring the amount of iodine which formed by reaction of peroxides with iodide ion. The liberated iodine was then titrated against a solution of sodium thiosulphate, $Na_2S_2O_3$. Starch solution was used as an indicator

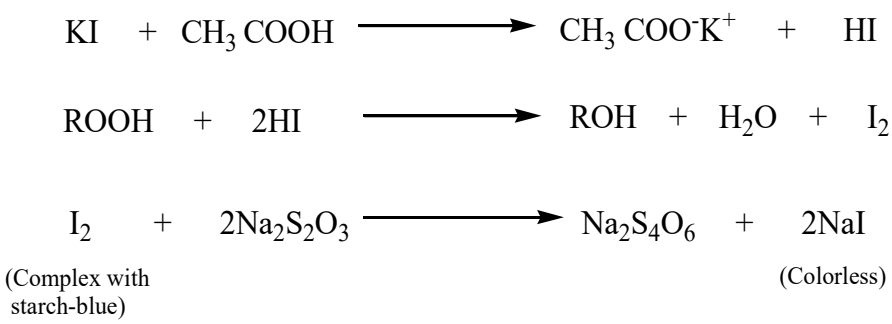


Figure 3.2: Reaction for peroxide value determination

Reagent and solution preparation

Reagents and solutions were prepared beforehand before proceeding for further analyses. Solution prepared were acetic acid – chloroform solution, saturated potassium iodide solution, sodium thiosulphate solution with, and starch solution.

Acetic acid-chloroform solution was prepared by mixing three parts by volume of glacial acetic acid with two parts by volume of chloroform. The potassium iodide was prepared by using boiled water. The solution must remain saturated. Sodium thiosulphate 0.05N solution was prepared in standard volumetric flask and standardized before used. Starch solution was prepared by mixing 1g of starch powder with 20 mL of water. The mixtures were mixed and added to the 100 mL of boiling water and leave boiling for 3 minutes.

Procedure

About 2.5 g of samples accurately weighed into the 250 mL Erlenmeyer flask. Then 15 mL acetic acid- chloroform solution was added. The mixture was then swirled until the sample dissolved in solution. 0.5 mL of saturated potassium iodide is then added using graduated pipette. The solution was then swirled again for 1 minute. Then, add 15mL of distilled water into the sample mixture. When the mixture was homogenised, 0.5 mL of starch was added and stirred again slowly. The samples were then titrated with 0.05N sodium thiosulphate solution by adding it gradually and with constant and vigorous shaking. Titration while shaking the flask vigorously continued until near the end-point to liberate all iodine from the chloroform layers. The thiosulphate solution is then, must drop wisely until the blue colour just disappeared. Blank determination of reagent was conducted parallel with the determination. The blank was determined by using the same steps without the oil sample.

Expression of the results

The peroxide value is expressed in miliequivalent of active oxygen per kilogram of samples were calculated as follows:

$$\text{Peroxide Value} = \left[\frac{(V_s - V_b) \times N \times 1000}{W} \right] \text{meq } O_2 \text{/kg}$$

Where;

V_s is the volume in millilitres, of the sodium thiosulphate solution of normality N, used for determination;

V_b is the volume in millilitres of sodium thiosulphate solution used for blank test;

W is weight in grams of test portion;

N is the normality of sodium thiosulphate solution.

3.2 Computational Method

In this study, three type of homo-chain TAGs i.e tripalmitic (to represent palm olein), trioleic (to represent canola oil) and trilinoleic (to represent safflower oil) were used as the neutral molecule and also as the free radicals species except

for tripalmitic. All theoretical calculations were performed using the quantum mechanical software package of Gaussian09 (Frisch *et al*., 2009) at the theoretical level of DFT/6-31G (d,p) and the model of molecule where drawn using gaussview 05 (Figure 3.3)

The optimization can be categorize into three groups ie single species (either TAGs, TAGs COO radicals, antioxidants (BHA, BHT, TBHQ, PG) or fatty acids (PA, SA, OA, LA) , bimolecular complexes (TAGs with antioxidants at the position of $H_{ß}$ and COO allylic and TAGs with fatty acids at the same two positions) and termolecular complexes (TAG trioleic $H_{ß}$ with TBHQ and fatty acids; TAG trioleic C8 OO with TBHQ and fatty acids; and TAG trilinoleic C9OO with TBHQ and fatty acids). The molecular structure can be referred in the Appendix A.

The optimizations were performed to the minimum potential energy at the selected theoretical level to obtain the physical parameter such as dipole moment, SCF energy and interaction energy. Once the optimization structure of the complex achieved, the selected structure were then underwent the potential energy scan at the lower level of STO-3G by imposing the constraints and rotating the smaller species(such as antioxidants of fatty acids) by the total amount of 350˚ (35 * 10 / rotation) through the hydrogen bond or otherwise stated. If there exist another potential energy minimum, the whole system will be re-optimized starting from the minimum energy structure obtained. For the bimolecular and tri-molecular

complexes, the interaction energy was calculated by subtracting the total individual SCF energies from the complex SCF energy (Sherrill *et al*., 2009):

$$\text{Interaction Energy}, \Delta\varepsilon = SCF\ (complex) - \sum_{i=1} component$$

However, due to the very small values of interaction energy, units used are kJ/mol instead of hartree/particle. The interaction energy was then corrected using counterpoise procedure. It was done due to the incompleteness of the LCAO basis set which will cause an artificial shortening of intermolecular distances and concomitant artificial strengthening of the intermolecular interaction that is known as "basis set superposition errors" (BSSE). System which consist of two subsystems A and B, when A is treated on its own, isolated, only the basis orbitals in the A system are used to describe it. When A couples to B, the total basis set available for A is larger (more complete), since also the orbitals in B can be used to describe A. This gives rise to an artificial attraction, since when A and B get closer each system can lower its energy by utilizing the basis functions of the other system. The BSSE can be neutralized by adding the counterpoise (cp) correction (Boys and Bernardi, 1970):

$$E = E_{AB} + E^{\text{cp}}$$

where E_{AB} is the total energy of system AB and E is the counterpoise corrected energy. The counterpoise correction is obtained through

$$E^{cp} = (E_A - E_{AB}) + (E_B - E_{AB})$$

where E is the energy of system A in the AB basis, which is obtained by putting so-called ghost orbitals at the atomic positions in system B. The counterpoise correction is readily extended to several subunits, A_i, through

$$E^{cp} = \sum_i (E_{Ai} - E_{Ai\,Ci})$$

where C_i is the A_i complement, i.e. the atoms not in region A_i. In ATK it is possible to label a set of atoms as ghost atoms. Ghost atoms have no charge and no mass, but they have basis orbitals, defined by whichever element and basis set that defines the atom if it were not a ghost. This will compute the total energy of the vacancy configuration with the atom(s) that form the vacancy as ghost orbitals, instead of actually removing them from the configuration.

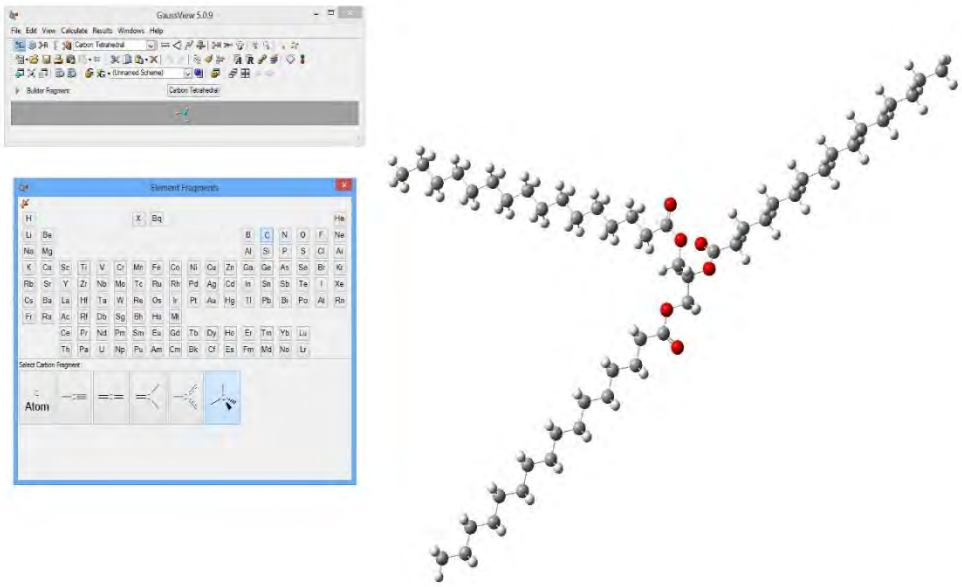


Figure 3.3 : Molecule drawn using Gaussview 05 (Dennington *et al*., 2009).

CHAPTER 4

RESULTS AND DISCUSSION ON QUANTUM MECHANICAL STUDIES

4.1 Interaction between Antioxidants and $H_ß$ of TAGs

Interaction between antioxidant and $H_ß$ TAG were studied theoretically using Gaussian 09. Table 4.1 shows the total electron energies (SCF energy) of antioxidants studied (BHA, BHT, TBHQ,PG) while Table 4.2 to Table 4.4 shows SCF energy and interaction energy between $H_ß$ of TAG tripalmitic, trioleic, trilinoleic with antioxidant respectively. PG has the highest interaction energy among all the three types of TAG exceeding 34kJ/mol. The interaction energy for TBHQ, BHA and BHT are lower compared to PG. Dunn, 2005 stated that PG showed a good physical compatibility and was the most effective antioxidant in their studies. For instance TBHQ, 21 kJ/mol; BHA, 21 kJ/mol; BHT, 15 to 23 kJ/mol. Results also shows that TBHQ and BHA expressed similar value of interaction energy. The lowest interaction was observed in the presence of BHT. Detailed analyses made on the interaction value of antioxidants with all three types of TAGs, higher interaction value was observed in PG and BHT on TAG trilinoleic (Table 4.4) as compared to other TAGs system. However, TBHQ and BHA exhibit no preferences on any types of TAGs.

Table 4.1: Total electronic energy (SCF energy) of antioxidants

Antioxidants	SCF Energy,au
BHA	-579.26529770
BHT	-661.31705552
TBHQ	-539.96004079
PG	-764.43668643
PY	-457.91342283

Table 4.2 :SCF and interaction energies between $H_{ß}$ of TAG Tripalmitic and antioxidants (BHA/BHT/TBHQ/PG)

Molecule/ Complexes	SCF energy, au	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol
TAG TRIPALMITIC	-2454.07616526			
TAG TRIPALMITIC + PG	-3218.53633060	-61.64	26.96	-34.68
TAG TRIPALMITIC + TBHQ	-2994.05366427	-45.84	24.83	-20.54
TAG TRIPALMITIC + BHA	-3033.35841751	-44.51	23.80	-20.71
TAG TRIPALMITIC + BHT	-3115.40664936	-35.26	17.65	-17.68

Table 4.3 :SCF and interaction energies between $H_{ß}$ of TAG Trioleic and antioxidants (BHA/BHT/TBHQ/PG)

Molecule/ Complexes	SCF energy, au	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol
TAG TRIOLEIC	-2686.26262001			
TAG TRIOLEIC + PG	-3450.72275473	-61.56	27.14	-34.43
TAG TRIOLEIC + TBHQ	-3226.24035283	-46.45	24.86	-21.13
TAG TRIOLEIC + BHA	-3265.54505800	-45.00	23.75	-21.25
TAG TRIOLEIC + BHT	-3347.59343243	-36.12	21.35	-14.84

Table 4.4 :SCF and interaction energies between Hß of TAG Trilinoleic and antioxidants (BHA/BHT/TBHQ/PG)

Molecule/ Complexes	SCF energy, au	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol
TAG TRILINOLEIC	-2682.55624792			
TAG TRILINOLEIC + PG	-3447.01803935	-65.91	26.86	-39.05
TAG TRILINOLEIC +TBHQ	-3222.53364075	-45.56	24.70	-20.39
TAG TRILINOLEIC + BHA	-3261.83836835	-44.17	23.89	-20.28
TAG TRILINOLEIC + BHT	-3343.88878782	-40.65	18.06	-22.66

4.2 Interaction between Fatty Acids and Hß of TAGs

All types of fatty acids tested reduced the possibility of TAG rearrangements which can lead to the formation of fatty acids. This could be predicted by large magnitude of interaction energy between fatty acid and TAG where is between 21 to 32 kJ/mol. Based on the results, the interaction energy between FAs and unsaturated TAG (TAG Trioleic, TAG Trilinoleic) were similar between 27 to 32 kJ/mol. Tri-saturated TAG (tripalmitics) expressed the smallest interaction energy which is between 21 to 25 kJ/mol.

The total electronic energy of FAs involved in this study is tabulated in Table 4.5. Oleic acid exhibits the most significant effect with TAG tripalmitic with the value of 25.16 kJ/mol and saturated shows to have the lowest interaction energy (Table 4.6). The order of interaction can be described as follow: Oleic acid >

Linoleic Acid ~Palmitic acid ~Stearic acid. Linoleic acid (21.88 kJ/mol), palmitic acid (21.72 kJ/mol) and stearic acid (21.78 kJ/mol) exhibited almost little or no preferences in their interaction values.

Table 4.5: Total electronic energy (SCF energy) of fatty acids

Fatty acids	SCF Energy, au
PA	-779.52384939
SA	-858.15687953
OA	-856.91942176
LA	-855.68440059

Table 4.6: SCF and Interaction energies between Hß of TAG Tripalmitic and fatty acid

Molecule/ Complexes	SCF energy, au	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol
TAG TRIPALMITIC	-2454.07616526			
TAG TRIPALMITIC + PA	-3233.61644188	-43.13	21.41	-21.72
TAG TRIPALMITIC + SA	-3312.24937643	-42.88	21.10	-21.78
TAG TRIPALMITIC + OA	-3311.01328969	-46.48	21.32	-25.16
TAG TRIPALMITIC + LA	-3309.77699893	-43.15	21.22	-21.88

Highest interaction value of oleic acid with TAG was observed to have close resemblance with the TAG tripalmitic which also exhibited the highest interaction energy (Table 4.7). However, interaction energy of oleic acid and TAG trioleic is higher which is 27.37 kJ/mol compared to 25.16 kJ/mol for oleic acid with TAG tripalmitic. The order of interaction energy between FAs and TAG trioleic is

described as follows: SA (32.267 kJ/mol) > PA (32.10 kJ/mol) ~ OA (27.37 kJ/mol) ~ LA (27.13 kJ/mol).

The higher interaction energy for oleic with both TAG tripalmitic and TAG trioleic compared to other fatty acids were believed to be able to reduce the chances of TAGs rearrangement which then will reduce the FFA formation.

Table 4.7: SCF and Interaction energy between $H_{ß}$ of TAG Trioleic and fatty acids

Molecule/ Complexes	SCF energy, au	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol
TAG TRIOLEIC	-2686.26262001			
TAG TRIOLEIC + PA	-3465.80507905	-48.86	16.76	-32.10
TAG TRIOLEIC + SA	-3544.43799326	-48.56	16.30	-32.26
TAG TRIOLEIC + OA	-3543.20132211	-50.62	23.26	-27.37
TAG TRIOLEIC + LA	-3541.96546961	-48.44	21.26	-27.13

Different trend was observed for TAG trilinoleic system where the highest interaction energy was seen with stearic acid (29.46 kJ/mol). However, the interaction are not much different compared with other fatty acids (LA= 27.12 kJ/mol, PA = 27.19 kJ/mol & OA= 27.18 kJ/mol). These theoretical results can be used to predict that stearic acid is able to reduce FFA formation of the high TAG trilinoleic vegetable oils.

Table 4.8 :SCF and interaction energies between Hß of TAG Trilinoleic with fatty acids

Molecule/ Complexes	SCF energy, au	Interaction Energy (uncorrected) kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected) kJ/mol
TAG TRILINOLEIC	-2682.55624792			
TAG TRILINOLEIC + PA	-3462.09856168	-48.48	21.29	-27.19
TAG TRILINOLEIC + SA	-3540.73210166	-49.82	20.36	-29.46
TAG TRILINOLEIC + OA	-3539.49410266	-48.40	21.23	-27.18
TAG TRILINOLEIC + LA	-3538.25913927	-48.55	21.37	-27.12

4.3 Interaction in the Tri-species Systems

Theoretical studies on tri-species systems were only performed on Hß TAG trioleic with TBHQ and four different fatty acids; palmitic, stearic, oleic and linoleic. Hß of TAG trioleic was selected to represent other TAGs (tripalmitic and trilinoleic) due to its interaction energy with antioxidants or fatty acids are of the same magnitude as of other TAGs. (Hß TAGs with antioxidants: 45 to 46 kJ/mol; Hß TAGs with fatty acids: 48 to 49 kJ/mol). Even though the theoretical shows that PG has the highest interaction with Hß trioleic, TBHQ was chosen based on its performance which is almost the same as the other two antioxidants, BHA and BHT. PG was not selected due to the difficulties in comparing theoretical results with experimental. Another factor for TBHQ be selected for tri-species calculation is due to TBHQ has the highest interaction with TAG $C_8OO•$ radical and the second highest interaction energy with TAG $C_9OO•$ radical.

Analyses on the interaction energy of the selected tri-species system ($H_ß$ TAG trioleic + TBHQ + FAs) has shown that oleic acid has the highest interaction which is -36 kJ/mol while other fatty acids show lower and almost similar interaction energy which is around -33 kJ/mol.

The interaction energy between TBHQ and $H_ß$ TAG trioleic in the presence of fatty acids has dropped by an amount of 6 kJ/mol. The highest reduction occurred when the system contain palmitic acid (97.81%) followed by stearic (93.31%), linoleic (71.36%) and finally oleic acid (58.60%). It could be further concluded that the saturated fatty acids such as palmitic and stearic gave larger impact on TBHQ performance compared to unsaturated fatty acids (oleic and linoleic). These effects can be expressed for vegetable oil which is rich with TAG trioleic such as canola oil. Detailed analyses on the investigated tri-species system are shown in Table 4.9.

Table 4.9: Interaction energy between TAG trioleic $H_{ß}$ + TBHQ + FAs (PA, SA, OA, LA)

Complexes	SCF energy (a.u)	Interaction Energy (uncorrected) kJ/mol	BSSE kJ/mol	Interaction Energy (corrected) kJ/mol	% reduc-tion
TAG TRIOLEIC + TBHQ + PA	-4005.77651646	-78.78	45.76	-32.56	97.81
1) TAG TRIOLEIC + TBHQ	-3226.23811812	-40.58			
2) TAG TRIOLEIC + PA	-3465.79977436	-34.93			
TAG TRIOLEIC + TBHQ + SA	-4084.40949519	-78.65	44.51	-33.67	93.31
1) TAG TRIOLEIC + TBHQ	-3226.23804098	-40.38			
2) TAG TRIOLEIC + SA	-3544.43299642	-35.44			
TAG TRIOLEIC + TBHQ + OA	-4083.17338168	-82.18	45.61	-36.11	58.60
1) TAG TRIOLEIC + TBHQ	-3226.23818174	-40.75			
2) TAG TRIOLEIC + OA	-3543.19663499	-38.31			
TAG TRIOLEIC + TBHQ + LA	-4081.93701701	-78.65	44.96	-33.18	71.36
1) TAG TRIOLEIC + TBHQ	-3226.23821948	-40.85			
2) TAG TRIOLEIC + LA	-3541.96041881	-35.18			

4.4 Analyses on the Interaction of TAG Trioleic $C_8OO•$ Radical and Antioxidants

Theoretical studies on TAG trioleic $C_8OO•$ radical with antioxidants shows that TBHQ has the highest interaction energy compared to BHA, BHT and PG. The order of interactions is as follows: TBHQ (22.78 kJ/mol) > BHA (22.77 kJ/mol) > PG (14.46 kJ/mol) > BHT (10.40 kJ/mol). Based on the results, interaction energy of TBHQ is closely similar with BHA while PG shows similar performance with BHT.

Analyses on the compatibility of dipole moment between TAG trioleic $C_8OO•$ radical and antioxidant were also performed on the same system. For this case, even though PG's dipole moment (3.49 D) is the closest to $C_8OO•$ radical dipole moment (5.33 D) but the interaction energy between the molecules are the lowest with the reduction value of 8 kJ/mol compared to TBHQ and BHA. This indicates that the dipole moment alone cannot be used to predict the performance of antioxidants. However, the analyses on the combination of dipole moment and interaction energy parameters, the TBHQ (2.60 D) and BHA (2.43 D) is expected to show the best performance in scavenging the $C_8OO•$ radical in the trioleic-rich vegetable oil such as canola. Due to the same reason, BHT is expected to show the poorest performance due to the low in interaction energy (10.40 kJ/mol) and larger differences in dipole moment that can be clearly seen in Table 4.10.

Previous studies (Wang *et al*., 2011; Dun, 2005; Rodiguez *et al*., 1993 and Ryu, 2009) has proved that the best antioxidant in inhibit oxidation process is TBHQ while BHT has shown the poorest in reducing peroxide formation. However, all previous studies indicate that BHA, BHT, TBHQ and PG are able to reduce oxidation and had been widely used as food additives to retard oxidative degradation (Wanasundana and Shahidi, 2005; Agustine and Berry, 1983).

Table 4.10: Physical parameters of the complexes between TAG trioleic $C_8OO•$ radical and antioxidants

System/Complexes	SCF Energy, a.u	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol	Dipole moment, D	R1 (OO• --- H-O AO)	I1 H-O (AOs)
TAG TRI OLEIC C_8O-OH	-2836.607469				3.8639		0.7579
TAG TRI OLEIC $C_8OO•$	-2835.970113				5.3268		
BHA	-579.2652298				2.4339		0.7392
BHT	-661.3170552				2.0540		0.7064
TBHQ	-539.9600408				2.5974		0.7388
PG	-7644366864				3.4933		0.7130
TAG TRI OLEIC $C_8OO•$ + BHA	-3415.247449	-31.79	8.84	-22.77	8.7822	1.91699	0.6741
TAG TRI OLEIC $C_8OO•$ + BHT	-3497.295593	-22.12	11.79	-10.40	7.9501	1.93448	0.6669
TAG TRI OLEIC $C_8OO•$ + TBHQ	-3375.942668	-32.86	9.61	-22.78	7.8476	1.91583	0.6745
TAG TRI OLEIC $C_8OO•$ + PG	-3600.415378	-22.52	8.07	-14.46	6.2553	1.91357	0.6564

*R1= Bond length in Å; I1= Bond Index

4.5 Analyses on the Interaction between TAG Trioleic $C_8OO•$ Radical and Fatty Acids

Comparison on the interaction energy for all four fatty acid revealed that palmitic, stearic and oleic shows almost the same strength of which is about 22-23 kJ/ mol while linoleic (polyunsaturated) gave the lowest interaction, 20.18 kJ/mol. If the interaction is calculated to the nearest hundredth and tenth, the order of the interaction is: palmitic acid (23.17) > oleic acid (22.93) > stearic acid (22.03). Detailed calculation can be referred to Table 4.11.

Table 4.11: Physical parameters of the two-species system TAG trioleic $C_8OO•$ radicals and fatty acids

System/Complexes	SCF Energy a.u	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol	Dipole moment, D	R2 (OO• --- H-O-C=O FA)	I1 H-O (AOs)	I2 H-O-C=O (FAs)
TAG TRI OLEIC C_8O-OH	-2836.607469				3.8639		0.7579	
TAG TRI OLEIC $C_8OO•$	-2835.970113				5.3268			
PALMITIC ACID	-779.5238494				1.3901			0.7186
STEARIC ACID	-858.1568795				1.3898			0.7186
OLEIC ACID	-856.9194194				1.5011			0.7186
LINOLEIC ACID	-855.6844006				1.5910			0.7186
TAG TRI OLEIC $C_8OO•$ + PA	-3615.505513	-30.33	7.15	-23.17	6.9974	1.85710		0.6481
TAG TRI OLEIC $C_8OO•$ + SA	-3694.138532	-30.30	8.27	-22.03	6.4867	1.85574		0.6475
TAG TRI OLEIC $C_8OO•$ + OA	-3692.901017	-30.15	7.23	-22.93	7.0581	1.85851		0.6483
TAG TRI OLEIC $C_8OO•$ + LA	-3691.665073	-27.72	7.49	-20.18	7.2133	1.84890		0.7065

*R1= Bond length in Å; I1= Bond Index of OH in antioxidant; I2= Bond index of OH in fatty acid

4.6 Analyses on the Effect of Fatty Acids on the performance of TBHQ to Scavenge the $C_8OO•$ radical

Analyses on the total interaction energy revealed that PA and LA has almost similar total energy (E_{tot} (PA) =30.00; (E_{tot} (LA) =29.38kJ/mol. Similar to the pair of OA and SA, (E_{tot} (OA) 28.12; E_{tot} (SA) = 28.27 kJ/mol. The higher the interaction energy means, the higher the stabilization energy of the complexes. The high stabilization can be related with extra time or chance for the H transfer to occur.

Analyses performed on the interaction energy between $C_8OO•$ radical with TBHQ in the presence of fatty acids show that the presence of any fatty acids will reduce the antioxidant (TBHQ) performance. However, the magnitude reductions are smaller compared to the case of $H_ß$ of TAG trioleic. Order of interaction energy reduction for $C_8OO•$ radical and TBHQ with fatty acids is OA (77.2%) > SA (72.58%) > PA (70.03%) > LA (59.65%).

Table 4.12: Physical parameters of transition state complexes of TAG Trioleic $C_8OO•$ radicals with antioxidants (TBHQ) and Fatty acids (PA, SA, LA, OA)

System/Complexes	SCF Energy a.u	Interaction Energy (uncorrected), kJ/mol		BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol	Reduction in Interaction (%)	Dipole moment, D	R1 (OO• --- H-O AO)	R2 (OO• --- H-O-C=O FAs)	I1 H-O (AOs)	I2 H-O-C=O (FAs)
TAG TRI OLEIC $C_8OO•$ + TBHQ + PA	-4155.475065	-55.30	(1)	24.83	-30.00	70.03		1.93473	2.0811	0.681	0.687
TAG TRI OLEIC $C_8OO•$ + TBHQ	-3375.941308	-29.29					7.6514				
TAG TRI OLEIC $C_8OO•$ + PA	-3615.503925	-26.16					5.2527				
Interaction TBHQ + PA		0.15									
TAG TRI OLEIC $C_8OO•$ + TBHQ + SA	-4234.107572	-53.92	(4)	25.19	-53.46	72.58	7.0056	1.93223	2.06008	0.6813	0.6851
TAG TRI OLEIC $C_8OO•$ + TBHQ	-3375.940809	-27.98					7.6116				
TAG TRI OLEIC $C_8OO•$ + SA	-3694.136671	-25.41					5.2549				
Interaction TBHQ + SA		-0.54									
TAG TRI OLEIC $C_8OO•$ + TBHQ + OA	-4232.870192	-54.14	(3)	25.55	-53.67	72.2	7.0407	1.92992	2.05896	0.6808	0.6858
TAG TRI OLEIC $C_8OO•$ + TBHQ	-3375.941179	-28.95					7.4422				
TAG TRI OLEIC $C_8OO•$ + OA	-3692.899368	-25.82					5.3595				
Interaction TBHQ + OA		0.64									
TAG TRI OLEIC $C_8OO•$ + TBHQ + LA	-4231.635577	-55.20	(2)	25.30	-54.68	59.65	6.9349	1.93465	2.05838	0.6815	0.6846
TAG TRI OLEIC $C_8OO•$ + TBHQ	-3375.941049	-28.61					7.441				
TAG TRI OLEIC $C_8OO•$ + LA	-3691.664675	-26.68					5.2911				
Interaction TBHQ + LA		0.09									

R1 = Bond length between OO• and OH of antioxidant; R2 = Bond length between OO• and OH of Fatty acid; I1= Bond index of OH from antioxidant; I2= Bond index of OH from fatty acid

4.7 Analyses on the Interaction of TAG Trilinoleic $C_9OO•$ Radical and Antioxidants

Theoretical studies on the interaction between $C_9OO•$ radical and antioxidant show that BHT have the highest interaction value which is 32.80 kJ/mol and the lowest is PG which is 25.70 kJ/mol. However, the interaction energy dissimilarities are small for BHT (32.80 kJ/mol), TBHQ (32.31 kJ/mol), and BHA (32.00 kJ/mol). Detailed data are tabulated in Table 4.13.

4.8 Analyses on the Interaction of TAG Trilinoleic $C_9OO•$ Radical and Fatty Acids

Interaction on TAG trilinoleic $C_9OO•$ radical with fatty acid were also studied. Results of the theoretical studies are shown in Table 4.14. Studies on the interaction between TAG $C_9OO•$ radical with four different fatty acids showed that OA has the highest interaction (25.98 kJ/mol) followed by PA (23.32 kJ/mol), LA (23.07 kJ/mol) and SA (22.54 kJ/mol). Theoretical results of these studies can be used to predict higher peroxide value for TAG trilinoleic rich vegetable oil in the presence of SA compared to PA. The same pattern was also observed in TAG trioleic $C_8OO•$ radical cases where OA shows the highest interaction energy.

Table 4.13: Physical parameter of the transition state complexes between TAG trilinoleic $C_9OO•$ radicals and antioxidants

SYSTEM	SCF Energy, a.u	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol	Dipole moment, D	R1 (OO• --- H-O AO)	I1 H-O (AOs)
TAG TRI LINOLEIC C_9O-OH	-2832.913417				3.3601		0.7593
TAG TRILINOLEIC $C_9OO•$	-2832.275861				1.9101		
BHA	-579.2652298				2.4339		0.7392
BHT	-661.3170552				2.0540		0.7064
TBHQ	-539.9600408				2.5974		0.7388
PG	-764.4366864				3.4933		0.7130
TAG TRI LINO $C_9OO•$ + BHA	-3411.55328	-32.00	8.93	-22.89	7.2388	1.90973	0.6731
TAG TRI LINO $C_9OO•$ + BHT	-3493.60541	-32.80	16.19	-16.67	5.2076	1.93534	0.6630
TAG TRI LINO $C_9OO•$ + TBHQ	-3372.24821	-32.31	9.04	-22.80	7.5124	1.91087	0.6734
TAG TRI LINO $C_9OO•$ + PG	-3596.72234	-25.70	15.50	-10.19	1.8998	1.95477	0.6606

R1= Bond length in Å; I1= Bond index of OH in antioxidant

Table 4.14: Physical parameters of the transition state complexes between TAG trilinoleic $C_9OO•$ and fatty acids

System/complexes	SCF Energy, a.u	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol	Dipole moment, D	R2 (OO• -- H-O-C=O FA)	I2 H-O-C=O (FAs)
TAG TRI LINOLEIC C_9O-OH	-2832.913417				3.3601		
TAG TRILINOLEIC $C_9OO•$	-2832.275861				1.9101		
PALMITIC ACID	-779.5238494				1.3901		0.7186
STEARIC ACID	-858.1568795				1.3898		0.7186
OLEIC ACID	-856.9194194				1.5011		0.7186
LINOLEIC ACID	-855.6844006				1.5910		0.7186
TAG TRI LINO $C_9OO•$ + PA	-3611.81386	-37.16	13.84	-23.32	1.8098	1.82385	0.6408
TAG TRI LINO $C_9OO•$ + SA	-3690.44447	-30.79	8.25	-22.54	4.1060	1.84777	0.6459
TAG TRI LINO $C_9OO•$ + OA	-3689.20847	-34.62	8.64	-25.98	4.0445	1.85086	0.6459
TAG TRI LINO $C_9OO•$ + LA	-3687.97214	-31.18	8.06	-23.07	4.0111	1.84275	0.6451

R2= Bond length in Å between radical and fatty acid; I2= Bond index of OH in fatty acid

4.9 Analyses on the Effect of Fatty Acids on the performance of TBHQ to Scavenge $C_9OO•$ radical

Theoretical results on transition state complexes of TAG trilinoleic $C_9OO•$ radicals with TBHQ and fatty acids are tabulated in Table 4.15. Analyses showed that the utilization of various fatty acids gave no significant different on the tri-species complexes. If the calculation are made to the closes hundredth and tenth of kJ/mol, the order of interaction can be listed as follows. OA (43.80 kJ/mol) > SA (43.79 kJ/mol) > SA (69.49 kJ/mol) > PA (43.34 kJ/mol). Analysis also revealed that any fatty acid applied to this system shows only small reduction in interaction energy between $C_9OO•$ radical and TBHQ which is only about 7-21%. The order of reduction is as follows, OA (21.85) > PA (12.19) > LA (9.82) > SA (6.83).

These theoretical results could be used to predict that the performance of TBHQ in rich TAG trilinoleic should not be affected very much by any fatty acids (PA, SA, OA and LA) added to the system.

Table 4.15: Physical parameters of transition state complexes of TAG Trilinoleic $C_9OO•$ radicals with antioxidants (TBHQ) and Fatty acids (PA, SA, LA, OA)

System/ Complexes	SCF Energy, a.u	Interaction Energy (uncorrected), kJ/mol	BSSE, kJ/mol	Interaction Energy (corrected), kJ/mol	Reduction in interaction Energy, %	Dipole moment, D	R1 (OO• --- H-O AO)	R2 (OO• --- H-O-C=O FA)	I1 H-O (AOs)	I2 H-O-C=O (FAs)
TAG TRI LINO $C_9OO•$ + TBHQ + PA	-4151.78614	-69.28	25.48	-43.34	12.19	5.5695	1.94938	1.92942	0.6834	0.6600
TAG TRI LINO $C_9OO•$ + TBHQ	-3372.24753	-30.54				6.8218				
TAG TRI LINO $C_9OO•$ + PA	-3611.81552	-41.50				2.9070				
Interaction TBHQ + PA		2.75								
TAG TRI LINO $C_9OO•$ + TBHQ + SA	-4230.41925	-69.49	25.24	-43.79	6.83	5.0923	1.94824	1.92989	0.6835	0.6603
TAG TRI LINO $C_9OO•$ + TBHQ	-3372.24748	-30.40				6.4059				
TAG TRI LINO $C_9OO•$ + SA	-3690.44862	-41.68				2.6654				
Interaction TBHQ + SA		2.60								
TAG TRI LINO $C_9OO•$ + TBHQ + OA	-4229.181818	-69.57	25.30	-43.80	21.85	5.4857	1.94761	1.93409	0.6831	0.6606
TAG TRI LINO $C_9OO•$ + TBHQ	-3372.247406	-30.20				6.767				
TAG TRI LINO $C_9OO•$ + OA	-3689.211187	-41.76				3.0016				
Interaction TBHQ + OA		2.40								
TAG TRI LINO $C_9OO•$ + TBHQ + LA	-4227.946831	-69.65	25.50	-42.63	9.82	5.6746	1.93012	1.94832	0.6831	0.6602
TAG TRI LINO $C_9OO•$ + TBHQ	-3372.247510	-30.48				6.8773				
TAG TRI LINO $C_9OO•$ + LA	-3687.976345	-42.23				2.7733				
Interaction TBHQ + LA		3.05								

R1 = Bond length between OO• and OH of antioxidant; R2 = Bond length between OO• and OH of Fatty acid; I1= Bond index of OH from antioxidant; I2= Bond index of OH from fatty acid

CHAPTER 5

RESULTS AND DISCUSSION ON PALM OLEIN

5.1 Oxidation of Palm Olein

Triglycerides of palm olein (PO) have an equivalent percentage of saturated and unsaturated fatty acids. The highest fatty acid composition of PO is palmitic acid followed by oleic acid. In vegetable oils, free fatty acid occurs naturally at a very low amount. A few studies have shown that production of free fatty acid contributes to objectionable odour, flavour and other characteristic. The formation of free fatty acids is due to the splitting of the triglyceride molecule at the ester linkage. Total acid number (TAN) is the analyses that are used to measure the amount of acids in vegetable oil. The level of free fatty acids elevates further during processing, storage, frying or cooking (Kreivaitis *et al*., 2013).

In this study, the TAN of the treated PO was analysed according to the AOCS Cd 3A-63 standard method. Samples of PO were taken at every 0 day and after 1, 3, 6, 10 and 15 days for analyses.

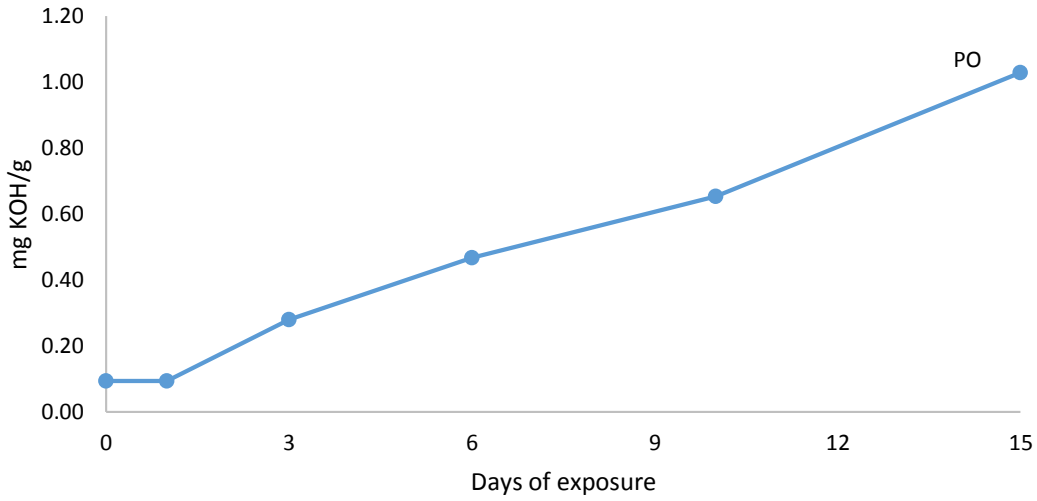


Figure 5.1: Total acid number (TAN) development of PO treated at 60˚C

Figure 5.1 shows an acid development in PO during heat treatment at 60˚C. The sample shows that the TAN start to escalate on the third day of heating and the increment continues until the final day (day 15). This is evidently caused by continuation of hydrolysis throughout the heating process (Fox and Stachowiak, 2007)

Oxidation process of PO was also measured using PV analyses. PV were measured in miliequivalent of peroxide per kilogram of sample for each sampling period are shown in Figure 5.2. Oil samples were taken on day zero (0) which is before the heat treatment start and after 1, 3, 6, 10 and 15 days.

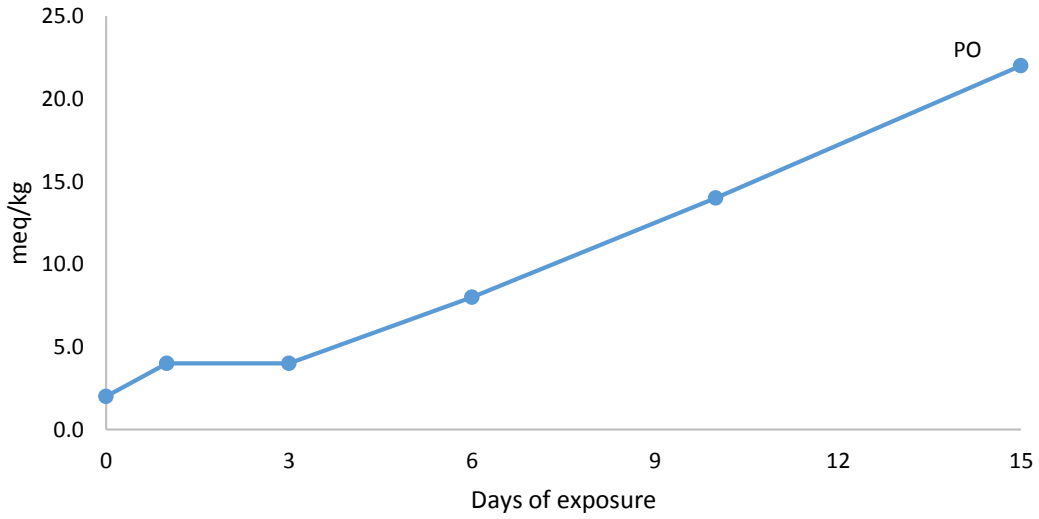


Figure 5.2: Peroxide value (PV) development of PO treated at 60˚C

Result shows that PV of PO increased over time. The increment of the value started since the first day of heat treatment. The value remains constant and starts to rise after third day of heat treatment. These analyses proved that oxidation occurred at this temperature. Temperature that was used in this study reflects the storage and transportation temperature. According to Frankel (1993), storage at this temperature is recognized as having the least limitation, and results correlate well with evaluation of actual shelf life.

According to results from this 15 days study of PO, heat treatment on the oil tends to degrade as indicated by peroxide and TAN. The results in this work have confirmed the literature information that vegetable oil storage promotes a rise in the PV and TAN. To obtain a highly stable vegetable oil during storage, it is

necessary to take antioxidant to help to delay the oxidation process thus protect oil from degradation.

5.2 Oxidation of Palm Olein in the Presence of Antioxidants

Since the results of oxidation of PO studies (Figure 5.1 and 5.2) proved that the deterioration of oil occur with time at 60°C, the PO samples were then tested with 4 types of synthetic antioxidant (chain breaking radical scavenger group) to observed the oxidation trend in the presence of antioxidant. The experimental procedures are still the same and the PO samples without the addition of antioxidant will become the control set for this studies.

PV and TAN were measured for all mixtures of PO with antioxidant. The results of the analyses displayed in Figure 5.3 for TAN results and Figure 5.4 for PV.

5.2.1 TAGs Decomposition

Overall studies on the effect of antioxidant on TAG decomposition shows that all antioxidant except PG gave lower TAN value compared to PO without antioxidants. It is also observed that BHA, BHT and TBHQ exhibited similar

performance in reducing TAG decompositions of PO. The reduction of TAN can be related with theoretical calculation (Table 4.2 and 4.3) where it shows a strong interaction (>35 kJ/mol) between antioxidant and H_{β} TAG tripalmitic/trioleic. This strong interaction had prevented rotamer formation which could lead to TAG hydrolyses and form fatty acids.

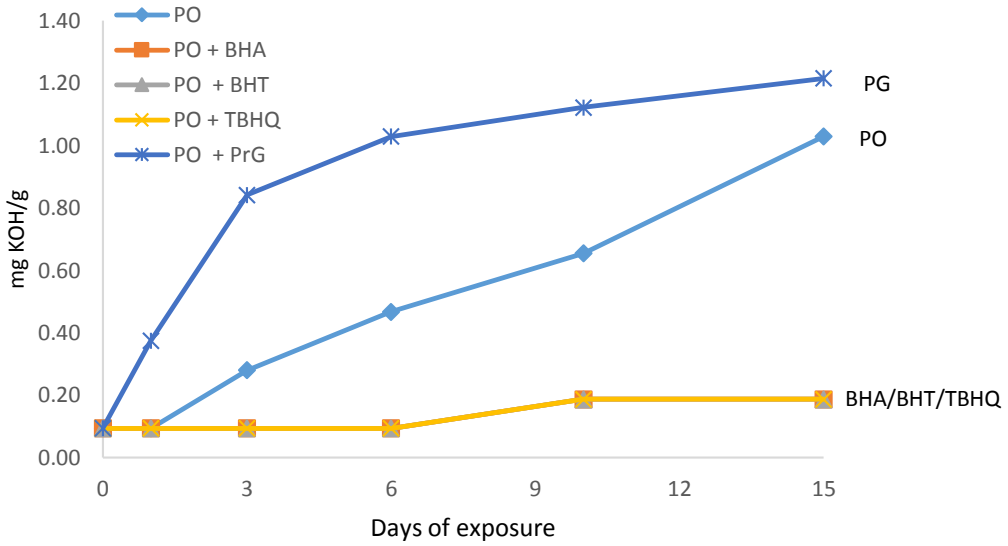


Figure 5.3: Total acid number of PO samples with four different types of antioxidants: BHA, BHT, TBHQ and PG

Total acid number (Figure 5.3) shows that three out of four antioxidants used in this study manage to protect the PO from H_{β} TAG hydrolyses which releases fatty acid from the triacylglycerides. BHA, BHT and TBHQ managed to maintain the TAN of 0.09 mg KOH/g from the first day of the heat treatment until the sixth day and slight increment to 0.19 mg KOH/g and maintain until day 15. Samples with PG show to increase significantly but this does not indicates that PG unable to protect the TAG from hydrolysis. In 2011, Karavalakis *et al*. studies the storage

stability on biodiesel blends treated with different antioxidants. Results from the studies found that, all samples those were treated with PG shows high TAN because of the acid nature of the component.

5.2.2 Peroxide Formation

Overall studies on the effect of antioxidant on peroxide formation shows that all antioxidants manage to reduce peroxide formation. Antioxidant such as BHT, TBHQ and PG exhibited ability in lowering the oxidation of PO significantly compared to BHA which was found to be the weakest. The order of antioxidant's performance in preventing or reducing peroxide formation in PO (good to poor) is as follows: TBHQ> BHT≈PG> BHA.

Figure 5.4 exhibit the results of PV of PO in the presence of antioxidants. As expected, all antioxidants used in this study manage to reduce the oxidation of PO. TBHQ gave the lowest PV followed by BHT, PG and lastly BHA. TBHQ express the ability to protect the oil from oxidation from the first day until the final day of heat treatment. Other three antioxidants show a slow increment day by day during the heat treatment process.

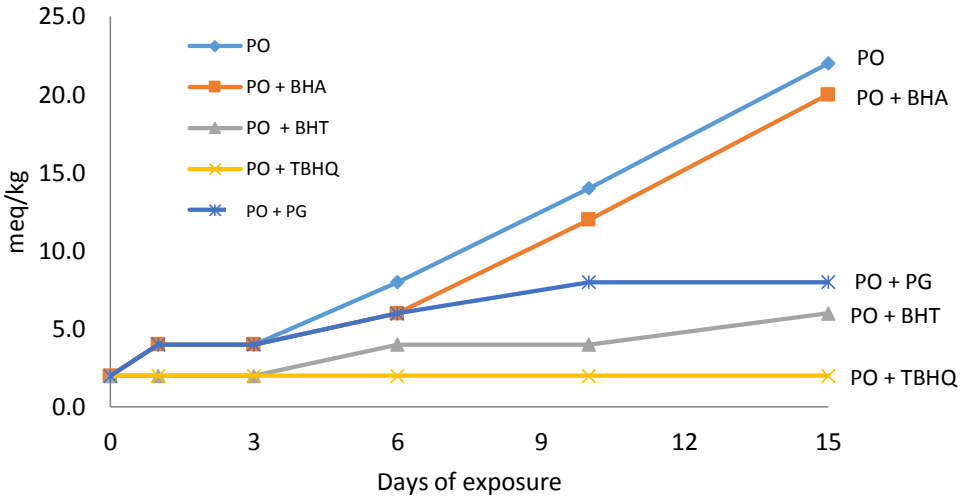


Figure 5.4: Peroxide value of PO samples with four different types of antioxidants: BHA, BHT, TBHQ and PG

Overall, PO with added antioxidant shows better oxidative stability. TBHQ were able to protect the oil from oxidation and hydrolysis of TAG compared to other three antioxidants. Even though PG was responsible in the increment of TAN, due to the formation of gallic acid, the antioxidant performance was good in delaying oxidation of PO. Gallic acid formed from PG still act as an antioxidant in preventing oil oxidation. BHA shown to be less effective antioxidant compared to other three antioxidants. This can be related to the property of BHA reported by Dunn (2005) which can invert and act as pro-oxidant at higher loading. As a conclusion, the best antioxidant for PO in preventing oxidation is TBHQ. The sequences of the antioxidant performance from good to poor in preventing oxidation are as follows; TBHQ > BHT > PG > BHA

The good performance of TBHQ could be associated with the theoretical findings where TBHQ shows the highest interaction energy (-22.78kJ/mol) compared to other three antioxidants. The similar performance between BHT and PG in the experiment can also be explained by the theoretical findings where the interaction energy between TAG trioleic $C_8OO•$ radical with both antioxidants are very similar which is BHT = -10.40.12 kJ/mol and PG = -14.46 kJ/mol.

The differences between the experimental and theoretical results were only seen on BHA where BHA antioxidant supposed to show similar performance with TBHQ as what had been calculated from the theoretical. This might be due to the effect of loading which was mention by Dunn, 2005 where BHA will act as pro-oxidant if added at higher loading.

5.3 Oxidation of Palm Olein in the Presence of Fatty Acids

5.3.1 TAGs Decomposition

Most vegetable oil contains low amount of free fatty acids (FFA). It was revealed that the free fatty acids that occur in vegetable oil are from triacylglycerides degradation (Paradiso *et al*., 2010). Previous research mentioned that FFA can act as pro-oxidant or as an antioxidant depend on types of fatty acids

and vegetable oil. This part of studies will focused on the effect of fatty acids on the oxidative stability of PO.

Measurements of TAN were conducted to monitor acid formation in oil samples. TAN for all samples before treatment is 0.09 mg KOH/g which was similar as TAN of the control. The addition of fatty acids (PA, SA, OA, LA) at any concentration (0.25%, 0.5%, 1%, 3%) reduced triacylglyceride decomposition compared to the oil without fatty acid. The TAN results of the PO samples which were heated in oven for 15 days at 60°C are shown in Figure 5.5 to Figure 5.8. After 15 days of heat treatment, all samples shows lower TAN which is between 0.09 mg/KOH to 0.37 mg/KOH compared to PO alone with the TAN of 1.03 mg/KOH. The phenomenon of the TAN reduction could be associated to the high molecular interaction between fatty acids and triacylglycerides which was calculated using Gaussian software (Table 4.2) where the value is around 43 to 45 kJ/mol. Results shown that OA is the best in reducing fatty acids formation at any concentration. For instance, at day 15th, the TAN was only 0.19 mg KOH/g for OA at concentration $\leq$ 1% compared to PO without any added fatty acid which is 1.03 mg KOH/g. Percentage reduction of TAN is about 80%. The order of increment of TAG hydrolyses are as follows: OA > PA > LA > SA. However, the results on the final days have shown that PA and LA shows similar effect.

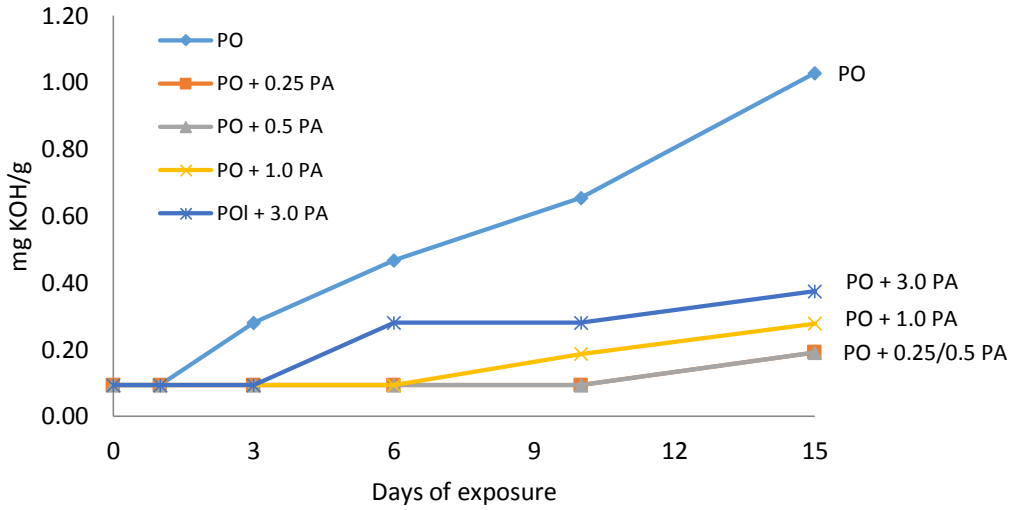


Figure 5.5: Total acid number (TAN) of PO samples with PA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

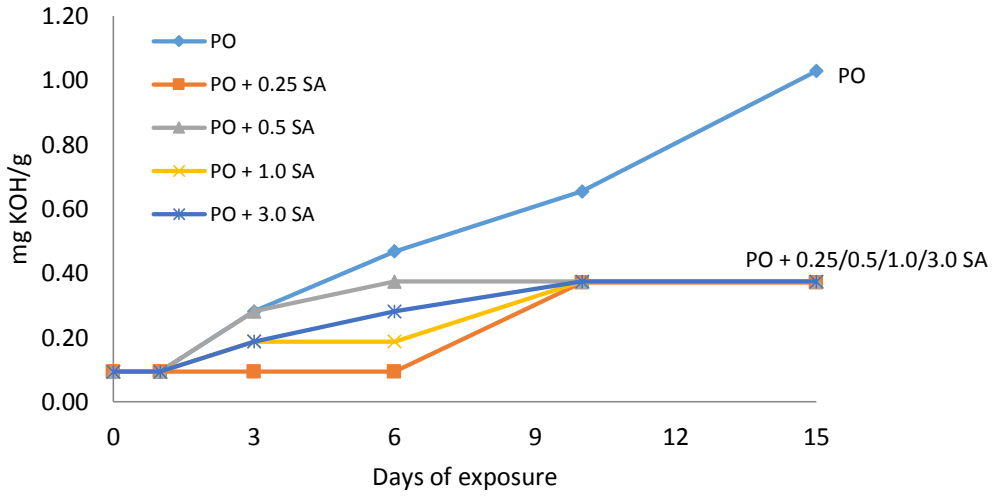


Figure 5.6: Total acid number (TAN) of PO samples with SA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

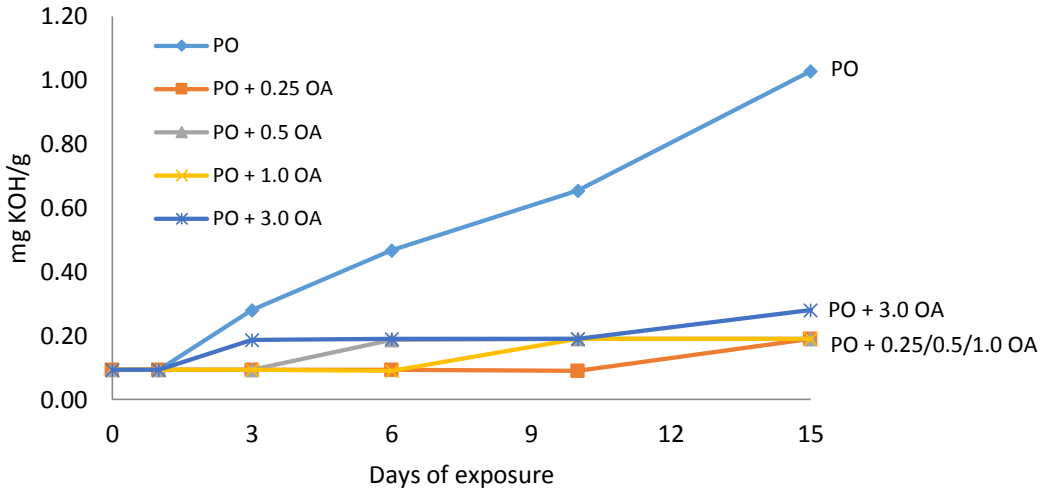


Figure 5.7: Total acid number (TAN) of PO samples with OA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

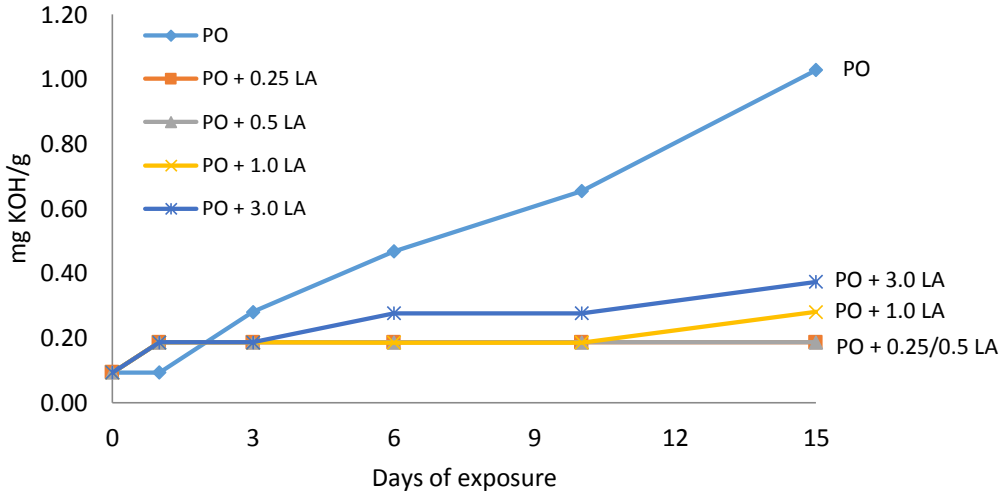


Figure 5.8: Total acid number (TAN) of PO samples with LA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

The PA at low concentration (0.25-0.5% w/w) is the best in reducing the TAG hydrolysis. SA shown to able inhibit the oil from degradation but not as an effective as palmitic where the TAN on the 15th day for samples with SA are all 0.37 mg KOH/g compared to palmitic which is in the range of 0.19-0.37 mg KOH/g.

Unsaturated, OA and LA, also shows the best result at lower loading which is around 0.25-1.0 w/w % where the TAN on the final day is in the range of 0.19 to 0.28 mg KOH/g. As mentioned, the fatty acids performance ranging from good to poor are expressed in an ordinal form as follows: OA > LA > PA > SA. The result on SA and PA is already predicted through theoretical evaluation where SA and PA shows very low interaction energy with H_β TAG tripalmitic, which is only at the amount of -22 kJ/mol compared to oleic, -25 kJ/mol.

5.3.2 Peroxide Formation

PV results of PO mixture with PA, SA, OA and LA were tabulated in Figure 5.9, 5.10, 5.11 and 5.12, respectively. An experimental study has shown that the addition of any fatty acids (PA, SA, OA and LA) at any concentration will promote peroxide formation. PA has shown the least pro-oxidant activity compared to other fatty acids while LA has shown the highest pro-oxidant effect on PO. Even though SA and OA has shown similar effects, further analyses on PV after 6 days revealed for higher concentration (more than 0.5%) oleic acid showed higher pro-oxidant effects compared to stearic acid. These experimental phenomena are supported by with theoretical findings discussed in section 4.5 where SA shows lower interaction compared to OA (Refer to Table 4.11).

Experimental results on PV are in good agreement with theoretical results where the order of the pro-oxidant properties is as follows: LA > OA ≈ SA > PA.

Both experimental and theoretical results support that the unsaturated fatty acids (linoleic and oleic acid) show higher pro-oxidant activity compared to the saturated fatty acids (PA and SA), and also polyunsaturated fatty acids gave higher pro-oxidant activity compared to monounsaturated (OA). These experimental and theoretical findings are in accordance with the previous studies on olive oil by Paradiso *et al.*, 2010.

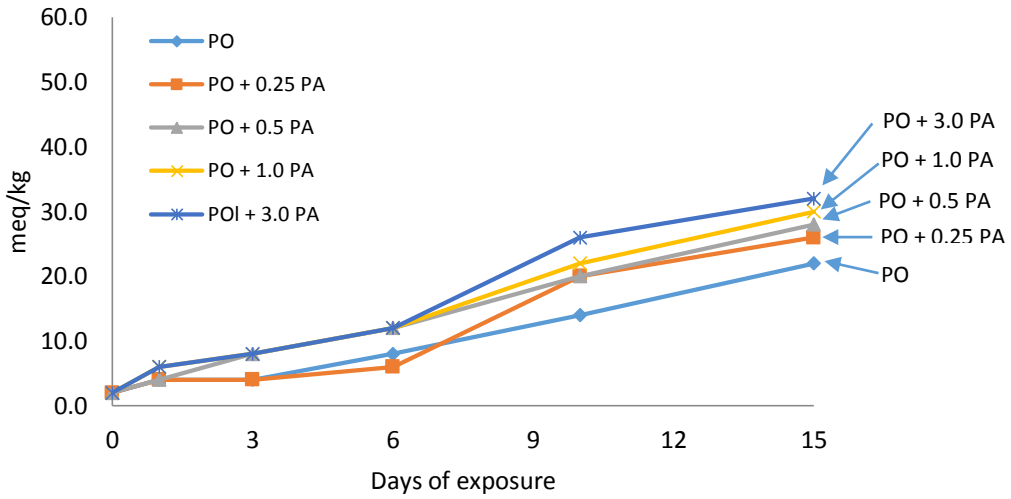


Figure 5.9: Peroxide value (PV) of PO samples with PA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

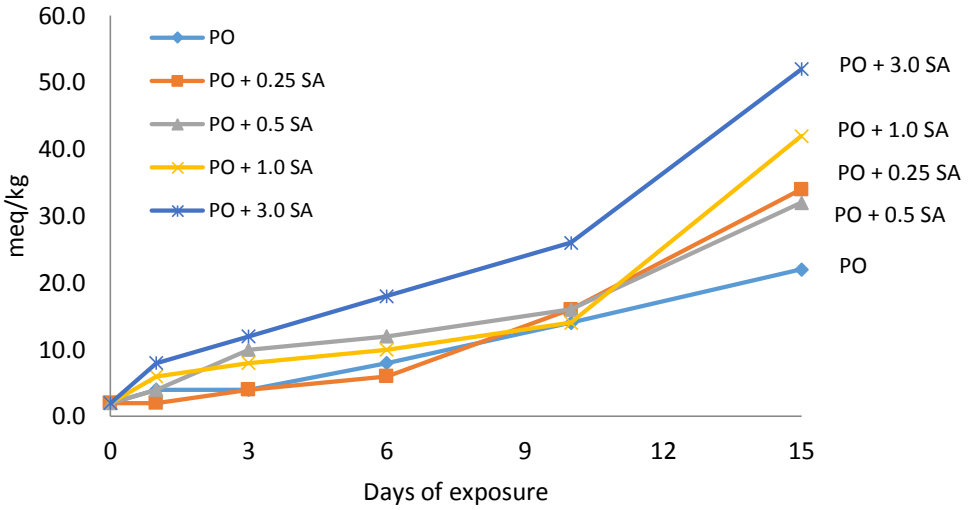


Figure 5.10: Peroxide value (PV) of PO samples with SA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

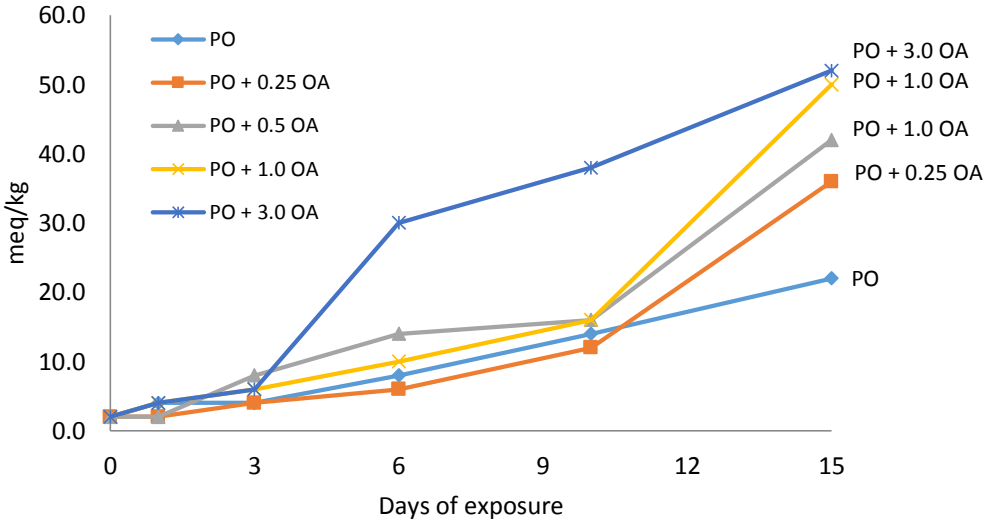


Figure 5.11: Peroxide value (PV) of PO samples with OA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

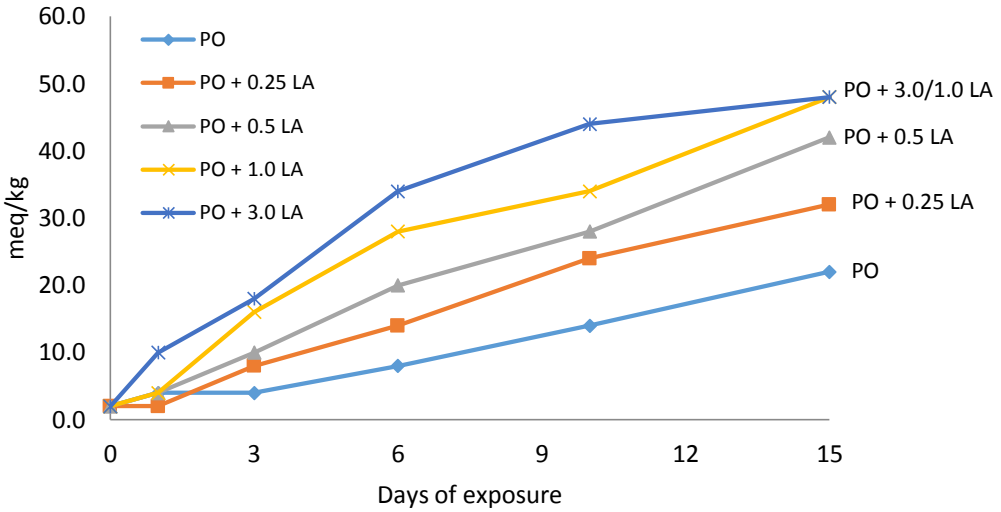


Figure 5.12: Peroxide value (PV) of PO samples with LA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

5.4 Effects of Fatty Acids on the Performance of Antioxidants

The use of antioxidant alone already proven to be able to delay the PO oxidation. Experimental results also revealed that fatty acids act as pro-oxidants towards PO which cause a drastic increment in PV. This subtopic will discuss the performance of antioxidants towards oxidative stability of PO when fatty acids were added to the systems.

On the TAGs decomposition:

Experimental studies on the performance antioxidant in inhibiting the TAG decomposition in the presence of fatty acids has shown that stearic acid gave

negative effects on BHA, BHT and TBHQ while the least effect was shown by linoleic acid.

Larger effects that were shown by SA can already be predicted expected based on its abrupt declining in interaction energy by as much as 73%. [Refer to interaction of tri-species complex between H_β TAG trioleic, TBHQ and fatty acids in Table 4.12]. The least affected is LA, and this experimental result is also supported by the least reduction in interaction energy which is about 59.65%. The order of the fatty acids in affecting the performance of antioxidant from the most to the least are as follows: SA>OA>PA>LA.

The negative effect shown by all four fatty acids on the performance of BHA, BHT and TBHQ are due to their differences in interaction energy between fatty acids and antioxidants. The theoretical calculation revealed that all antioxidants except PG has very low interaction energy with H_β of TAG compared to the other fatty acids. (I_{BHA} = -21.25; I_{BHT} = -14.84; I_{TBHQ} = -21.13; I_{PA}= -32.10; I_{SA} = -32.26; I_{OA}= -27.37; I_{LA} =-27.13). Please refer to Table 4.3 and Table 4.7.

In contrast to PG, all fatty acids have shown a synergistic effect where the TAN of the sample mixture (PO + PG + fatty acids) are lower compared to sample mixture of PO + PG only. The experimental results are in line with the theoretical findings where PG has higher interaction energy compared to all fatty acids used in this study (I_{PG} = -34.68 kJ/mol).

If the ranking were made from the least affected to the largest affected antioxidants, the order is as follows: PG> TBHQ> BHA> BHT. This results are in line/parallel to/in accordance to the order of interaction energy between H_β TAG trioleic and TAG Tripalmitic is as follows: TAG trioleic: PG (-34.43)>TBHQ (-21.13) ≈ BHA (-21.25) > BHT (-14.84). TAG Tripalmitic: PG (-34.68) > TBHQ (-20.54) ≈ BHA (-20.71) > BHT (-17.68). It is also worth to mention that this order is the reverse to the order of antioxidant only: BHA≈ BHT ≈TBHQ> PG (refer to subtopic 5.2.1)

It can be concluded that the calculated interaction energy of the tri-species complex can be used to measure the effect of fatty acids on the performance of antioxidants in inhibiting the TAG decomposition of PO.

On the Peroxide Formation:

These studies indicated that the saturated fatty acids (PA and SA) do not show any effect on the performance of all antioxidants tested (BHA, BHT, THQ, PG). In contrary, the unsaturated fatty acids, OA and LA, show negative effect on antioxidants performance especially on BHT, TBHQ and PG. The presence of any fatty acids, PA, SA, and OA does not give any negative effect on the performance of BHA with LA. However, the effects are not significant.

Antioxidant such as BHT, TBHQ and PG has shown almost the same characteristic towards the presence of fatty acids in the sample mixtures. The addition of saturated fatty acid (PA and SA) does not give any effect towards these three antioxidants. However, the performance of these antioxidants is decreasing in the presence of the unsaturated fatty acids (OA and LA).

BHA has shown interesting synergistic behaviour when fatty acids were added into the samples mixtures. BHA becomes a better antioxidant in the presence of unsaturated FAs (PA and SA). For instance, PV for the PO samples which contained only BHA on the 15th day is 20 meq/kg, in comparison to with the added PA or SA; PV has dropped to 16 and 18 meq/kg, respectively. This phenomenon can be related to the higher interaction energy between BHA and TAG trioleic $C_8OO\bullet$ radical -23 kJ/mol (refer to Table 4.10) which is a bit higher compared to the interaction energy with other fatty acids. (I_{PA} = -23; I_{SA}=-22.0; I_{OA}=-23; I_{LA}= -20).

5.4.1 Performance of BHA in Inhibiting Oxidation of Palm Olein in the Presence of Selected Fatty Acids

On the TAGs Decomposition:

Figure 5.13 displays the effect of adding palmitic acid at four different concentrations on the performance of BHA. Results showed only 0.5 % w/w of palmitic acid was able to reduce $H_{ß}$ TAG hydrolyses while others concentrations seem to show no effects.

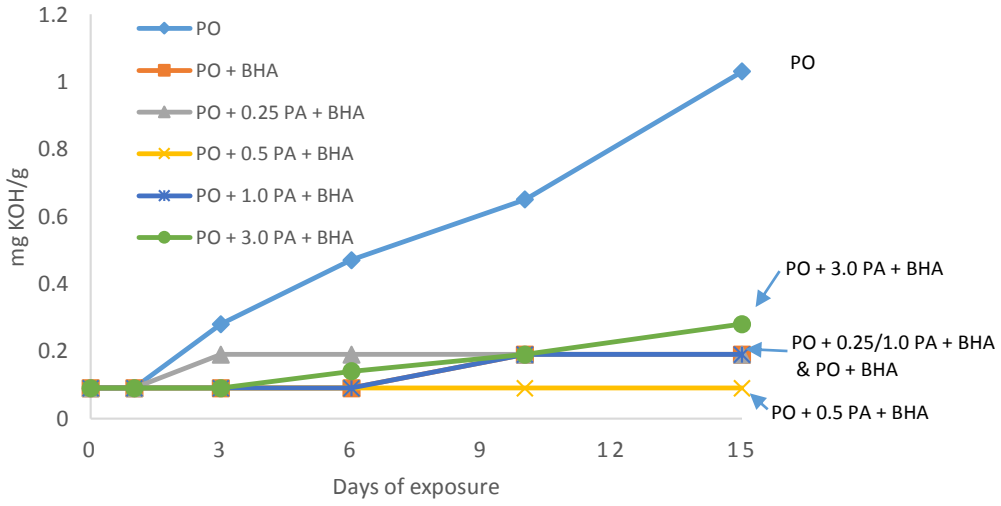


Figure 5.13: Effect of PA concentration on the TAN of PO in the presence of BHA

Variations of TAN results from the addition of SA to BHA are presented in Figure 5.14. The addition of SA seems to reduce the performance of BHA by increasing acid content the acid content of the mixture.

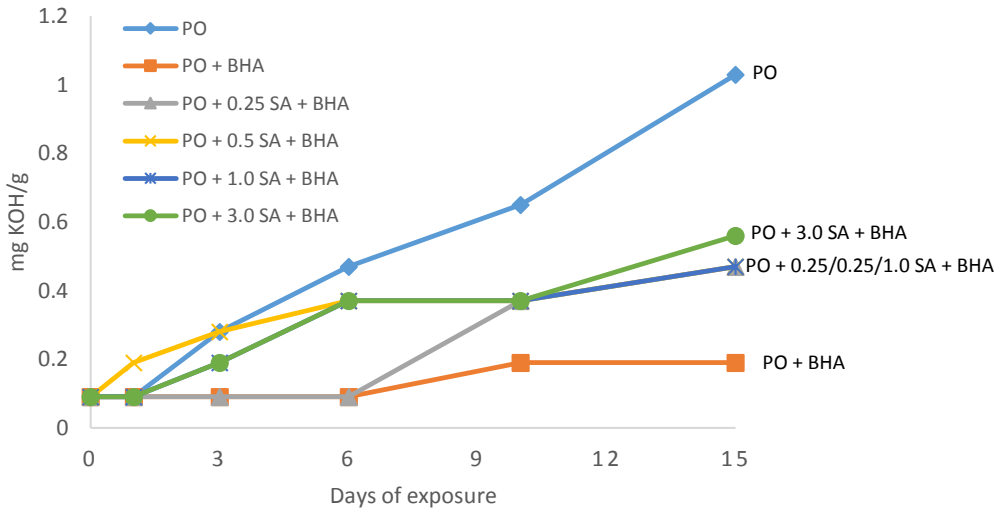


Figure 5.14: Effect of PA concentration on the TAN of PO in the presence of BHA

The performance of BHA in preventing $H_{ß}$ TAG hydrolyses in the presence of monounsaturated and polyunsaturated are shown in Figure 5.15 and Figure 5.16, respectively.

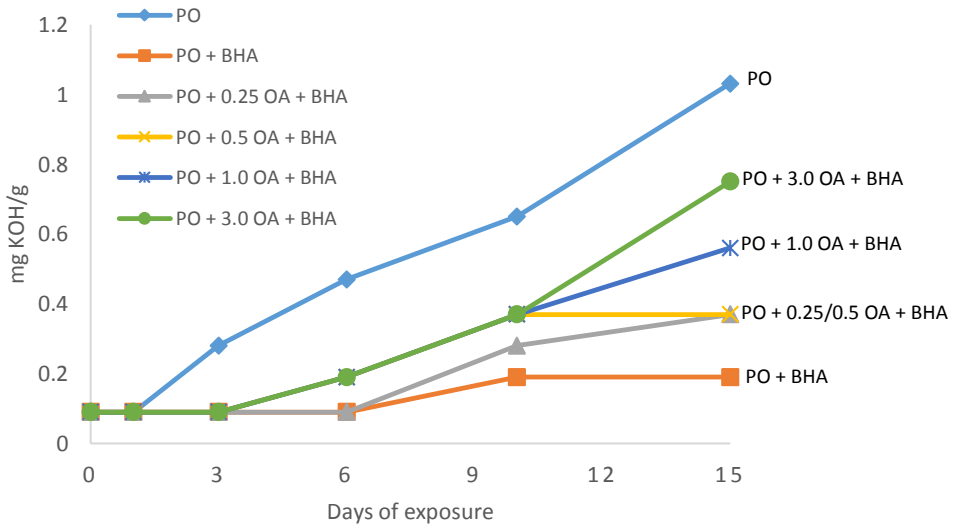


Figure 5.15: Effect of OA concentration on the TAN of PO in the presence of BHA

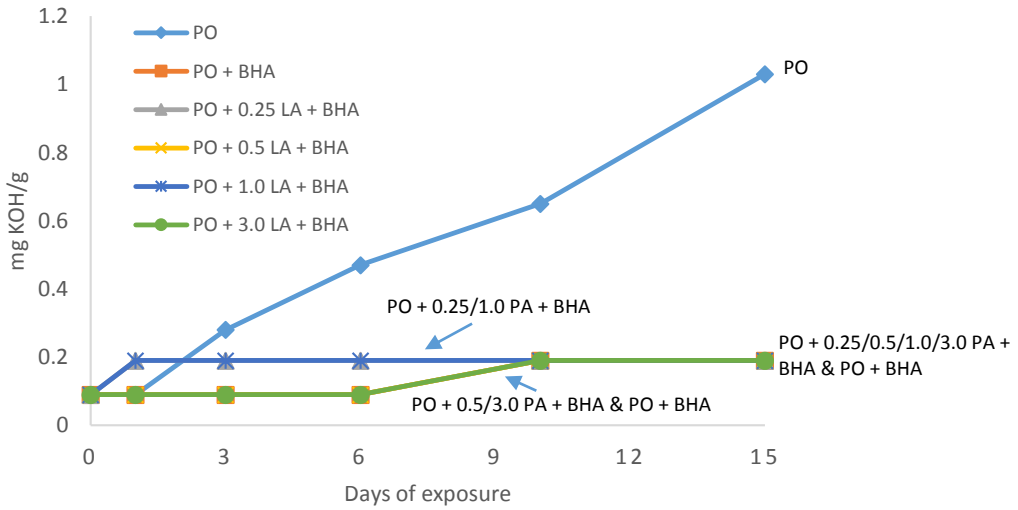


Figure 5.16: Effect of LA concentration on the TAN of PO in the presence of BHA

In the presence of monounsaturated (oleic acid), negative behaviour were seen at all four concentrations. However, the polyunsaturated fatty acid (linoleic acid), seems to shows to have no effect on the performance of BHA.

On the Peroxide Formation:

PV analyses on the mixture of PO, BHA and FAs (palmitic, stearic, oleic and linoleic) are shown in Figure 5.17 to 5.20.

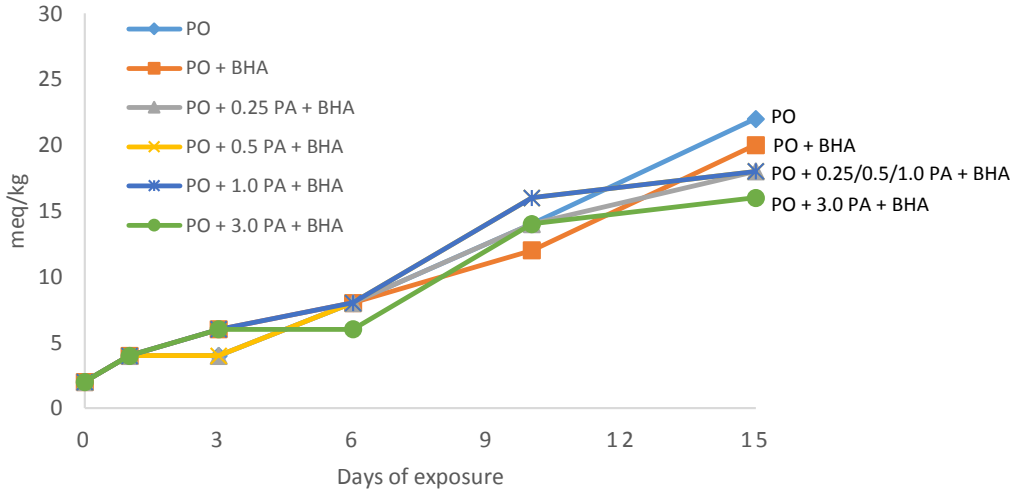


Figure 5.17: Effect of PA concentration on the PV of PO in the presence of BHA

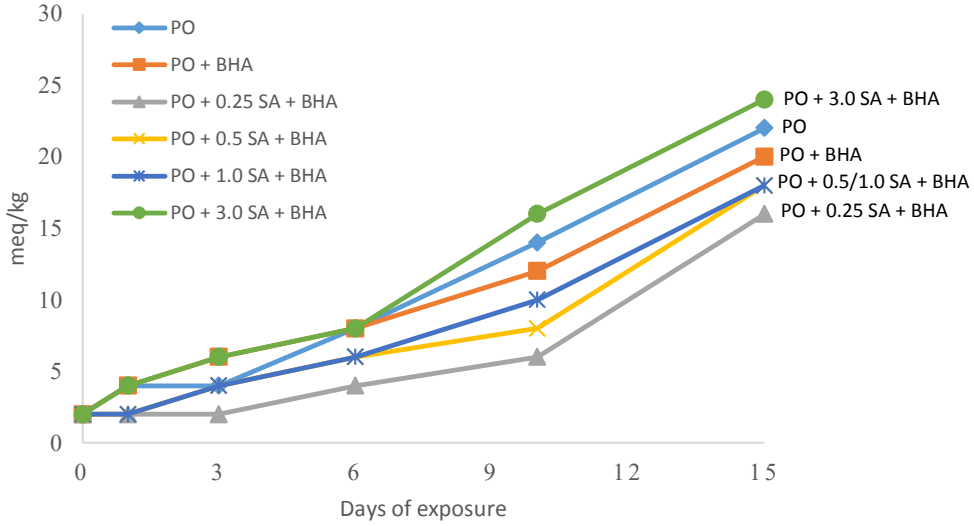


Figure 5.18: Effect of SA concentration on the PV of PO in the presence of BHA

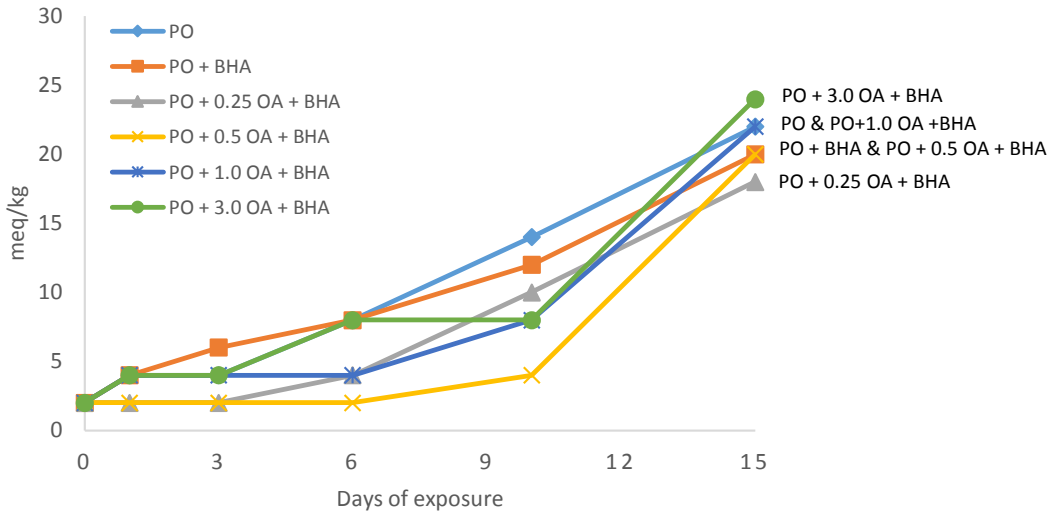


Figure 5.19: Effect of OA concentration on the PV of PO in the presence of BHA

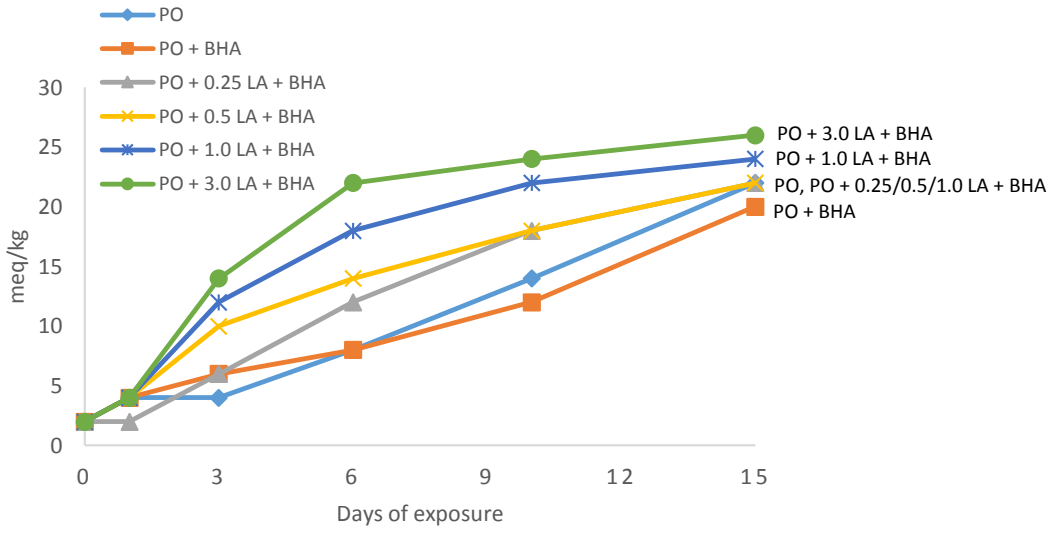


Figure 5.20: Effect of LA concentration on the PV of PO in the presence of BHA

Figure 5.17 shows that the performance of BHA increased when in the presence of PA at all concentrations tested. The addition of PA managed to hinder the peroxide formation of the oil samples better than BHA alone. In the cases of SA (Figure 5.18), similar trend were observed at 0.25 to 1.0% (w/w) loading where the

existence of SA manages to improve the BHA performances. However, at 3% loading, SA shows pro-oxidant behaviour. The addition of OA (Figure 5.19), at lower loading 0.25% (w/w) manages to reduce oxidation and improve BHA performance, while at higher loading, the pro-oxidant effect was observed especially towards the final days of the experimental period. Different case was observed when LA was added to BHA where pro-oxidant effect (Figure 5.20) lowered the performance of BHA.

The BHA performance in the presence of fatty acid ranging from good to poor in delaying $H_{ß}$ TAG hydrolyses are expressed in an ordinal form as follow; PA > LA > OA > SA. While if it were based on PV, the sequence are as follows: PA > SA > OA > LA

5.4.2 Performance of BHT in Inhibiting Oxidation of Palm Olein in the Presence of Selected Fatty Acids

BHT was discovered to be an excellent antioxidant due to its ability to prevent oil oxidation and TAG decomposition (Section 5.2). Under this subtopic, the discussion will be focused on the effect of added fatty acids towards the performance of BHT in protecting PO from oxidation and TAG degradation.

On the TAGs Decomposition:

The performance of BHT in hindering TAG H_{β} hydrolyses in the presence of PA, SA, OA and LA are shown in Figure 5.21 to Figure 5.24 respectively.

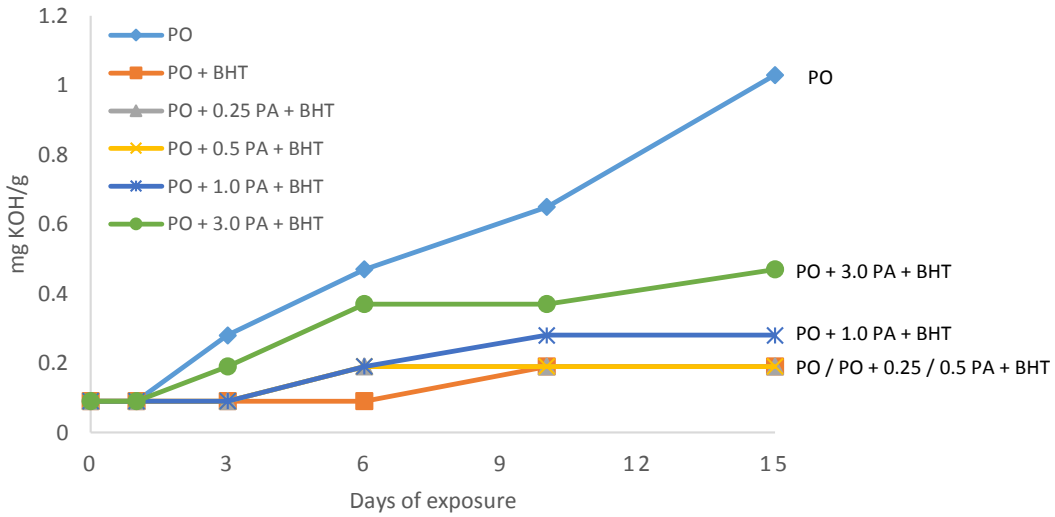


Figure 5.21: Effect of PA concentration on the TAN of PO in the presence of BHT

Results (Figure 5.21) revealed that at lower PA acid loading which is between 0.25 to 0.5 % (w/w), BHT was observed to be not very much affected by the presence of PA. However, at higher loadings, 1% and 3%, the fidelity of BHT seems to be lost. This can be seen by the increment of TAN after the 6^{th} day of exposure.

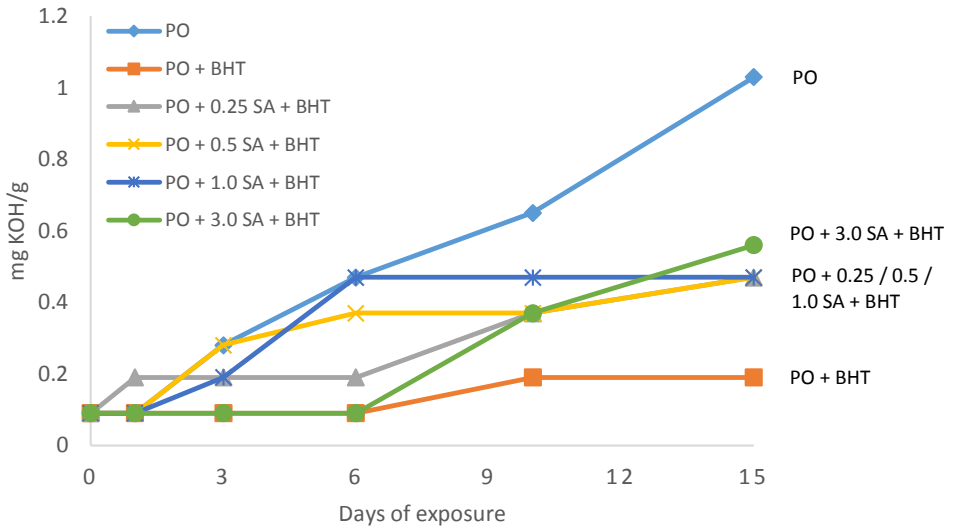


Figure 5.22: Effect of SA concentration on the TAN of PO in the presence of BHT

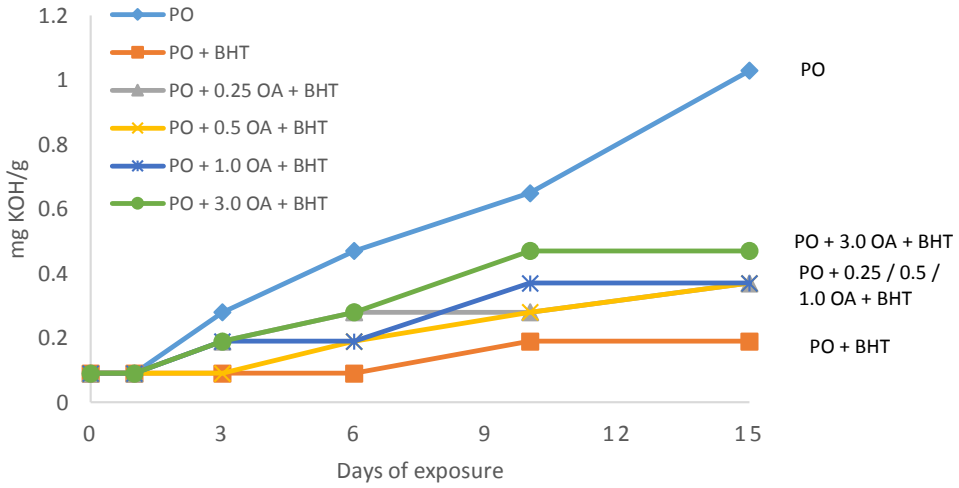


Figure 5.23: Effect of OA concentration on the TAN of PO in the presence of BHT

The addition of stearic (Figure 5.22) and oleic acid (Figure 5.23) evidently act as TAG H_{β} hydrolyses promoter hence to reduce BHT performance. These

results demonstrate that SA or OA will surely reduce the performance of BHT in preventing TAG degradation of PO.

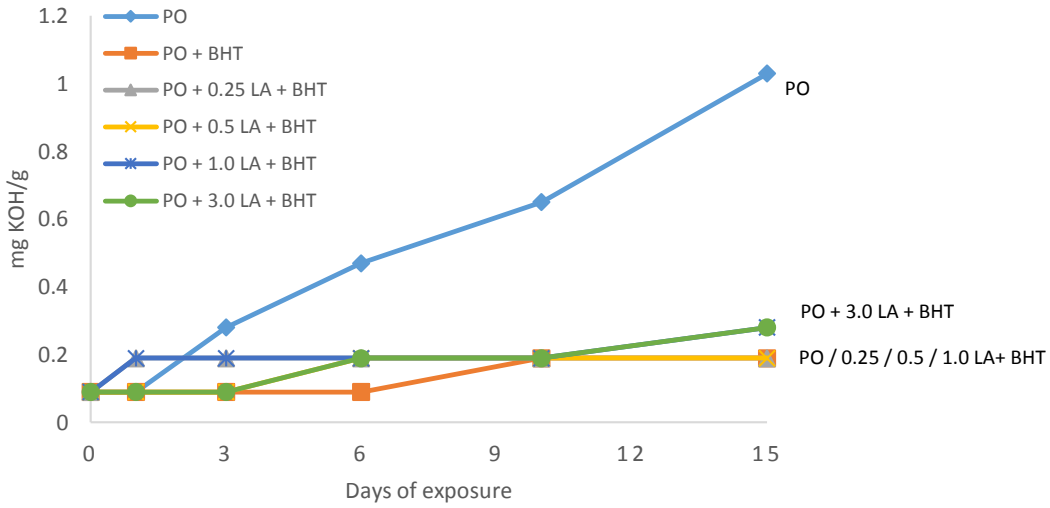


Figure 5.24: Effect of LA concentration on the TAN of PO in the presence of BHT

Addition of LA (Figure 5.24) shows similar effect as the addition of PA at lower loading between 0.25 and 0.5% (w/w). No effects were observed on BHT performances. At higher loading of 1% and 3% linoleic acid slight increment in TAN were recorded.

On the Peroxide Formation:

Variation of PV's of PO samples against the days of exposure for PA, SA, OA and LA with BHT are illustrated in Figure 5.25 to 5.28.

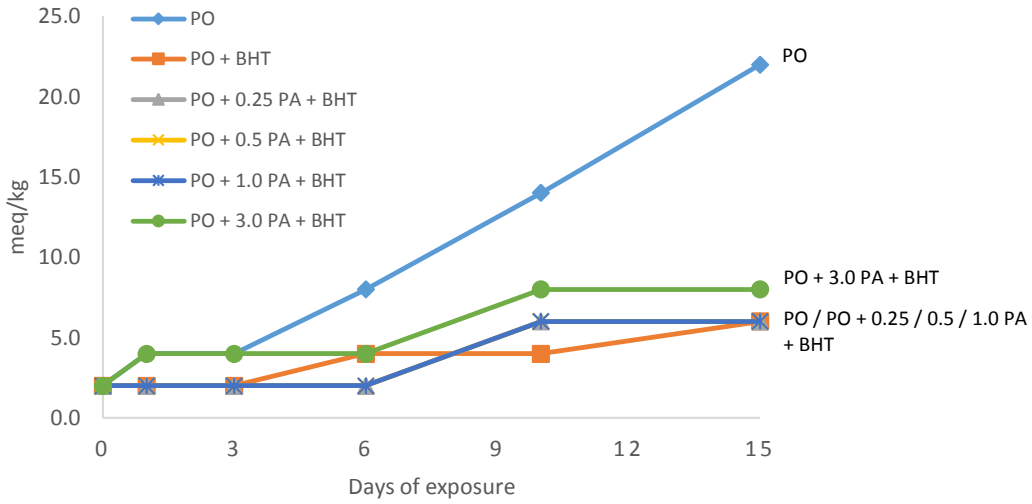


Figure 5.25: Effect of PA concentration on the PV of PO in the presence of BHT

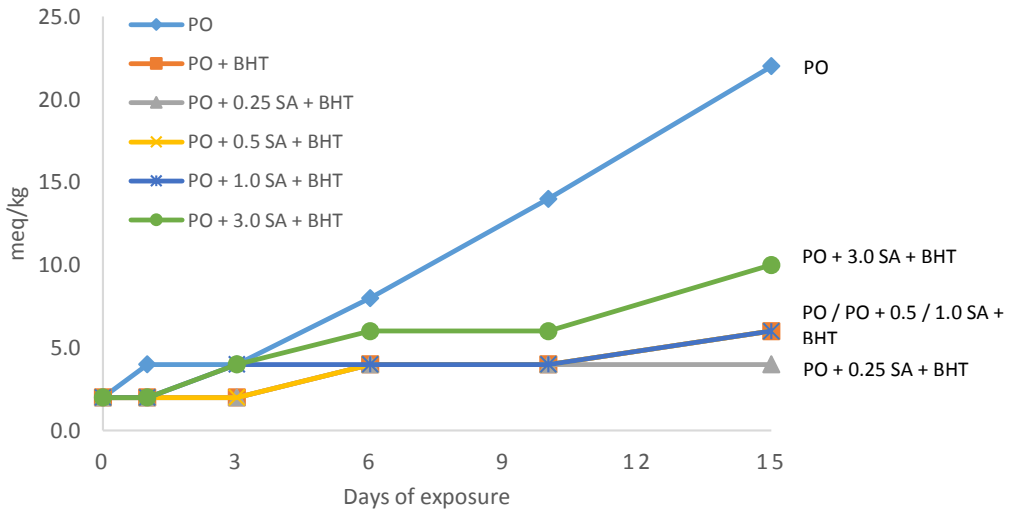


Figure 5.26: Effect of SA concentration on the PV of PO in the presence of BHT

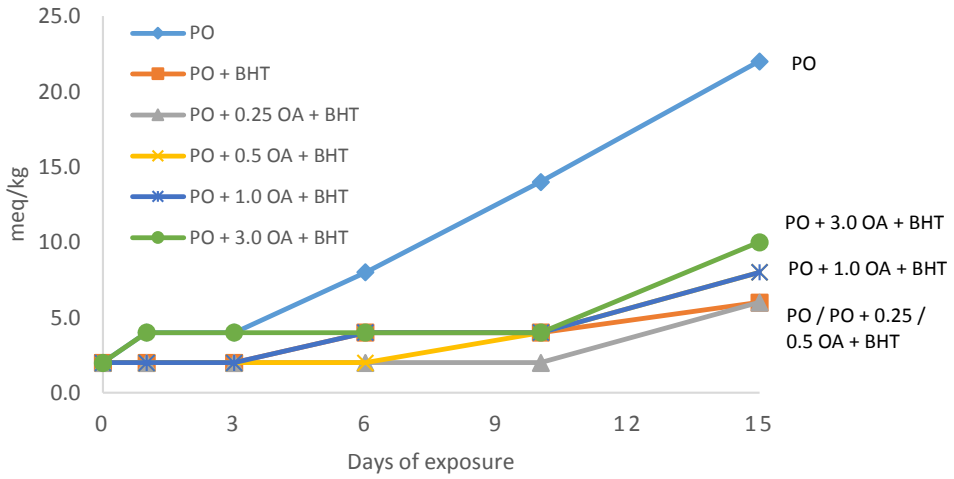


Figure 5.27: Effect of OA concentration on the PV of PO in the presence of BHT

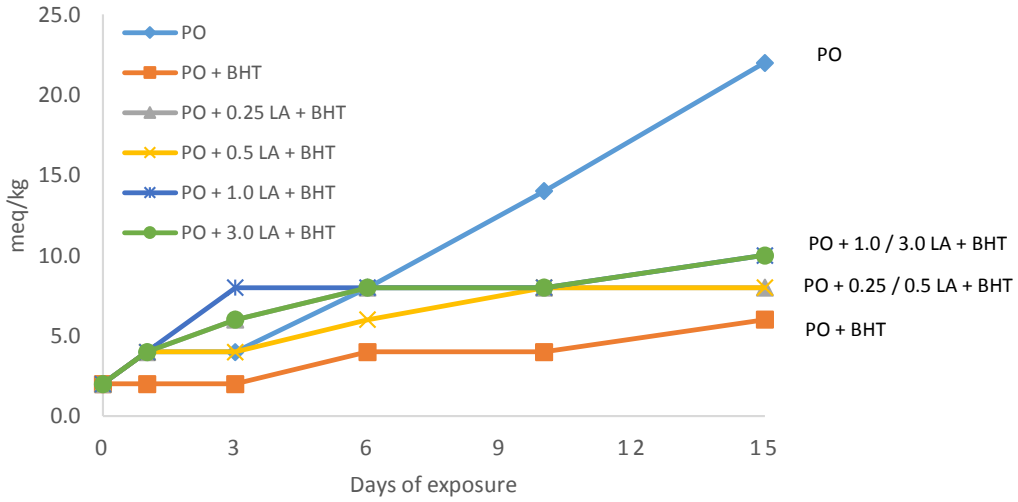


Figure 5.28: Effect of LA concentration on the PV of PO in the presence of BHT

PV analyses show that pro-oxidant effect were observed for samples with 1% to 3% (w/w) loading of OA and LA while all fatty acids at low concentration (0.25% w/w) shows to have no effect on BHT performance.

The sequence of fatty acid that affect the BHT antioxidant performance in delaying $H_{ß}$ TAG hydrolyses from good to poor can be expressed in ordinal form

as follows; PA > LA > OA > SA. While for the peroxide formation, the sequence was as follows: PA > SA > OA > LA. Interestingly, BHA shows sequential resemblance of performance with BHT. However, fatty acids added with BHT produced a good antioxidant combination compared to BHT alone. Almost in all cases, more negative effects were found compared to positive effect in protecting the oil.

5.4.3 Performance of TBHQ in Inhibiting Oxidation of Palm Olein in the Presence of Selected Fatty Acids

Previous research done on antioxidants performance repeatedly mentioned TBHQ as an excellent antioxidant (Dunn, 2005; Mohdaly *et al*, 2010 and Maia *et al*, 2011). Findings of this research studies proves the literature of previous findings in preventing the oil from degradation and oxidation (Figure 5.3 and Figure 5.4).

On the TAGs Decomposition:

Experimental studies on TBHQ performance in the presence of fatty acid were done and the TAN results are display in Figure 5.29 to 5.32 while PV results displayed in Figure 5.33 to 5.36.

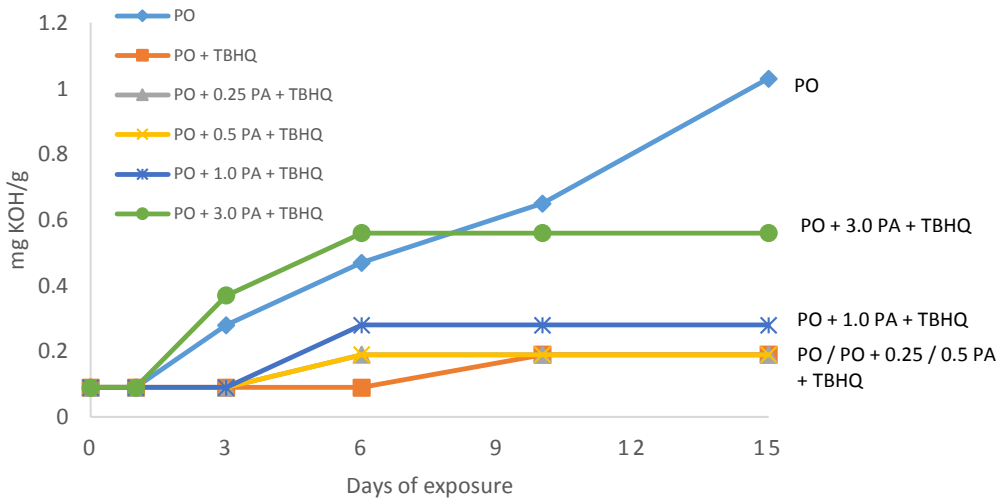


Figure 5.29: Effect of PA concentration on the TAN of PO in the presence of TBHQ

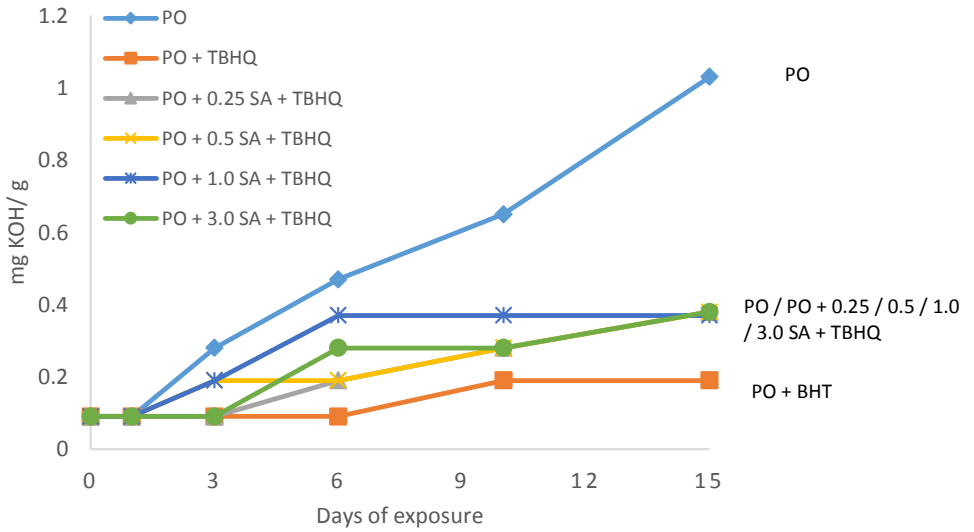


Figure 5.30: Effect of SA concentration on the TAN of PO in the presence of TBHQ

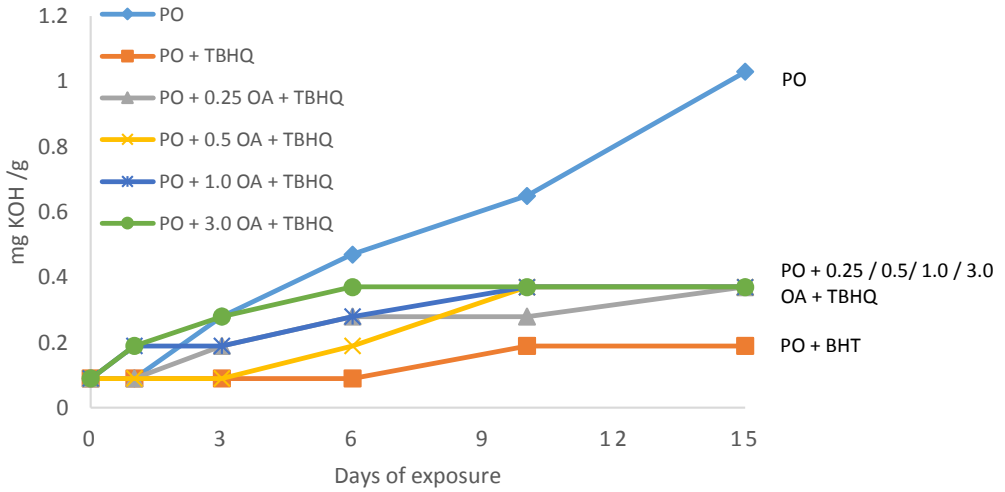


Figure 5.31: Effect of SA concentration on the TAN of PO in the presence of TBHQ

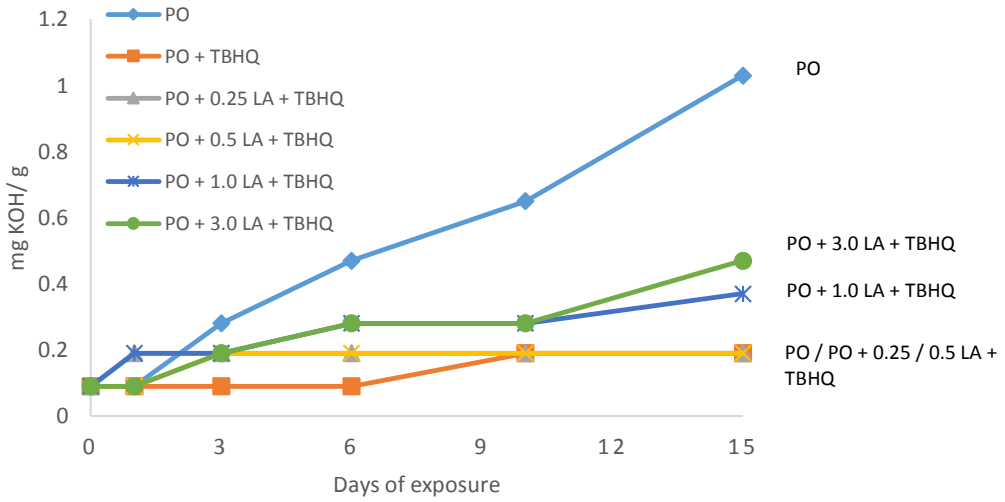


Figure 5.32: Effect of LA concentration on the TAN of PO in the presence of TBHQ

Figure 5.29, displays the effect of adding PA at four different concentrations on the performance of TBHQ. No effect were observed on TBHQ performance loaded with 0.25 to 0.5 % (w/w) palmitic acid, but when 1% and 3% loading of

palmitic acid were added to the mixture, TBHQ seems to be unable to hinder the $H_{ß}$ TAG.

Different phenomena were observed when SA and OA added in the mixture (Figure 5.30 and Figure 5.31). Combination of PO and TBHQ with stearic acid and combination of PO and TBHQ with oleic acid at all four concentrations exhibit a negative effect where the TAN increased starting on the 6^{th} day of heat treatment. A significance difference was observed between sample mixture of PO and TBHQ with and without SA and OA addition. Hence, the two combinations of TBHQ and SA and TBHQ and OA has depressed the TBHQ efficiency in preventing $H_{ß}$ TAG degradation/hydrolysis.

The addition of LA at 0.25 and 0.5% (w/w) shows no effect on TBHQ performance (Figure 5.32). However, the addition of LA at 1% and 3% (w/w) promotes TAG hydrolyses. Based on the TAN, linoleic and palmitic acid were shown to have similar effect on TBHQ performance.

On the Peroxide Formation:

PV analyses on the mixture of PO, TBHQ and FAs (palmitic, stearic, oleic and linoleic) are shown in Figure 5.33 to 5.36.

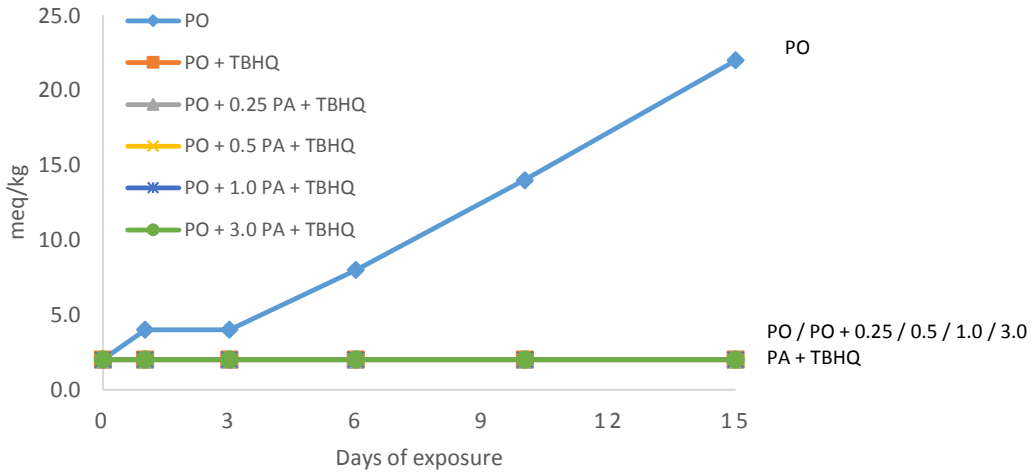


Figure 5.33: Effect of PA concentration on the PV of PO in the presence of TBHQ

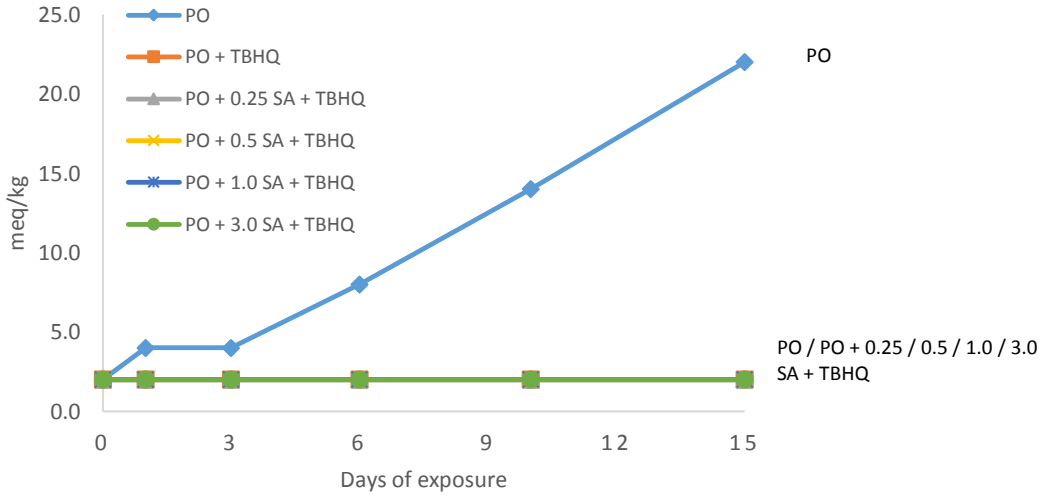


Figure 5.34: Effect of SA concentration on the PV of PO in the presence of TBHQ

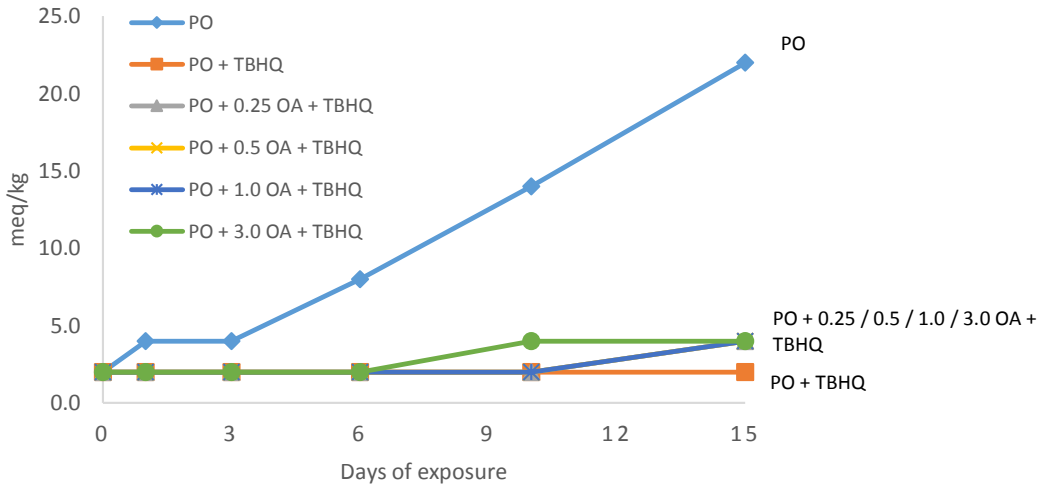


Figure 5.35: Effect of OA concentration on the PV of PO in the presence of TBHQ

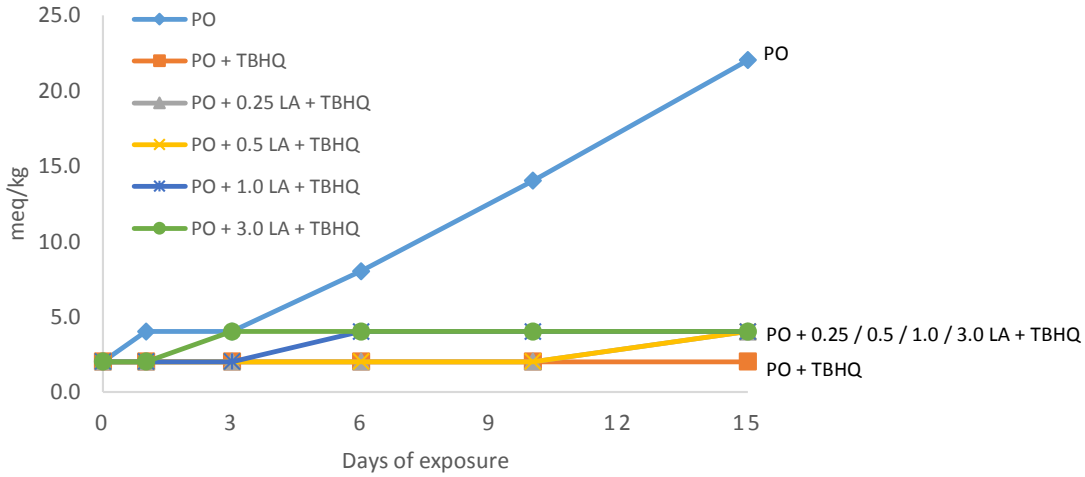


Figure 5.36: Effect of LA concentration on the PV of PO in the presence of TBHQ

The TBHQ performance in the presence of all fatty acids at various concentration has shown to be able to reduce peroxide formation. These combinations also gave the lowest PV compared to other combination of fatty acids and antioxidants (BHA, BHT, and PG). However, some combination of fatty acid

with TBHQ still shows an antagonistic it the TBHQ performance. To saturated acid, palmitic and oleic, had shown no effect on TBHQ performance while the unsaturated, oleic and linoleic acid has shown an antagonistic effect as the PV are higher compared to other combinations of the saturated fatty acids.

The sequence of fatty acids that affect the TBHQ performance in delaying TAG hydrolyses from good to poor are expressed in an ordinal form as follow: PA > SA > OA > LA. While if it were based on PV, the sequences are as follows: PA = SA > OA = LA

The fatty acid sequence based on antioxidant performance for TBHQ are different from BHT and BHA. Overall, TBHQ has shown a very good antioxidant property in preventing oxidation even in the presence of fatty acid at various concentrations.

5.4.4 Performance of PG in Inhibiting Oxidation of Palm Olein in the Presence of Selected Fatty Acids

On the TAGs decomposition:

The performance of PG in hindering TAG $H_{ß}$ hydrolyses in the presence of PA, SA, OA and LA are shown in Figure 5.37 to Figure 5.40 respectively.

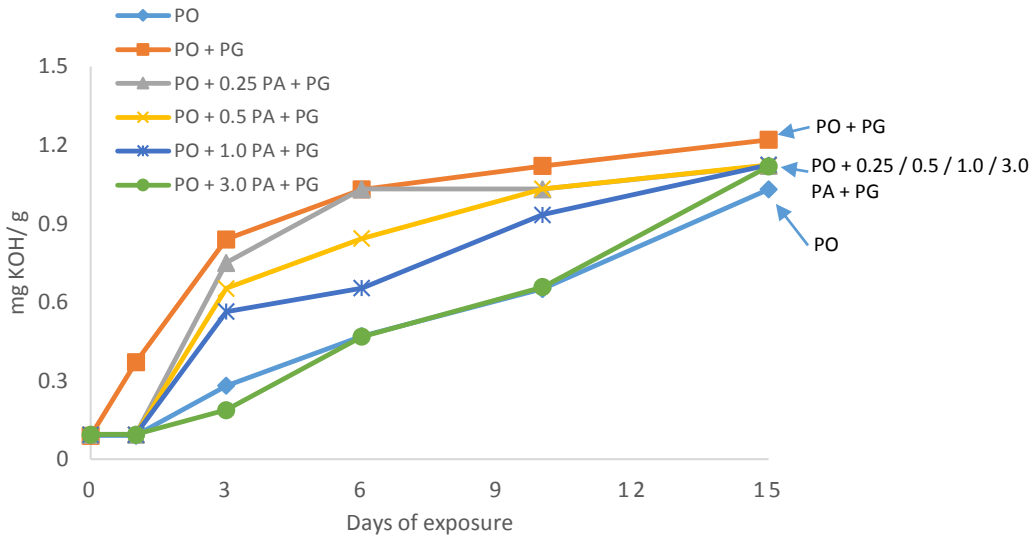


Figure 5.37: Effect of PA concentration on the TAN of PO in the presence of PG

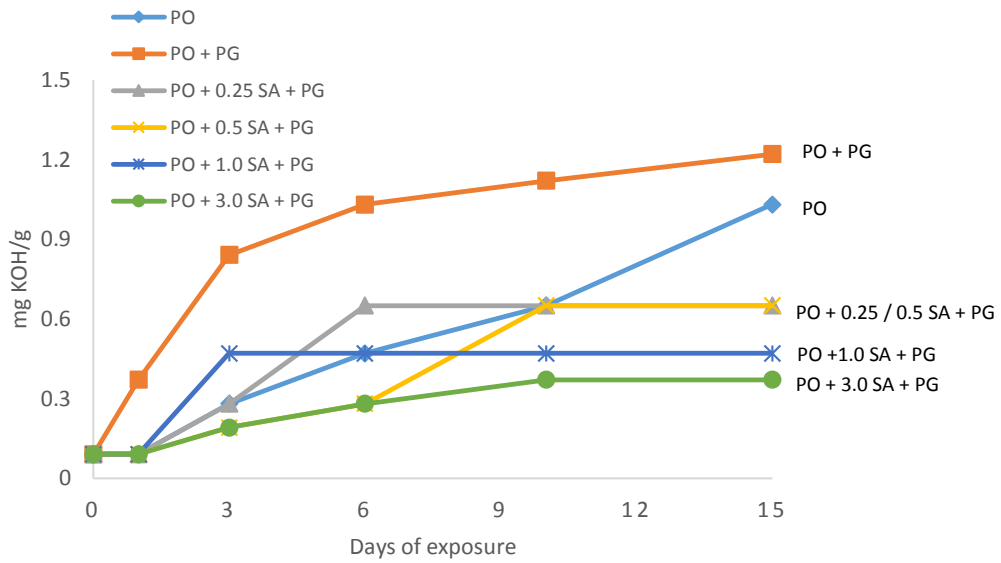


Figure 5.38: Effect of SA concentration on the TAN of PO in the presence of PG

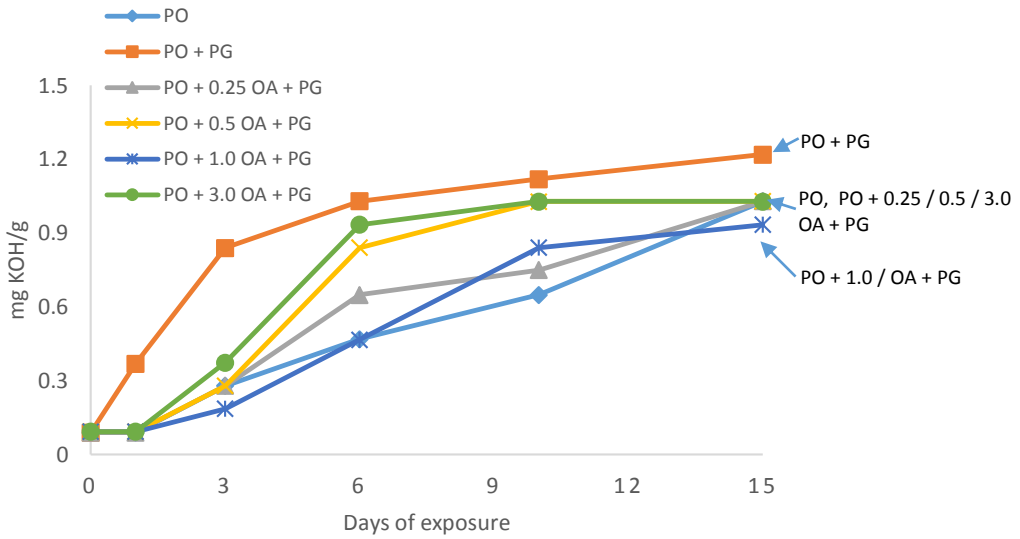


Figure 5.39: Effect of OA concentration on the TAN of PO in the presence of PG

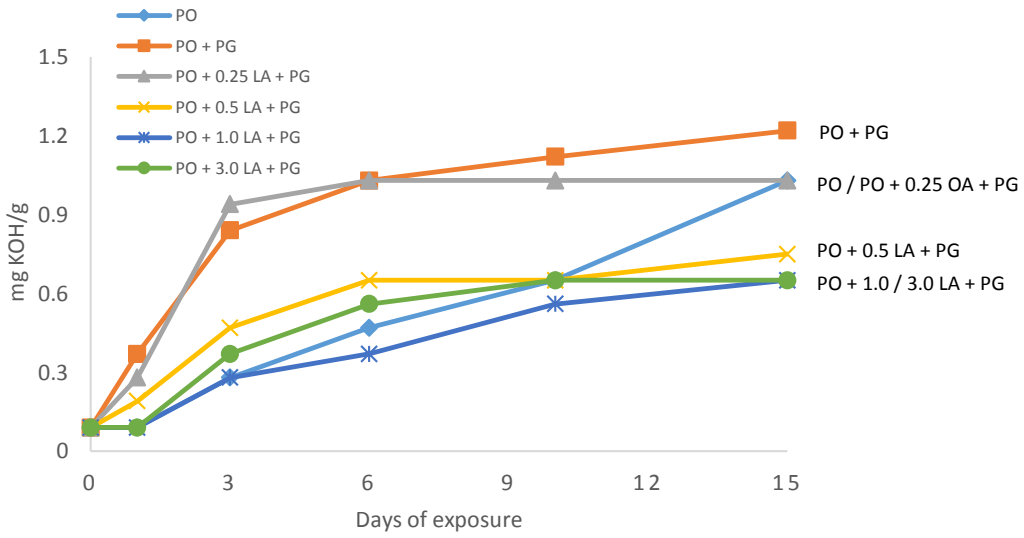


Figure 5.40: Effect of LA concentration on the TAN of PO in the presence of PG

Surprisingly, all fatty acids added at various concentration has shown to be able to improve PG performance where it manage to reduce the TAG hydrolyses compared to the mixture of PO and PG without addition of these fatty acids.

On the Peroxide Formation:

The performance of PG in hindering peroxide formation in the presence of PA, SA, OA and LA are shown in Figure 5.41 to Figure 5.44 respectively.

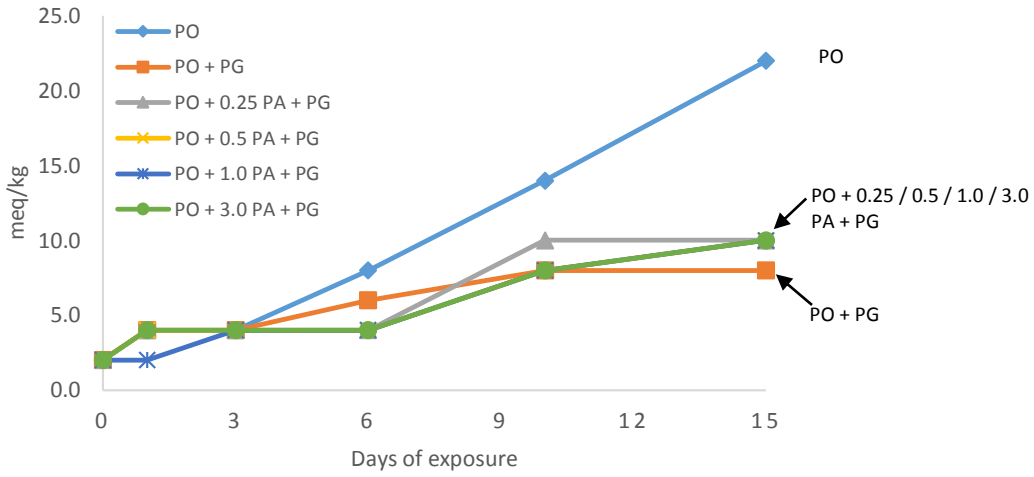


Figure 5.41: Effect of PA concentration on the PV of PO in the presence of PG

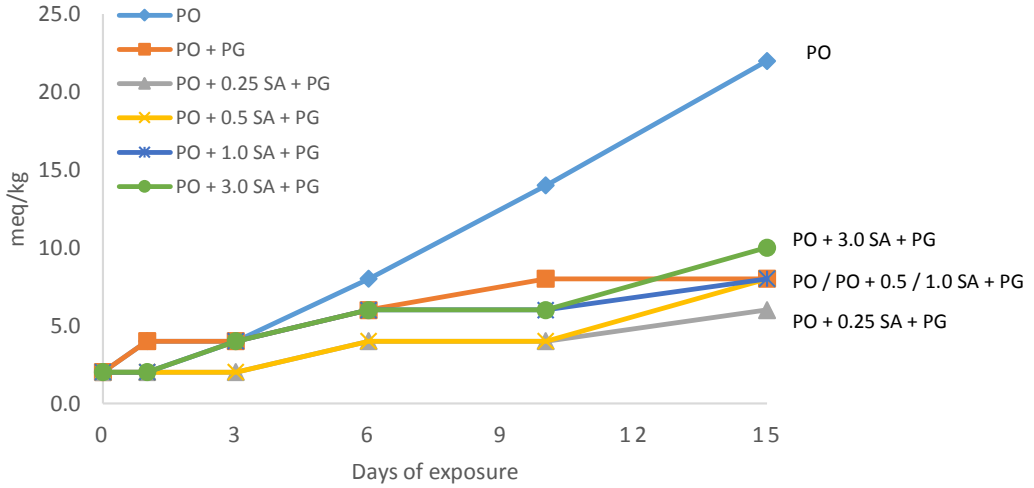


Figure 5.42: Effect of SA concentration on the PV of PO in the presence of PG

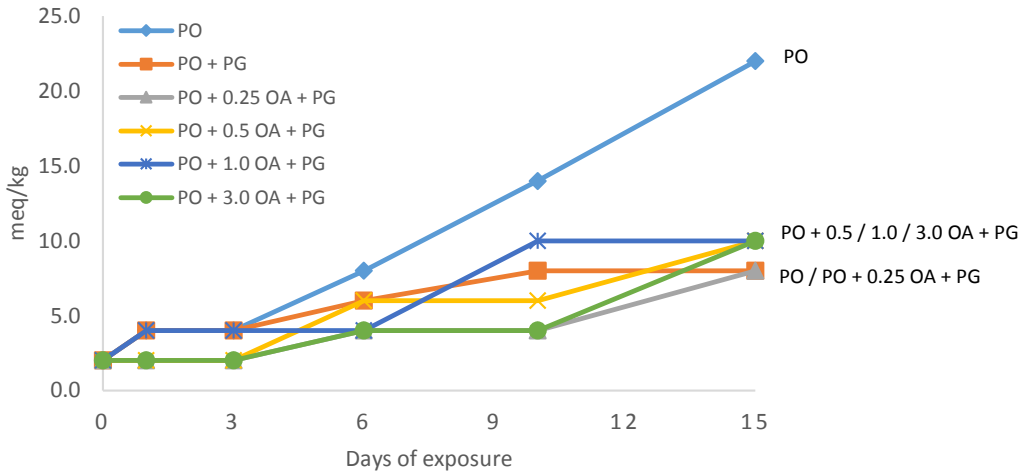


Figure 5.43: Effect of OA concentration on the PV of PO in the presence of PG

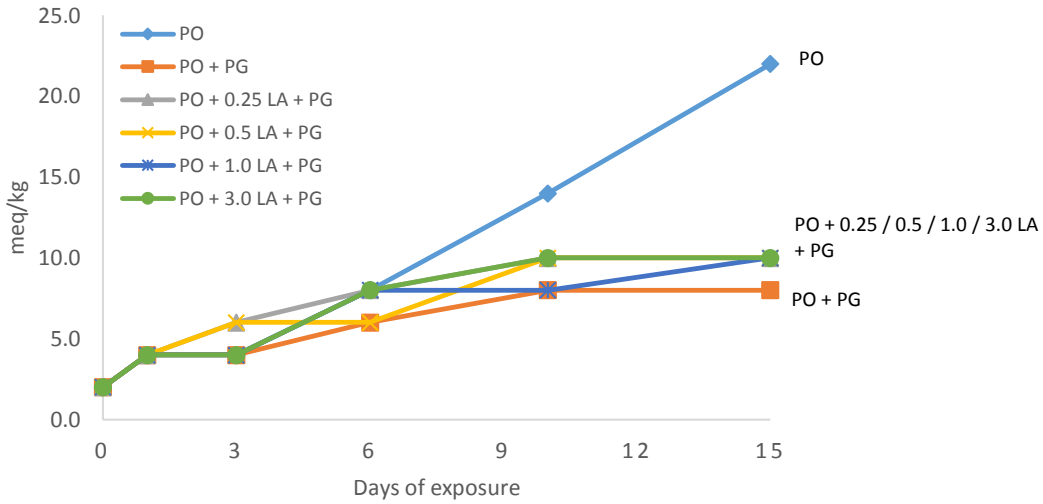


Figure 5.44: Effect of LA concentration on the PV of PO in the presence of PG

Analyses on PV revealed that pro-oxidant effect were shown for sample mixture with palmitic acid and linoleic acid. Addition of oleic acids (Figure 5.43) show pro-oxidant effect at 0.5-3% (w/w) loading. SA only exhibited pro-oxidant effect at 3% (w/w) loading. At low loading, SA does not interfere PG performance.

Even though combination of PG and fatty acid shows more pro-oxidant effect, the combination still manage to reduce the oil from oxidize.

The sequence of fatty acids that affect the PG performance in delaying TAG hydrolyses from good to poor are expressed in an ordinal form as follow: SA > LA > OA > PA. While for PV, the sequences are as follows: SA > OA > PA = LA

4.5 Summary

PO without any antioxidant exhibited more oxidative rancidity when heated compare with PO in the presence of antioxidants. All antioxidant added was proven to be very effective and superior performance when compared to PO without any added of antioxidants. Opposite behaviour was observed for PO with addition of fatty acids. All fatty acids added either saturated or unsaturated shows pro-oxidant effect towards PO. The higher the degree of unsaturated, resulted to higher pro-oxidant effect. However, fatty acids added to PO manage to decrease the TAN of the samples.

The combination of PO with fatty acids and antioxidant, various interactions occur from the combination where some combination had shown synergistic effect, some shows antagonistic while other show no reaction in controlling the antioxidants performance.

For oxidative stability which can be seen from PV results, TBHQ shows the best result followed by BHT, PG and finally BHA. TBHQ able to act as a good antioxidant in the presence of fatty acids. However, positive effect can only be seen between combination of saturated with TBHQ. The unsaturated fatty acids shows pro-oxidant effect hence reduce TBHQ performance. BHT also shows the same behaviour when combine with unsaturated fatty acids while combination of saturated fatty acids does not give any effect on BHT performance. However, addition of SA at 0.25% concentration to the samples improves BHT performance. PG which comes is third best after TBHQ and BHT show a pro-oxidant effect in the presence of PA, OA and LA especially when they are added 1% and 3% of w/w concentration. Positive effect can only be seen when SA added at 0.25% of concentration in PO-PG mixture. BHA exhibit the least ineffectiveness in controlling oxidation in the presence of FA shows good effect when combine with PA at all four concentration and OA at 0.25% concentration. Addition of LA has shown to act as pro-oxidant for PO where the BHA performances are weakens in the presence of LA.

TAN results shows that almost all fatty acid combination with antioxidant promotes hydrolyse of H_{β} TAG. Only a few reducing hydrolyses effect were seen while other combination give no effect on antioxidant performance. TBHQ and BHA shows similar performance but TBHQ seems to show the best antioxidant since it was able to give the lowest TAN content in most of its mixture in the

presence of certain fatty acids. Second most effective in antioxidant performance is the BHA followed by BHT and the last is PG. TBHQ performance in inhibiting TAG hydroxylation were clearly seen in the presence of SA and OA. PA and LA reduce TBHQ performance when added at 1 to 3% (w/w). Lower concentration than 0.5% (w/w) shows no effect on TBHQ performance. BHA also show the similar trend when SA and OA present in the PO-BHA mixture. Addition of LA and PA respectively shows no significant effect on BHA performance. BHT exhibit $H_{ß}$ TAG hydrolyses effect with SA, OA and at higher loading of LA and PA (1% and 3% w/w). No effects were seen on BHT performance when PA added at 0.25 and 0.5% concentration. PG's TAN shows the highest value compared to other samples. However, PG expressed more positive effect in reducing TAN with the fatty acid added. Fatty acids that show positive effect with PG are SA, OA and LA. Surprisingly, the increment of fatty acid added shows better improvement of PG performance. Only PA shows small effect on improving PG performance on delaying TAG hydrolyses.

CHAPTER 6

RESULTS AND DISCUSSION ON CANOLA OIL

6.1 Oxidation of Canola Oil

Canola oil (CO) is one of the most important sources of edible vegetable oils in the world. The development of genetically improved low-erucic acid canola varieties boosted the use of canola oil in food applications. The content of saturated fatty acids in canola is the lowest among all common vegetable oils. Fresh CO is odorless, bland in taste and light in color, however, it develops off-flavors during storage or upon heating. The oxidative deterioration of canola oil is similar to other vegetable oils and involves primarily autoxidative reactions which are accompanied by various processes having oxidative and non-oxidative characters.

In this study, the total acid number (TAN) of the treated canola oil were analysed according to the AOCS Cd 3A-63 method while peroxide value (PV) was analysed according to the AOCS Cd 8-53 method. Oil sample were taken at every 0 day and after 1, 3, 6, 10 and 15 days. TAN and PV results are shown in Figure 6.1 and 6.2 respectively.

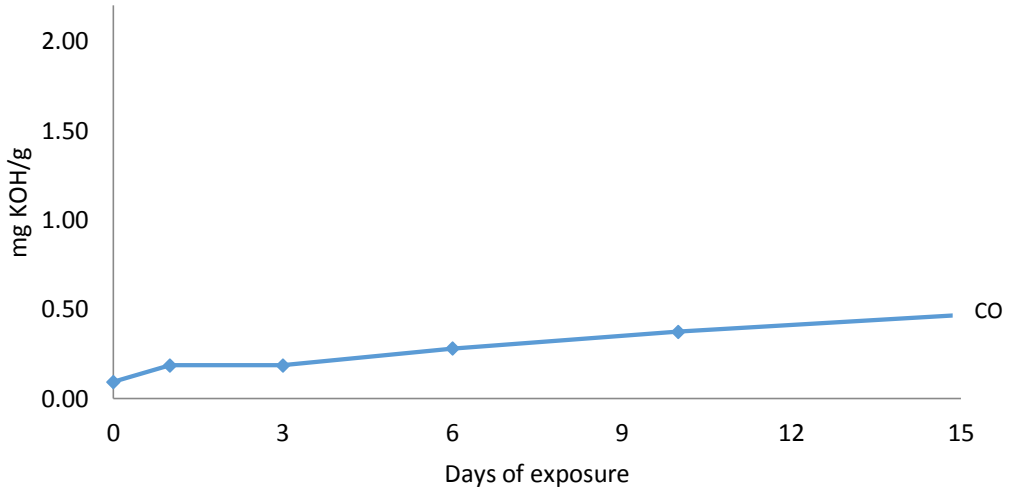


Figure 6.1: Total acid number development of CO treated at 60˚C

Figure 6.1 shows an acid development in CO during heat treatment at 60˚C. The sample shows that TAN of CO begins to increase on the first day of treatment and remained the same until the third day. After the third day of heating, the increment continues to rise until day 15. This is evidently caused by continuation of TAG hydrolysis throughout the heating process. The results trend is similar with oxidation of palm olein (Figure 5.1) and in fair agreement with those of Krevaitis *et al* (2013) and Yoshida *et al.* (1992) who assessed the oxidative deterioration of some oils.

Oxidation process on CO was measured using PV analyses. PV analyses which are plotted against days of exposure are shown in Figure 6.2.

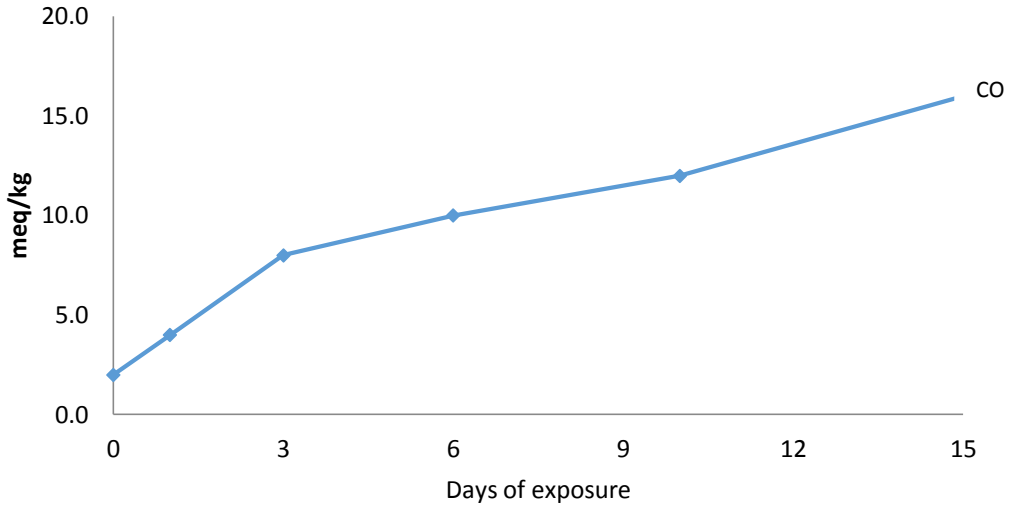


Figure 6.2: Peroxide value development of CO treated at 60˚C

PV of CO shows an increment over time. The gradual increment of the PV was observed from the first day until the fifteenth day of the experiments. These analyses proved that oxidation did occur at this temperature (60˚C) which also represent as actual shelf life for 15 months (Evans *et al.*, 1973). The increment of PV due to the storage of vegetable oils are also in a good agreement with the studies that was done by Catel *et al*. (2012) and Kreivaitis *et al*. (2013).

As a conclusion, heat treatment on the oil resulted to the degradation as indicated by peroxide and total acid numbers. The results in this work have confirmed the literature information that vegetable oil storage promotes the increment of the peroxide and total acid number (Obadiah *et al*., 2012; Das *et al*., 2009). To obtain a highly stable vegetable oil during storage, it is necessary to utilize antioxidant to assist in delaying the oxidation process thus protecting oil

from degradation. The next subtopic will discuss the oxidative stability of CO in the presence of antioxidant.

6.2 Oxidation of Canola Oil in the Presence of Antioxidants

Results on oxidation of CO (Figure 6.1 and 6.2) proved that the deterioration of oil occur with time at 60°C. The CO samples further tested with 4 types of synthetic antioxidants, BHA, BHT, TBHQ, to study the oxidation trend in antioxidants added vegetable oil. Similar experiments procedure performed was applied in this study. The CO samples without the addition of antioxidants were set as a control set for this studies.

PV and TAN were measured for all mixtures of CO with antioxidant. The results of the analyses are displayed in Figure 6.3 and 6.4, respectively.

6.2.1 TAG Decomposition

Comparatively, all antioxidants were observed to lower TAN content as compared to the CO without the addition of antioxidants except for PG. Higher TAN value in PG was due to high decomposition of PG to form gallic acid. Comparison between the three antioxidants used (BHA, BHT, and TBHQ), TBHQ shows the most effectiveness in reducing the decomposition of CO TAG followed

by BHT and BHA. Findings from the experiments agrees with theoretical calculations where the TBHQ expresses the higher interaction value with H_β TAG trioleic (I_{TBHQ}=-46.5; I_{BHA}= -45.0; I_{BHT}= -36.0). The difference in the reducing order between BHA and BHT were related factors such as temperature (60°C) and not at 0 K (theoretically) and other constituents in CO.

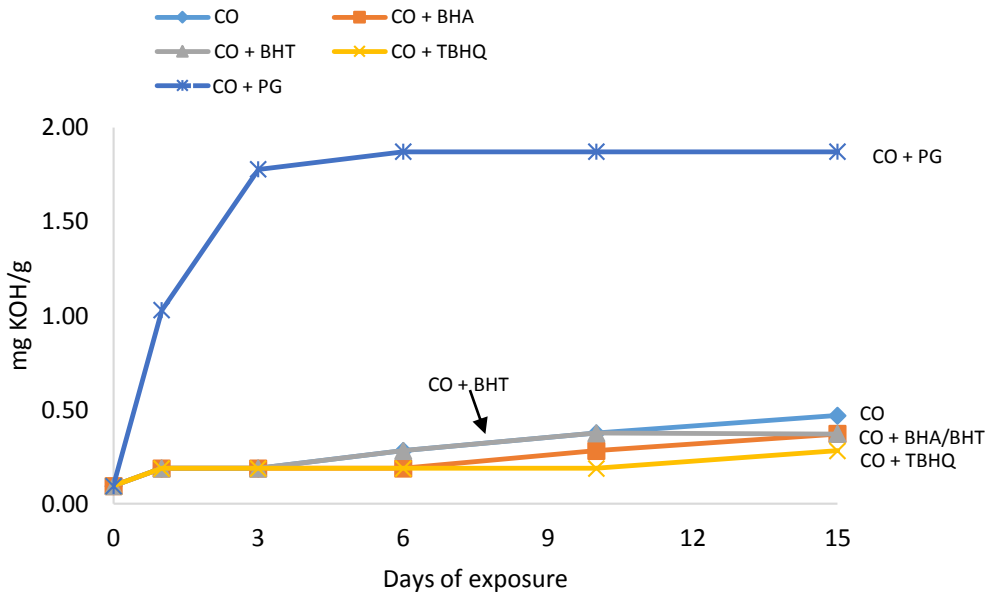


Figure 6.3: Total acid number of CO samples with four different types of antioxidants: BHA, BHT, TBHQ and PG

Total acid number results (Figure 6.3) shows that TBHQ, BHA and BHT manage to hinder fatty acid formation. TBHQ proves to be the most effective antioxidant in preventing acid formation/ hydrolysis of TAG $H_ß$ while PG shows the highest total acid number where the sample of CO containing PG increased from 0.09 to 1.87 mg/KOH. As mentioned before, PG shows high total acid number because of the acid nature of the component and the formation of gallic acid. The

order of antioxidants performance in inhibiting acid formation from good to bad are as follows: TBHQ > BHA> BHT > PG.

6.2.2 Peroxide Formation

Antioxidants used were able to inhibit autoxidation. However, TBHQ gave the lowest PV. Others such as BHA, PG and BHT gave similar PV reading even after 15 days of heat treatment. Analysis further concluded the antioxidants performances are in the following order: TBHQ> BHA> PG > BHT. Findings from the experiments agree with the value from the theoretical interaction energy calculation. Results of the energy interaction energy of TAG C_8OO radical and antioxidants could be utilised in predicting the antioxidant performance in preventing the formation of more peroxide group of oil that enriched with OA component such as CO. Order of the interaction energy yielded from the calculation are as follows: TBHQ (-22.78 kJ/mol) > BHA (-22.77 kJ/mol) > PG (-14.46 kJ/mol) > BHT (-10.40 kJ/mol).

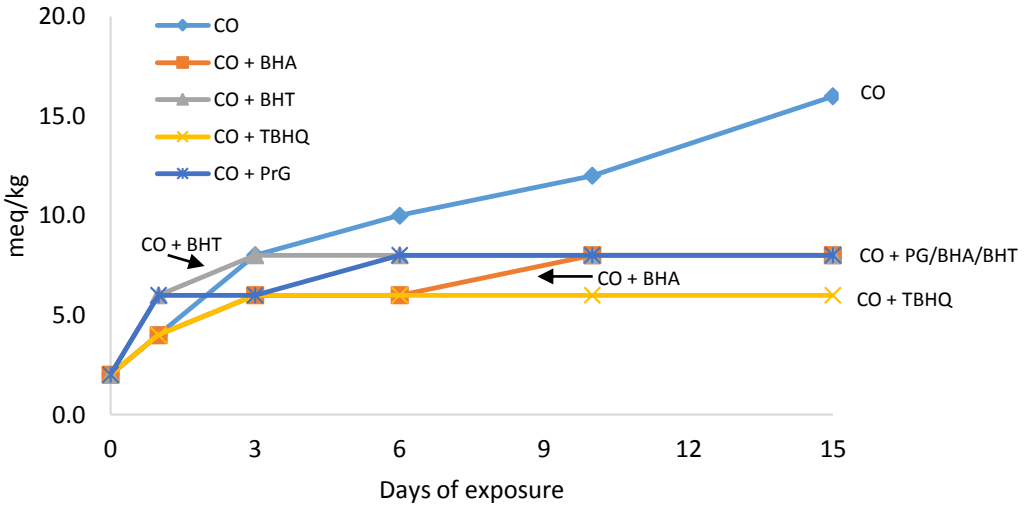


Figure 6.4: Peroxide value of CO samples with four different types of antioxidants: BHA, BHT, TBHQ and PG

Figure 6.4 display the experimental results of peroxide value of CO in the presence of antioxidants. As expected, all antioxidants used in this study manage to deliberate the oxidation of CO. From four antioxidants that were tested, TBHQ showed the lowest peroxide value (6.0 meq/kg) while others have shown the same performance since their peroxide value are all the same (8.0 meq/kg).

Overall, CO with the addition of antioxidants shows better oxidative stability. TBHQ has shown to be able to protect the oil from oxidation and hydrolysis of TAG compared to other antioxidants. Even though PG increase the AV of the oil drastically, the antioxidant performance in delaying oxidation of CO are quite effective. The trends of PG's results of CO are similar in palm olein. The gallic acid that was formed from the PG hydrolyses able to act as an antioxidant in preventing oil oxidation. BHA and BHT performance are almost similar either in

hindering the oxidation or TAG hydrolysis but BHT induction time is shorter compared to BHA. As a conclusion, the best antioxidant for CO in preventing oxidation is TBHQ.

6.3 Oxidation of Canola Oil in the Presence of Fatty Acids

6.3.1 TAGs Decomposition

Total acid number was performed to monitor fatty acid formation due to oil hydrolyses of the samples. Total acid number for all samples before treatment is 0.09 mg KOH/g which is same as total acid number of the control. Total acid number developments for all sample mixtures are shown in Figure 6.5 to Figure 6.8. CO contains high composition of OA (Przybylski *et al*, 2005; Ohara *et al*, 2009; Farhoosh and Samaneh, 2008). Jenab *et al* (2013) mention that the major TAG components of CO are OOO (49%) and LOO (30%). When hydrolysis of CO occurs, we believe that the most FFA produce will be OA.

Analysis performed on experimental data on the effects of additional FA to the decompositions of TAG forms FFA indicating the presence of unsaturated FA (OA and LA) on any tested concentrations ($\leq 3.0\%$) shows the ability in lowering TAG decompositions. Whereas, the presence on saturated FA (PA and SA) at lower concentration shown to have no effects decomposition rate of CO TAG. However,

the presence of PA and SA at higher concentrations of (≥ 1.0 %) shows to increase the decomposition rate.

Thorough further comparisons performed on OA and LA revealed that the addition of OA to CO exhibit synergistic behaviour compared to LA. The differences were evidently observed on day 15th for mixture of 0.25% and 0.5% for OA and LA. TAN for 0.25% OA = 0.09 mg KOH/g vs for 0.25% LA 0.19 mg KOH/g; TAN for 0.50% OA=0.19 mg/KOH/g vs TAN for 0.50% LA= 0.28 mg KOH/gram).

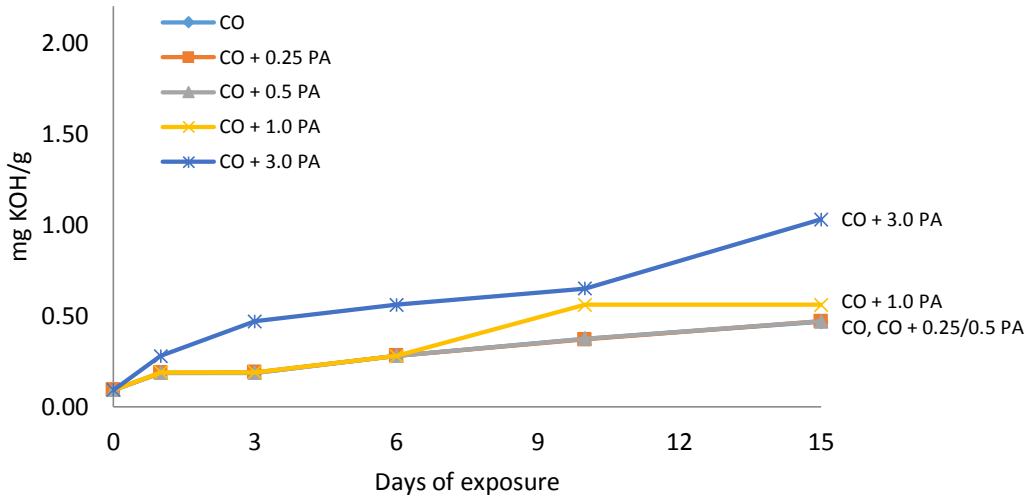


Figure 6.5: Total acid number of CO samples with PA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

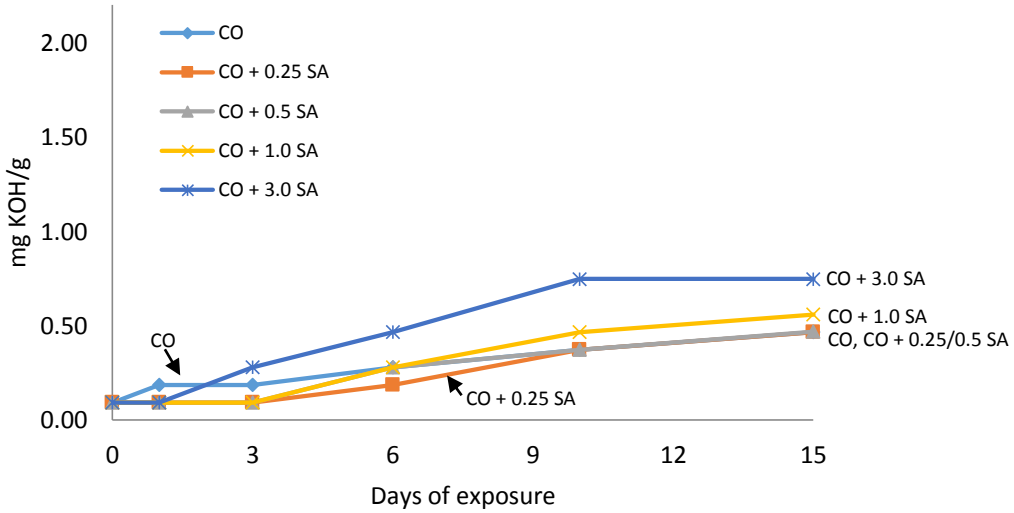


Figure 6.6: Total acid number of CO samples with SA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

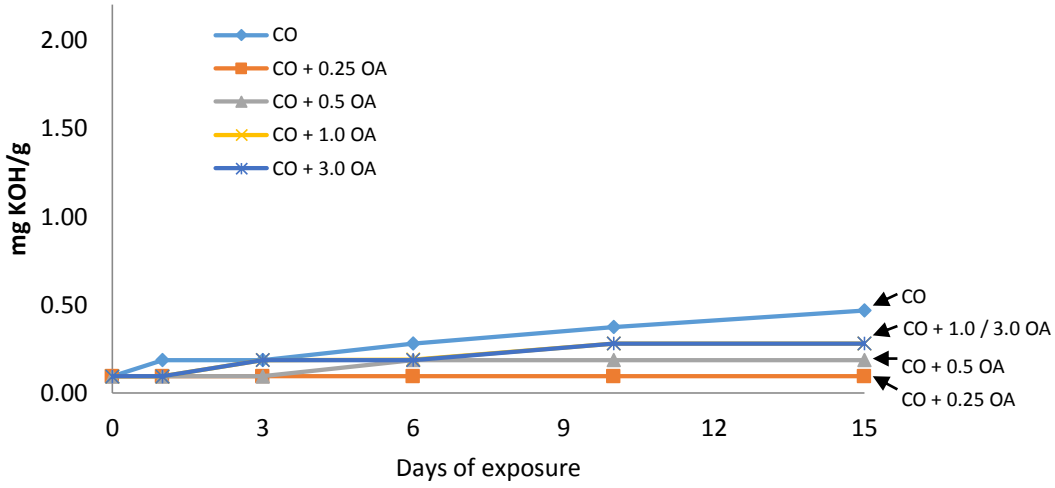


Figure 6.7: Total acid number of CO samples with OA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

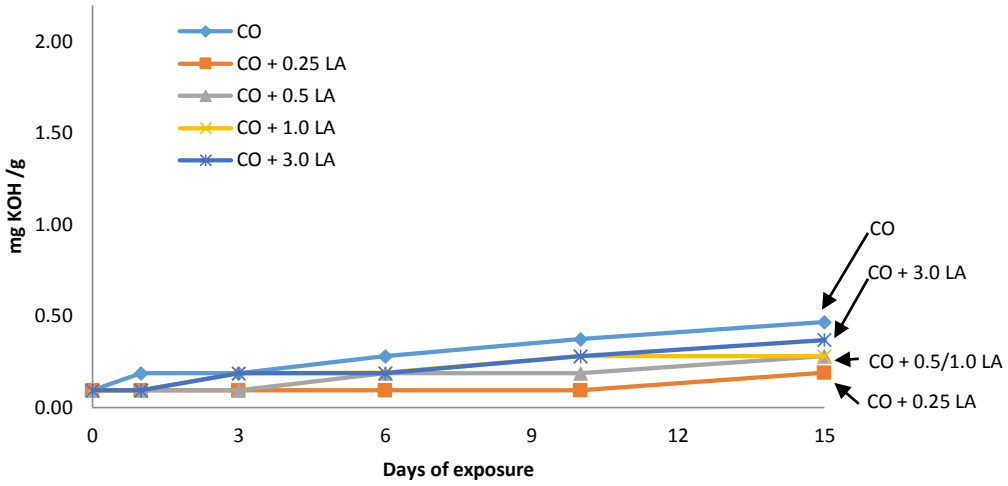


Figure 6.8: Total acid number of CO samples with LA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

The synergistic behaviour between CO with fatty acids was closely linked to the value of the interaction energy between the two species that is -.28 to 32 kJ/mol). As conclusion, OA that appears in CO due to TAG hydrolyses will not decay the oil. The conclusive order on the effects of fatty acid addition on the decomposition of TAG of canola is as follow: PA > SA > LA> OA.

6.3.2 Peroxide Formation

PV results of CO mixture with PA, SA, OA and LA were tabulate in Figure 6.9, 6.10, 6.11 and 6.12 respectively. The addition of PA to any concentration causes instant development of the peroxide group for CO. PV for the 15th day of heat treatment on CO without FA additions were only 16.0 meq/kg as compared to

PV for other sample added with PA ranging from 120.0 meq/kg (for 0.25% PA) to 148.0 meq/kg (for PA at 3.0%).

In contrast to the other FAs where the PV of samples added with FA were lower than the genuine/original/initial canola. In this case LA, OA and SA were able to prevent autoxidation. The orders on the FA addition towards the CO oxidation are as follows: PA > LA > OA > SA. The quantum mechanics calculation for this case on TAG trioleic $C_8OO•$ together with FAs shows all the three FAs PA, SA and OA possess similar interaction energy value which is around 22 to 23 kJ/mol. Whereas LA exhibit the lowest interaction energy (20 kJ/mol).

The drastic increasing number of peroxide with the presence of PA were elaborated by Paradiso *et al* (2010) and Aubourg (2001) which mentioned the short chains in FA foster the oxidation process in vegetable oil (olive oil) and marine lipids respectively.

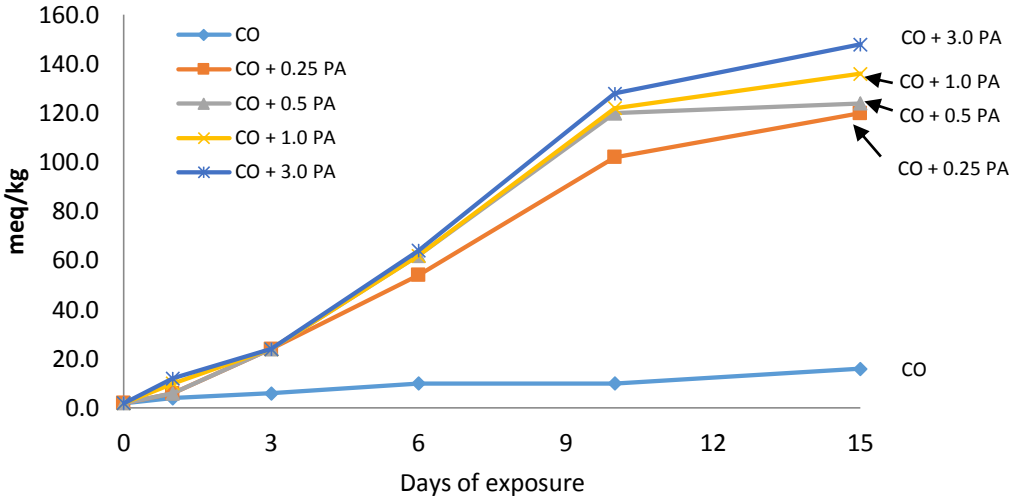


Figure 6.9: Peroxide value of CO samples with PA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

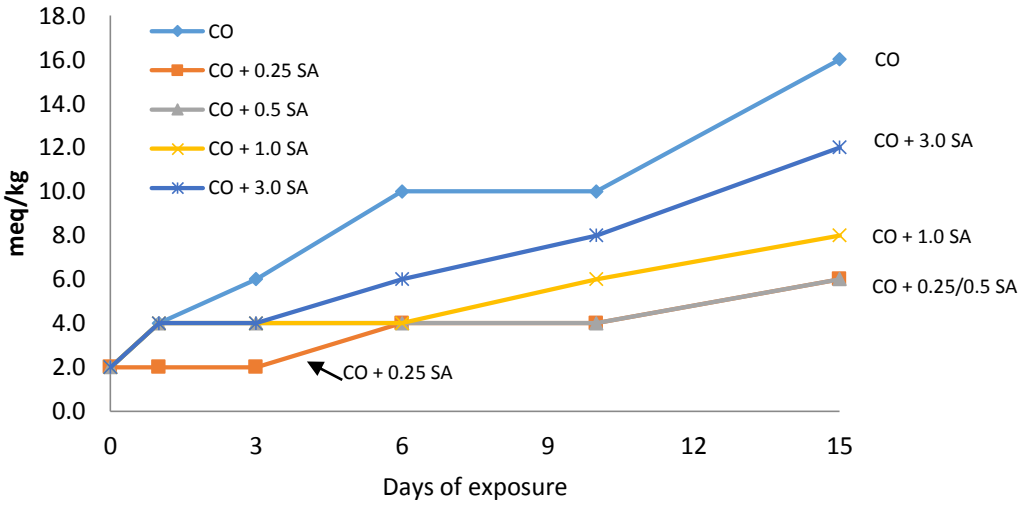


Figure 6.10: Peroxide value of CO samples with SA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

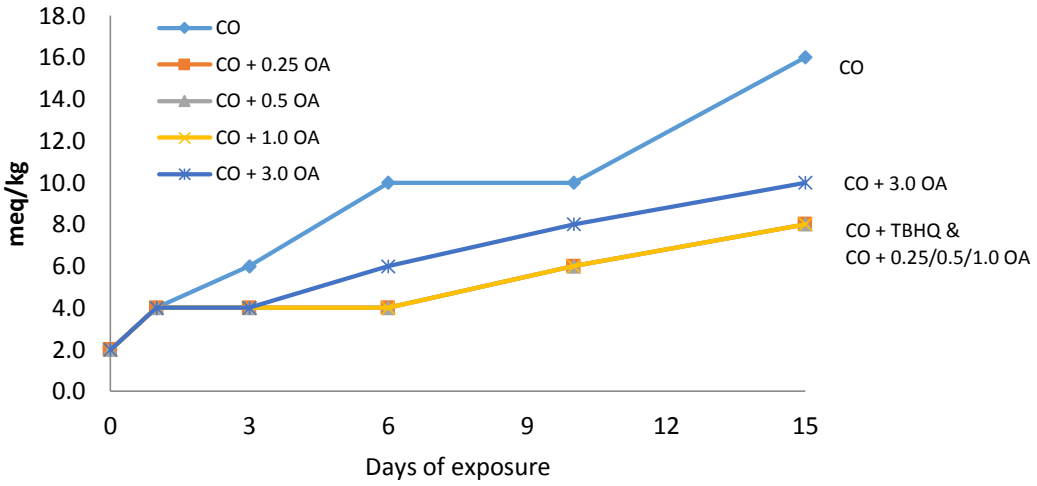


Figure 6.11: Peroxide value of CO samples with OA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

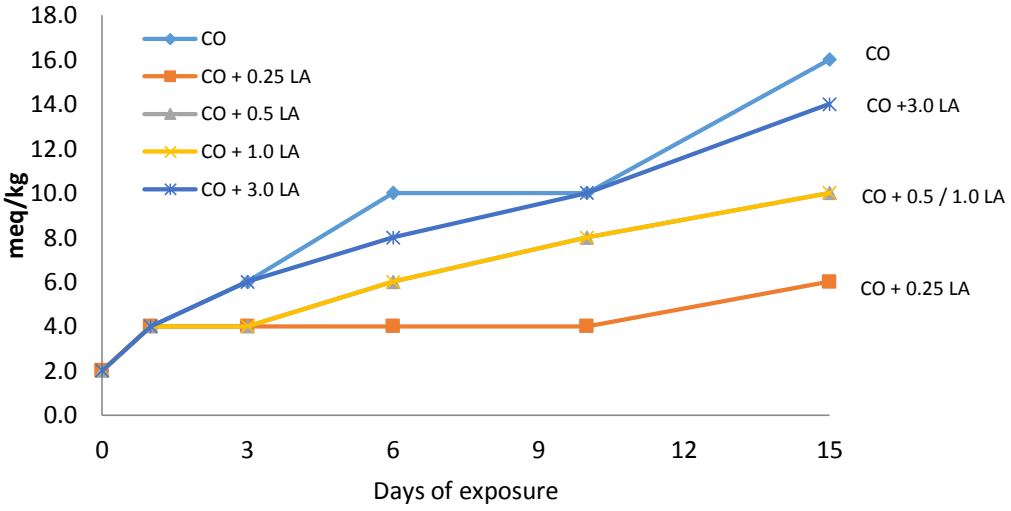


Figure 6.12: Peroxide value of CO samples with LA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w)

6.4 Effects of Fatty Acids on the Performance of Antioxidants

On the TAG Decompositions:

On the average antioxidants such as BHA, BHT and TBHQ will gradually reduce due to the saturated FAs (PA and SA), while OA and LA show to have no effect in reducing the TAG decomposition. Fatty acids added to PG were seen to form antioxidants which all together contributed to the reduction of TAG decompositions in canola.

It is worth to mention for all antioxidants tested (BHA, BHT, and TBHQ), LA has consistently expressed positive effect by producing antioxidants properties which reduce the decompositions of TAG. Comparisons between the interaction energy of FAs and H_β TAG trioleic explains the phenomena, where LA exhibit lower interaction energy; -27.13 kJ/mol as compared to the others FAs (I_{OA}= -27.37; I_{PA}= -32.10, I_{SA} = -32.26 kJ/mol). In short, weak interaction between LA and H_β TAG optimises the antioxidants function in preventing the decomposition process. Phenomena exhibited by the experiments performed could be linked to the theory of the interaction energy tri species system between TAG trioleic (H_β), antioxidants and FAs.

Defective effect on the antioxidants (BHA, BHT and TBHQ) performance with the presence of fatty acids SA > PA > OA > LA were directly link to the value dropped of the energy interaction between the H_β TAG and antioxidants used with presence of FAs. Calculations gave the exact similar order as the experiments observation saturated fatty acids shows higher reduction percentage compare to the unsaturated, however, calculations were only performed on TBHQ. PG has consistently exhibit improvements in preventing the decomposition of TAGs for canola together with the presence of all FAs. This phenomena were proven as PG exhibit the highest energy interaction value with H_β TAG trioleic which is -34.43 kJ/mol compared to other antioxidants TBHQ (-21.13 kJ/mol), BHA (-21.25 kJ/mol) dan BHT (-14.84 kJ/mol).

On the Peroxide Formation

The presence of FAs were shown to have no effect on the antioxidants performance except for the addition of PA to CO with the presence of BHA. Research shows the addition of FAs were the least effective on BHT and TBHQ. Evaluation on the inhibition period shows that TBHQ exhibit better performance compared to BHT.

The effectiveness on TBHQ performance is linked to the interaction energy where it shows to have the highest interaction energy with TAG trioleic $C_8OO\bullet$ which is -22.78kJ/mol (Refer to table 4.10).

Overall analysis shows that PA significantly effect a certain antioxidant performance (BHA, BHT, TBHQ and PG) compared to other FAs. This phenomena could also be linked to the nature of the interaction between $C_8OO•$ radical and FAs. (I_{PA} = -23.17 kJ/mol I_{SA} = -22.03 kJ/mol; I_{OA} = 22.93 kJ/mol I_{LA} = -20.18 kJ/mol).

6.4.1 Performance of BHA in inhibiting Oxidation of Canola Oil in the Presence of Selected Fatty Acids

On the TAG Decomposition:

Figure 6.13 to 6.15 respectively displays the effect of adding PA, SA, OA and LA at four different concentrations on the performance of BHA in inhibiting TAG hydrolyses.

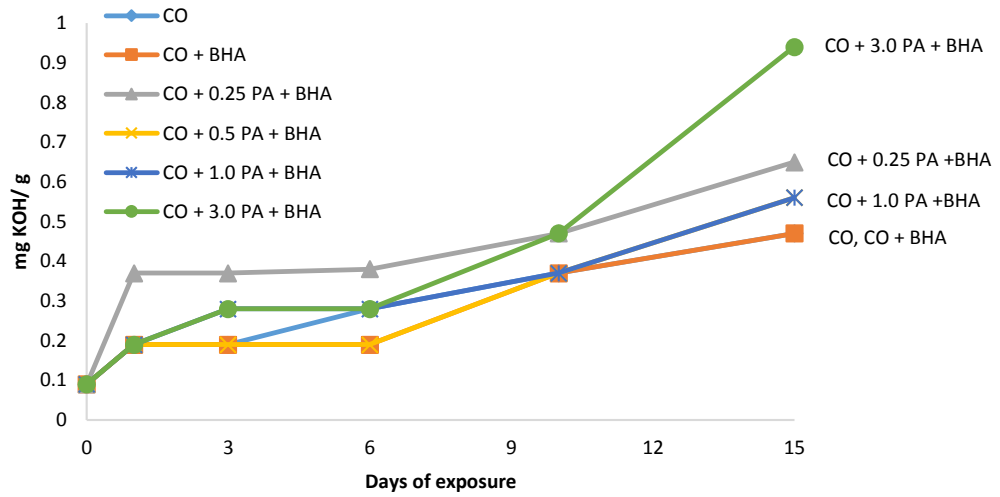


Figure 6.13: Effect of PA concentration on the TAN of CO in the presence of BHA

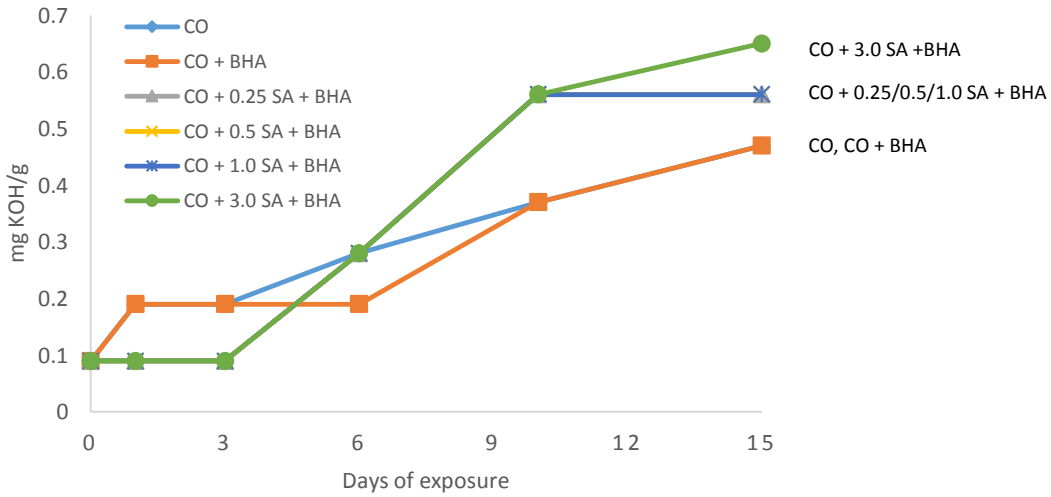


Figure 6.14: Effect of SA concentration on the TAN of CO in the presence of BHA

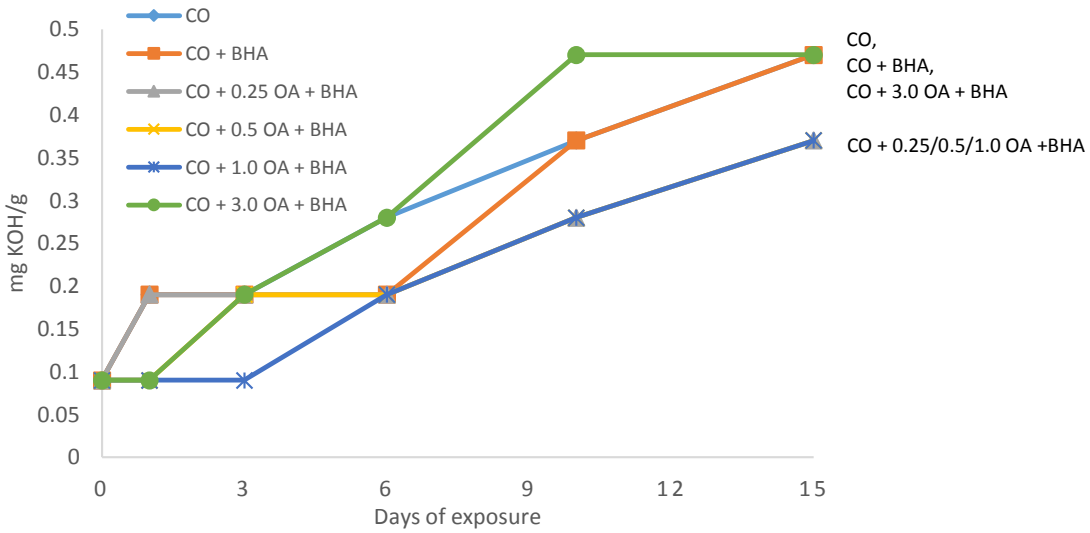


Figure 6.15: Effect of OA concentration on the TAN of CO in the presence of BHA

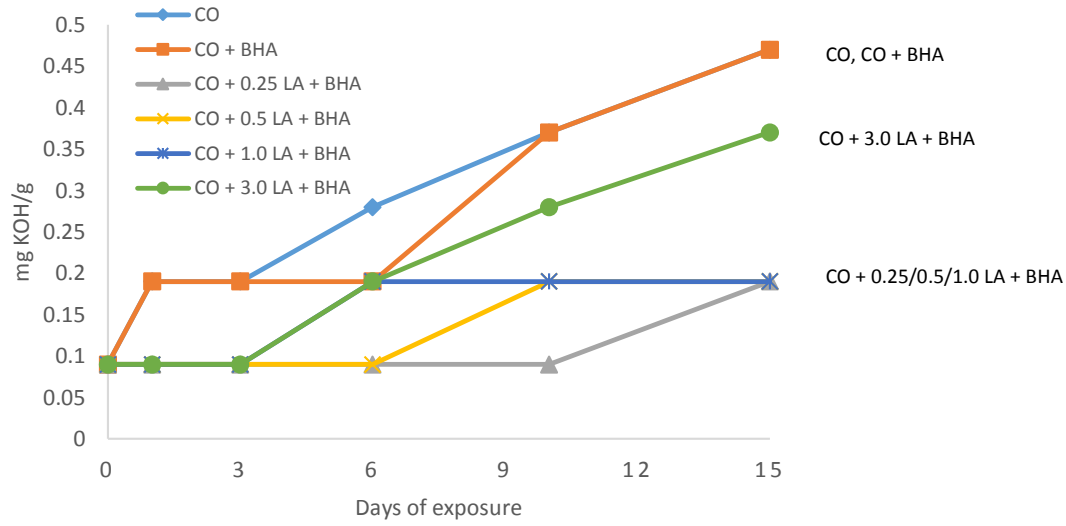


Figure 6.16: Effect of LA concentration on the TAN of CO in the presence of BHA

The unsaturated fatty acids exhibit proneness in exhibiting synergistic behaviour with BHA while the saturated shows an antagonistic behaviour with BHA. BHA performances are the best in the presence of LA followed by OA. The

reduction of BHA performance in delaying the TAG Hß hydrolyses occurred in the presence of PA and SA. The order of fatty acids in affecting the performance of BHA on reducing TAG Hß hydrolyses from the least to the most are as follows: LA > OA > SA > PA.

On the Peroxide Formation:

PV analyses on the mixture of CO, BHA and FAs (palmitic, stearic, oleic and linoleic) are shown in Figure 6.17 to 6.20.

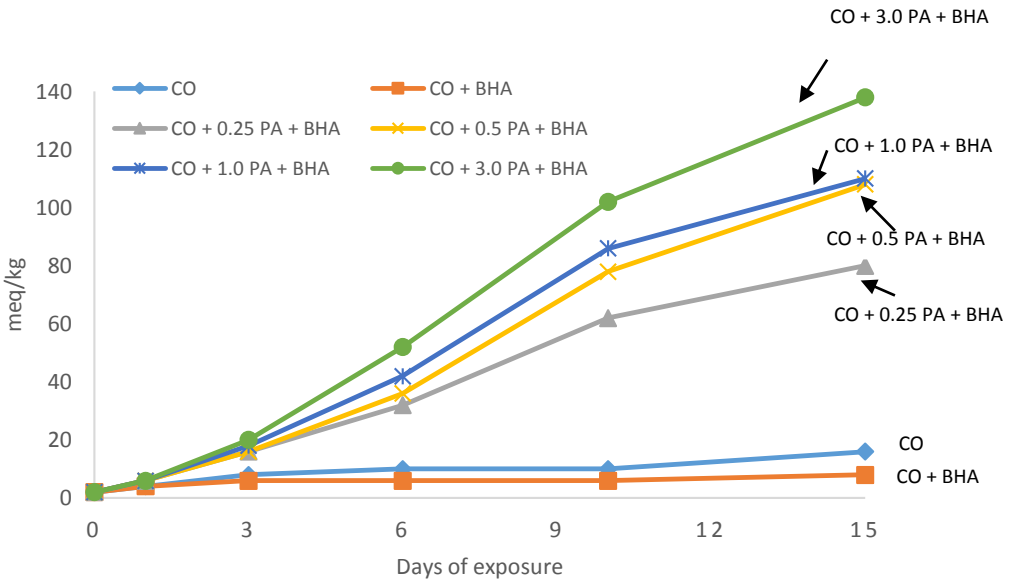


Figure 6.17: Effect of PA concentration on the PV of CO in the presence of BHA

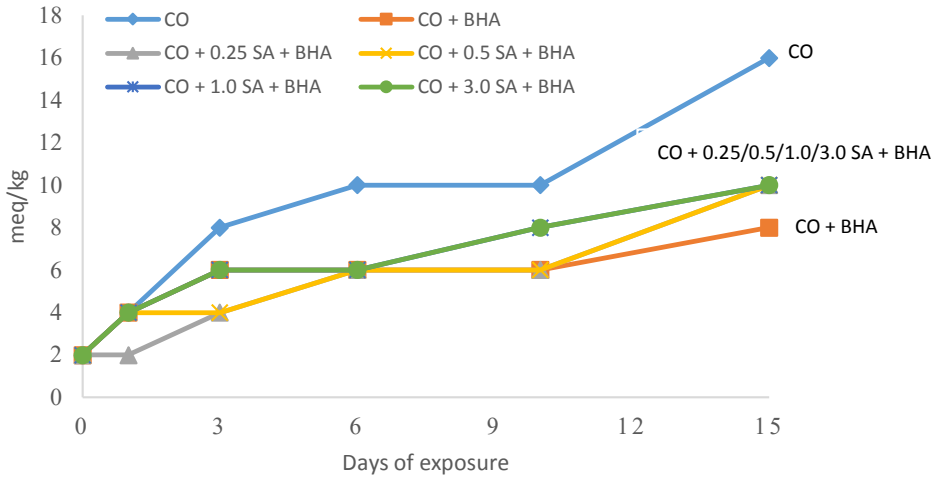


Figure 6.18: Effect of SA concentration on the PV of CO in the presence of BHA

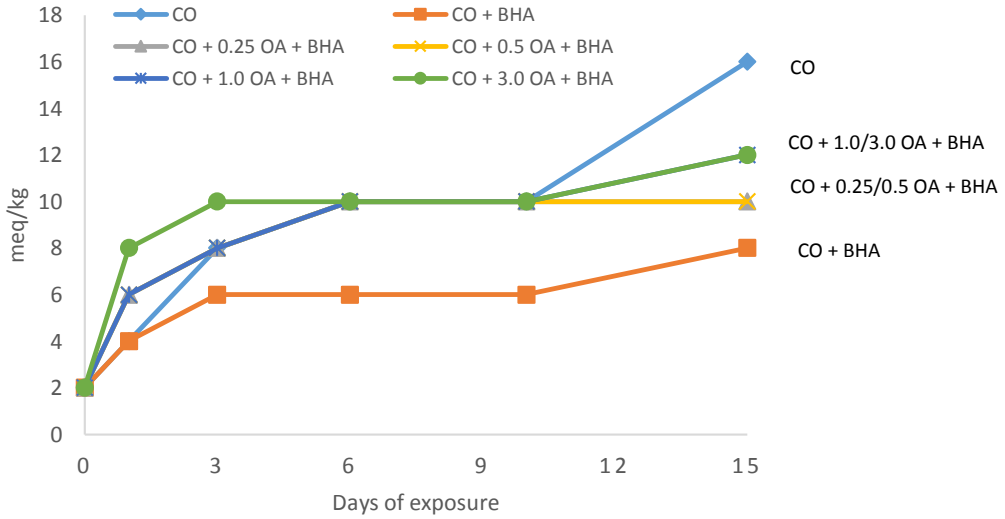


Figure 6.19: Effect of OA concentration on the PV of CO in the presence of BHA

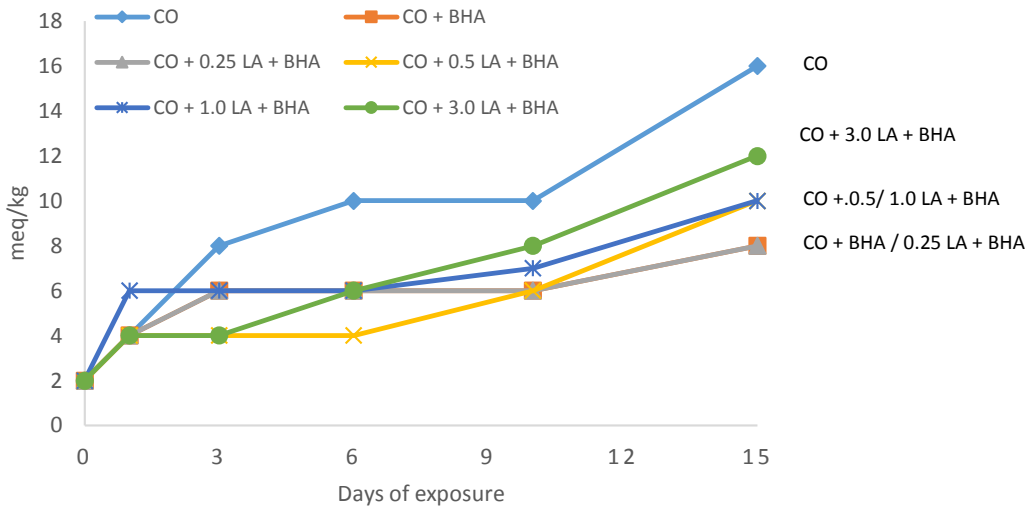


Figure 6.20: Effect of LA concentration on the PV of CO in the presence of BHA

PV analyses revealed that PA shows pro-oxidant effect where formation of peroxide value has shown to increase drastically (Figure 5.17). The higher the amount of PA added, the higher the peroxide formation in CO. SA, OA and LA exhibited synergistic behaviour with BHA where the combination of substances manage to delay the peroxide formation in CO. Out of these three fatty acids, LA shows the least peroxide formation followed by SA and OA. It was also observed that high fatty acid concentration resulted to reduce the synergistic performance of the combined antioxidant and fatty acid. The order of fatty acids in affecting the performance of BHA on reducing peroxide formation from the least to the most are as follows: LA > OA > SA > PA.

As a conclusion, the performance of BHA is the best in the presence of LA while PA shows to lower the performance of BHA. SA and OA show almost similar

effect on BHA performance in protecting CO from degradation and peroxide formation.

6.4.2 Performance of BHT in Inhibiting Oxidation of Canola Oil the Presence of Selected Fatty Acids

On the TAG Decomposition:

The performance of BHT in hindering TAG $H_{ß}$ hydrolyses in the presence of PA, SA, OA and LA are shown in Figure 6.21 to Figure 6.24 respectively. The presence of PA and OA does not show any effect on BHT performance (Figure 6.21 and Figure 6.23). SA shows antagonistic interaction on BHT (Figure 6.22). Concentration of SA at 0.5% to 3.0% (w/w) in the oil mixture was observed to effect the efficiency on BHT performance in preventing $H_{ß}$ hydrolyses. Synergistic interaction was found when LA added at lower concentration (0.25, 0.5and 1%) while at high concentration, 3% (w/w), no effect was found on BHT performance in delaying TAG hydrolyses. The BHT performance in the presence of fatty acid ranging from good to poor in delaying $H_{ß}$ TAG hydrolyses are expressed in an ordinal form as follow: LA > OA = PA > SA

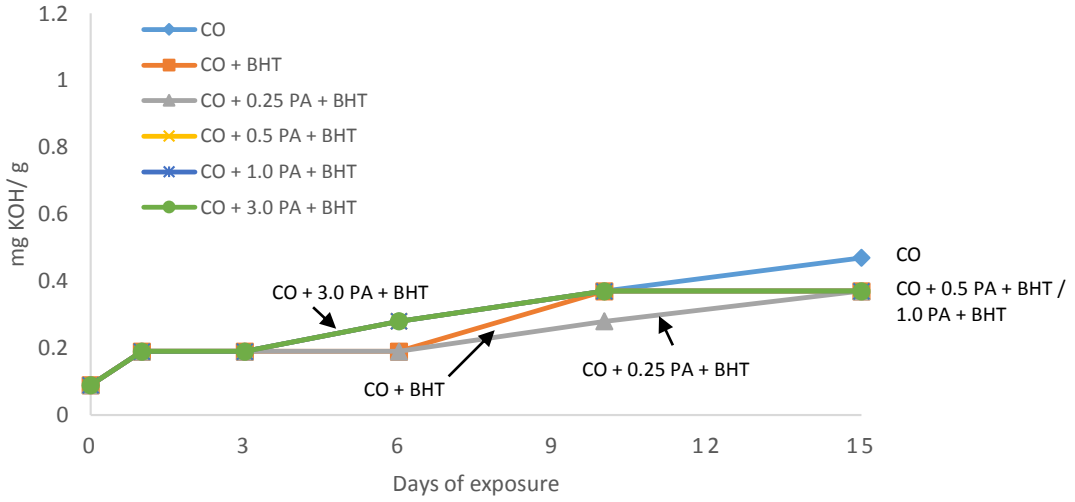


Figure 6.21: Effect of PA concentration on the TAN of CO in the presence of BHT

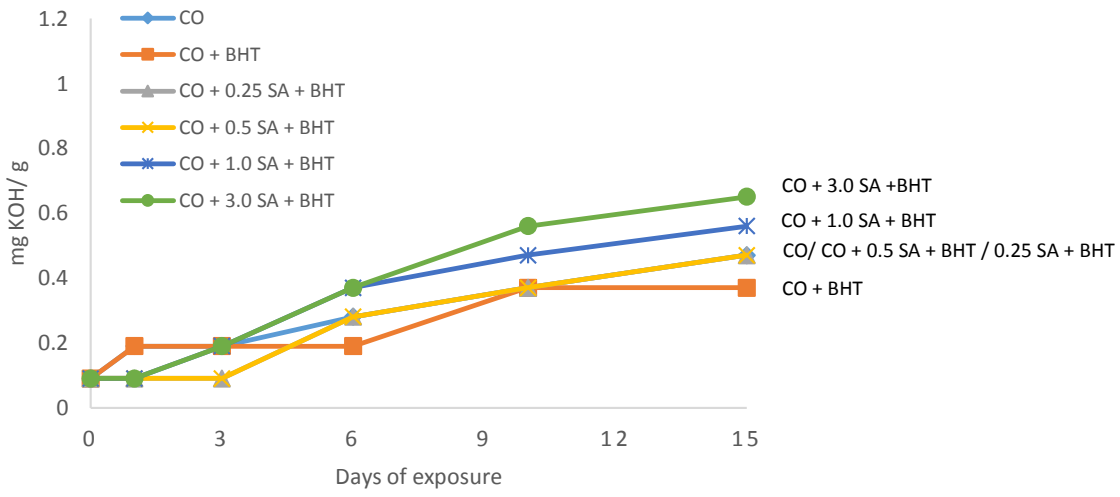


Figure 6.22: Effect of SA concentration on the TAN of CO in the presence of BHT

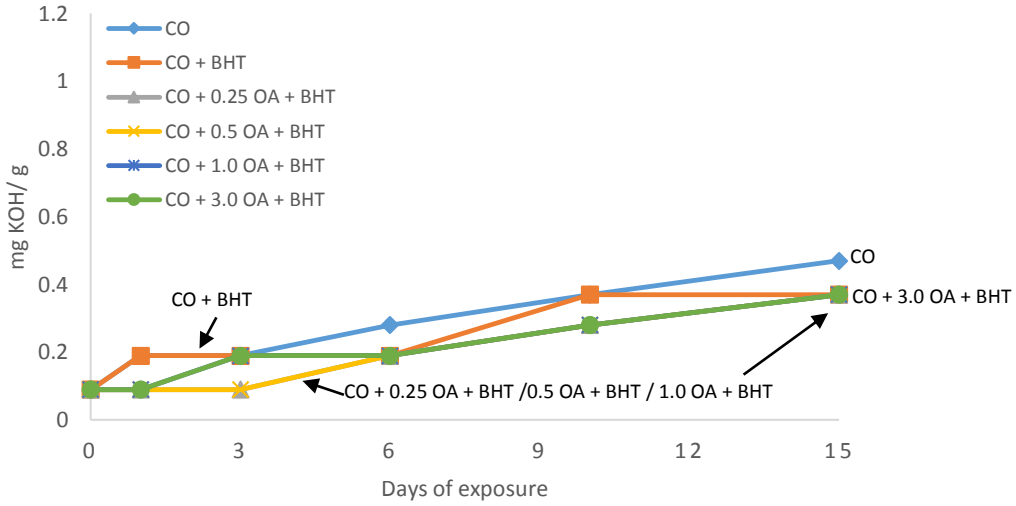


Figure 6.23: Effect of OA concentration on the TAN of CO in the presence of BHT

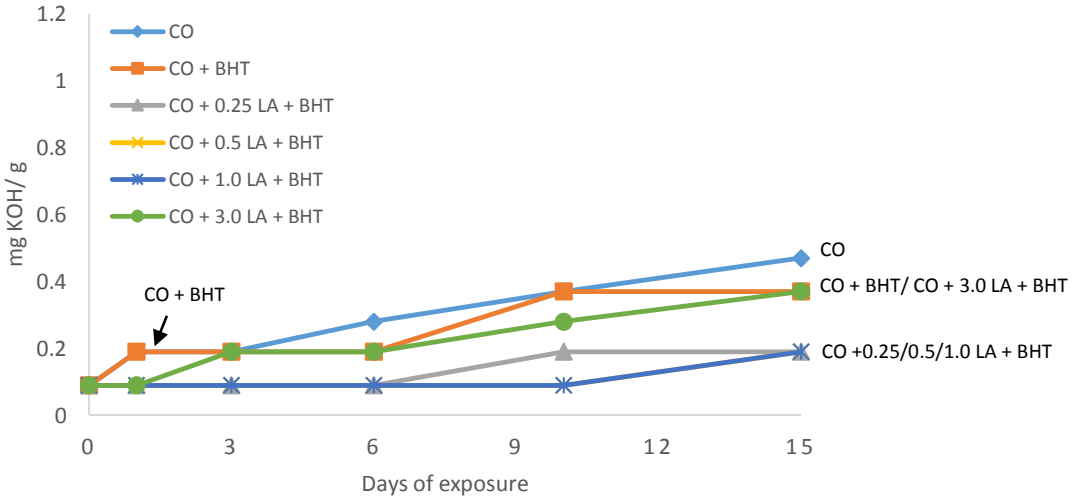


Figure 6.24: Effect of LA concentration on the TAN of CO in the presence of BHT

n the Peroxide Formation:

The performance of BHT in hindering peroxide formation in the presence of PA, SA, OA and LA are shown in Figure 6.25 to Figure 6.28 respectively. SA and LA were observed to improve BHT performance when added at 0.25, 0.5 and 1 % (w/w) (Figure 6.26 and Figure 6.28). SA and LA at 3% (w/w) concentration show no effect on BHT performance. The presence of OA at any loading (0.25% to 3.0%) has shown no effect on BHT performance (Figure 6.27) while the presence of PA (Figure 6.25) at any concentration shows an antagonistic interaction with BHT. Observation also revealed that BHT performances drastically reduced with the increment of PA concentration. The performance of fatty acid in reducing peroxide formation in degrading manner (best to worst) is as follows: LA = SA > OA > PA.

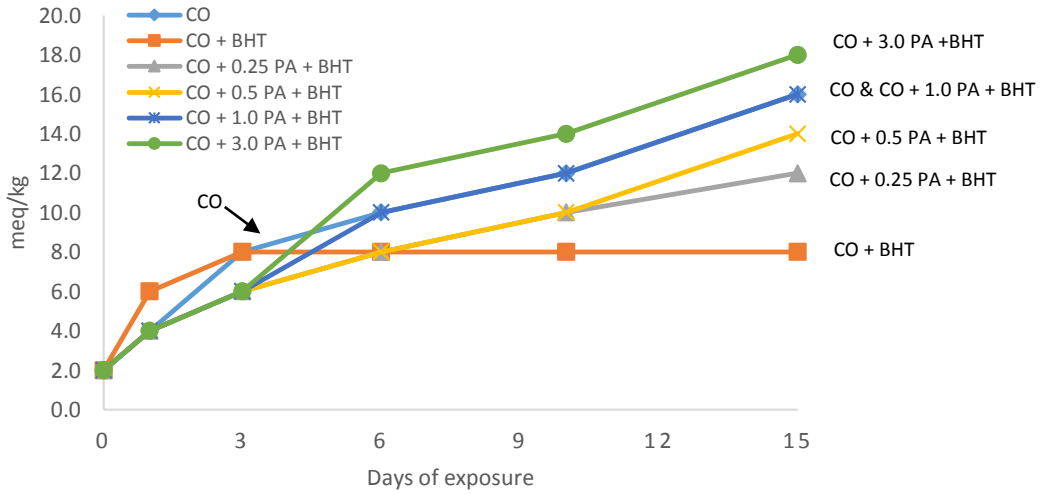


Figure 6.25: Effect of PA concentration on the PV of CO in the presence of BHT

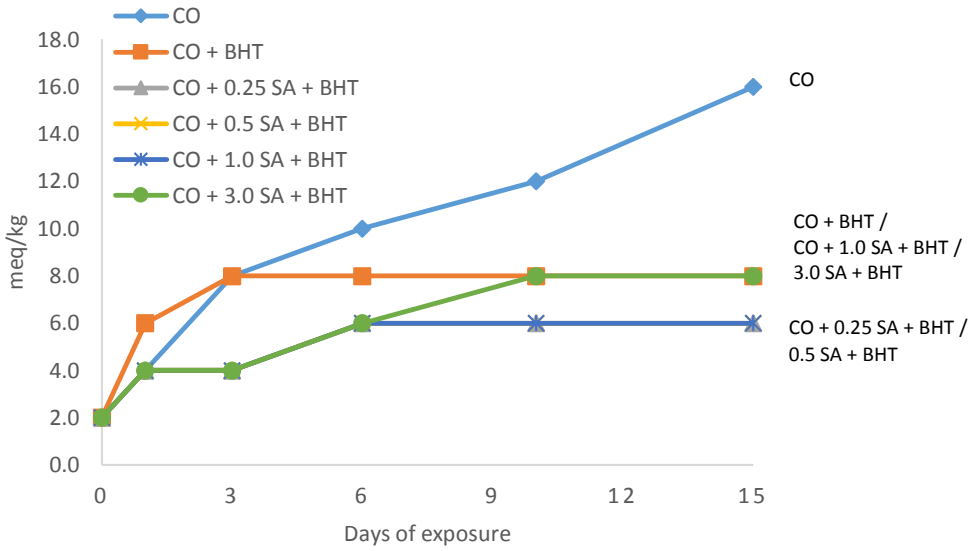


Figure 6.26: Effect of SA concentration on the PV of CO in the presence of BHT

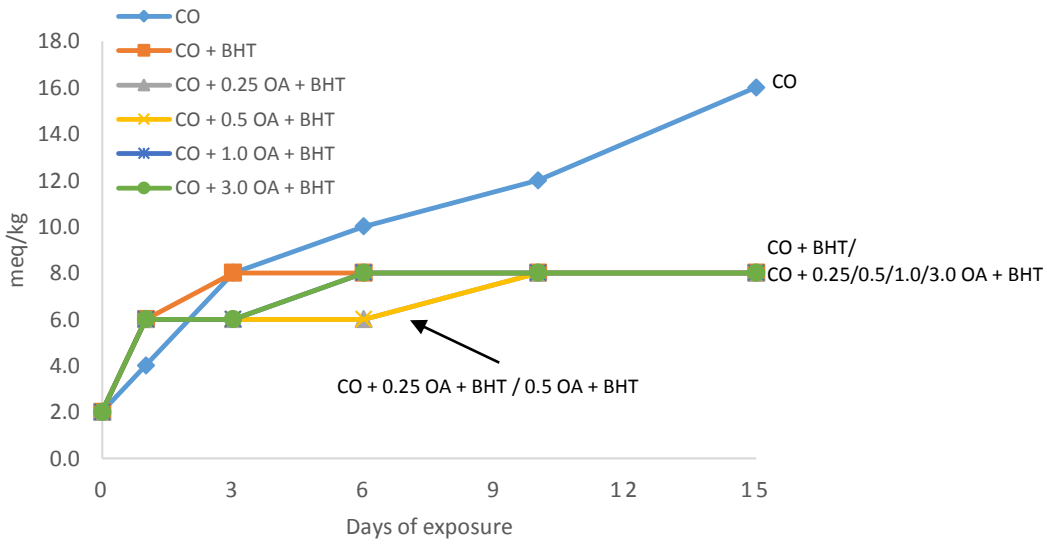


Figure 6.27: Effect of OA concentration on the PV of CO in the presence of BHT

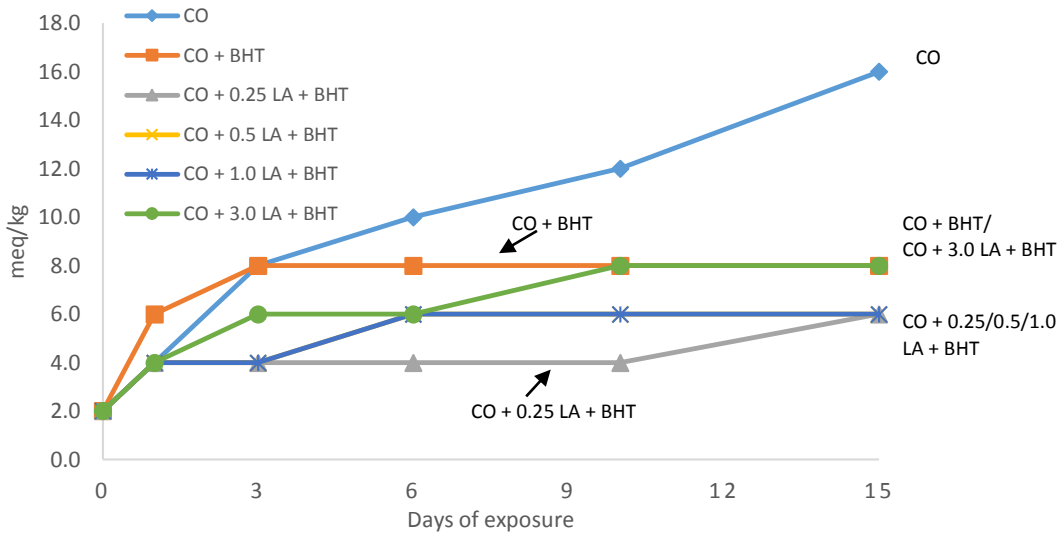


Figure 6.28: Effect of LA concentration on the PV of CO in the presence of BHT

Overall, the presence of LA shows improvement in BHT performance in delaying TAG hydrolyses and peroxide formation.

6.4.3 Performance of TBHQ in Inhibiting Oxidation of Canola Oil in the Presence of Selected Fatty Acids

On the TAG Decomposition:

The performance of TBHQ in hindering TAG H_{β} hydrolyses in the presence of PA, SA, OA and LAs are shown in Figure 6.29 to Figure 6.32. From the results, the presence of saturated (PA, and SA) were shown to lower the TBHQ performance (Figure 6.29, and Figure 6.30). LA shows to improve TBHQ performance when added at 0.25 and 0.5% w/w but at 3%, LA shows negative effect

on TBHQ performance (Figure 6.32). The higher concentration of fatty acid presence in the oil mixture, the lower the performance of TBHQ in preventing Hß hydrolyses. The performance of fatty acid in hindering TAG hydrolyses from good to worst are as follows: LA > OA > PA > SA

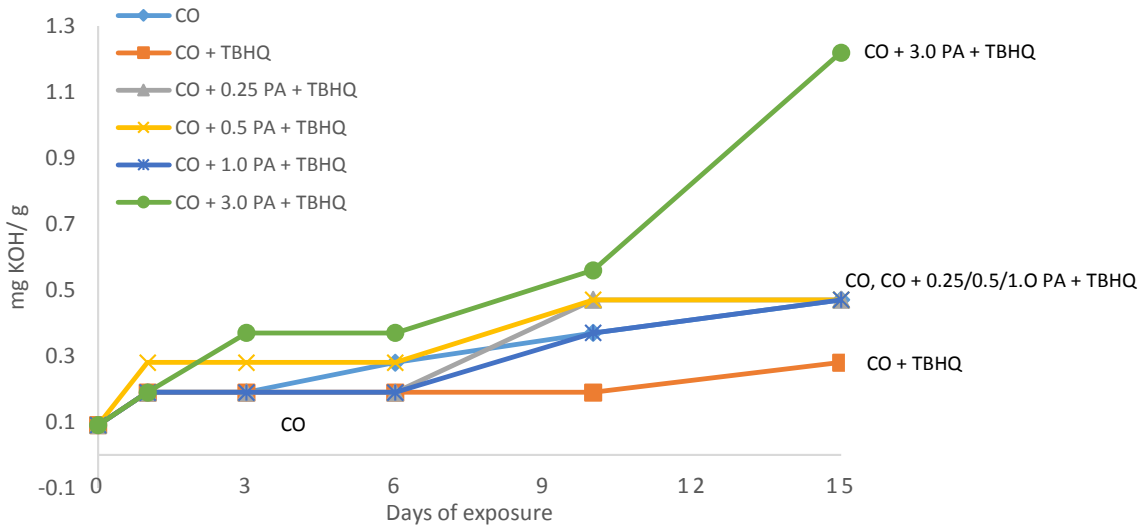


Figure 6.29: Effect of PA concentration on the TAN of CO in the presence of TBHQ

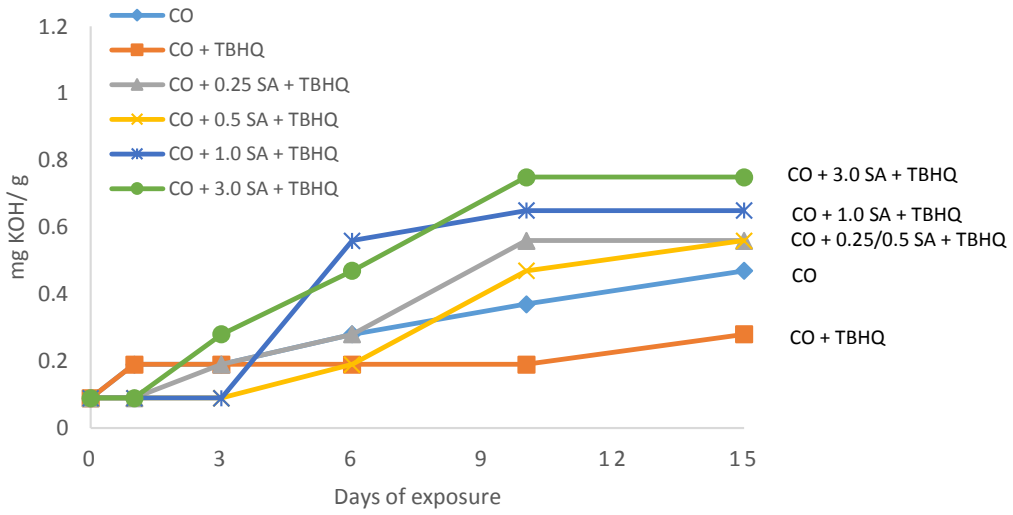


Figure 6.30: Effect of SA concentration on the TAN of CO in the presence of TBHQ

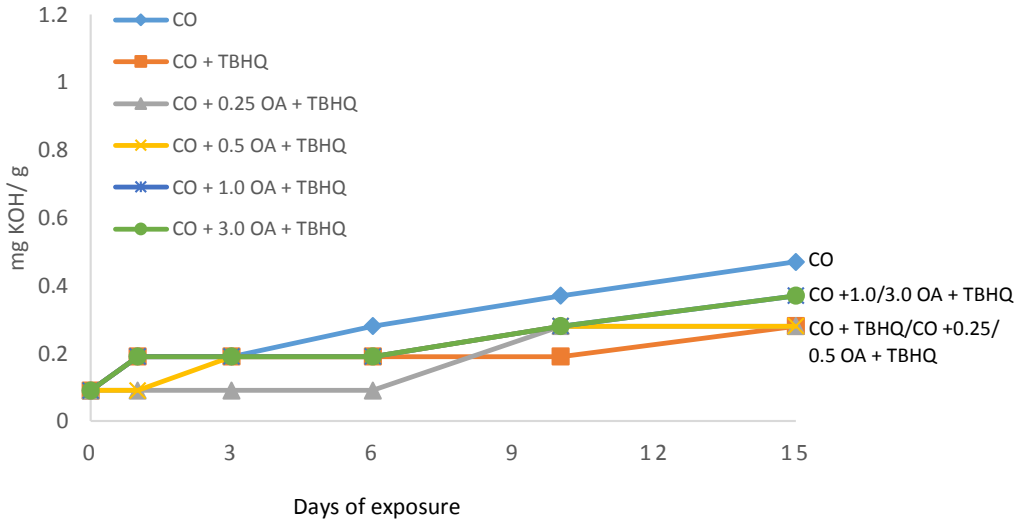


Figure 6.31: Effect of OA concentration on the TAN of CO in the presence of TBHQ

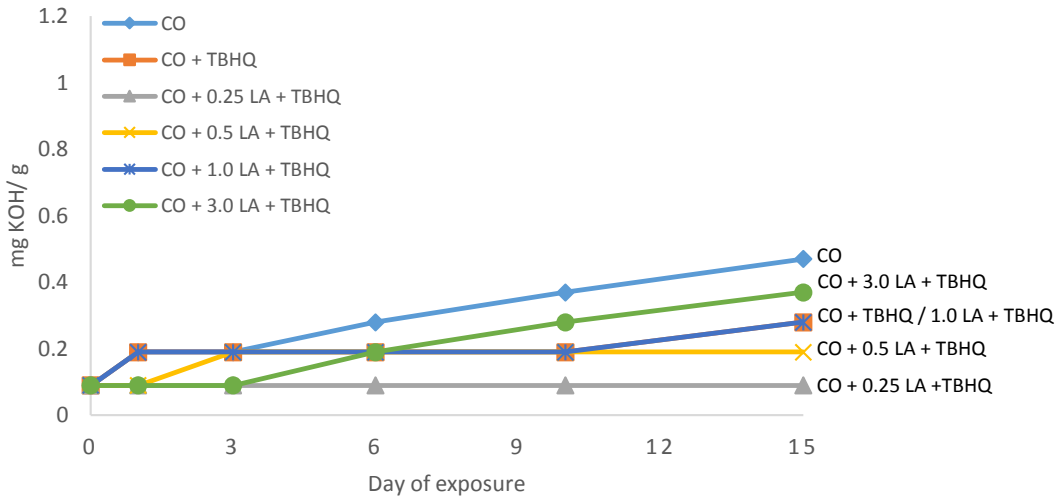


Figure 6.32: Effect of LA concentration on the TAN of CO in the presence of TBHQ

On the Peroxide Formation:

The performance of TBHQ in hindering peroxide formation in the presence of PA, SA, OA and LAs are shown in Figure 6.33 to Figure 6.36 respectively. In this case, the added fatty acids have shown to reduce TBHQ performance. The effect of fatty acids on TBHQ performance (good to bad) can be ranked as follows: OA > PA > LA > SA. The theoretical study was done on this tri species system. The theoretical studies are in line with the experimental results (Section 4.5)

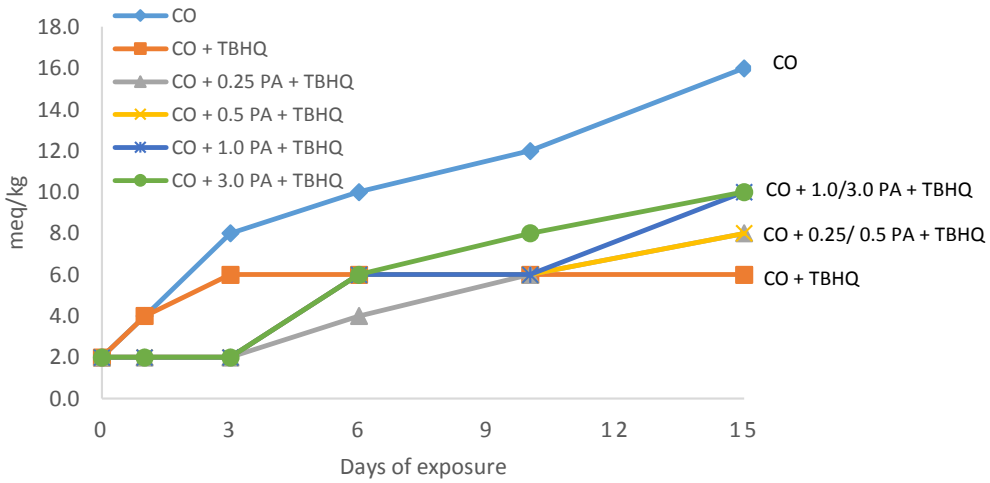


Figure 6.33: Effect of PA concentration on the PV of CO in the presence of TBHQ

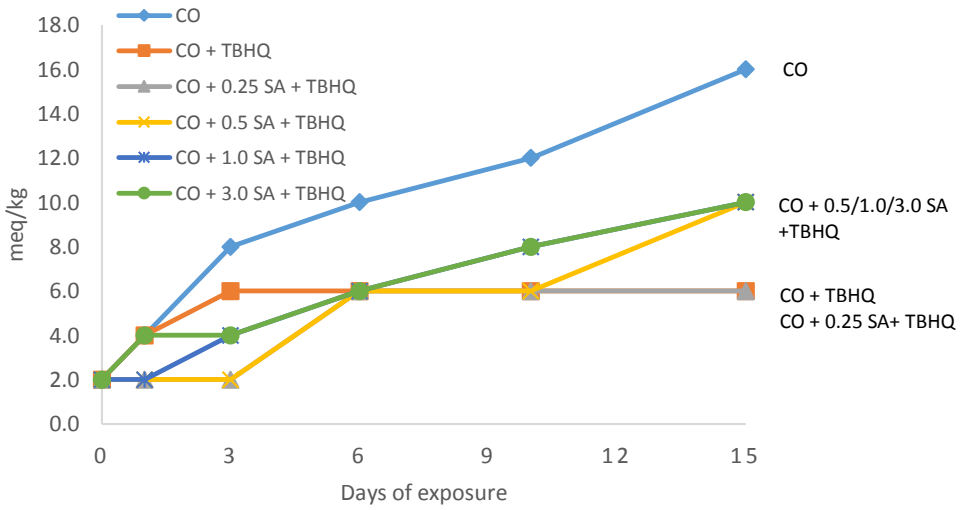


Figure 6.34: Effect of SA concentration on the PV of CO in the presence of TBHQ

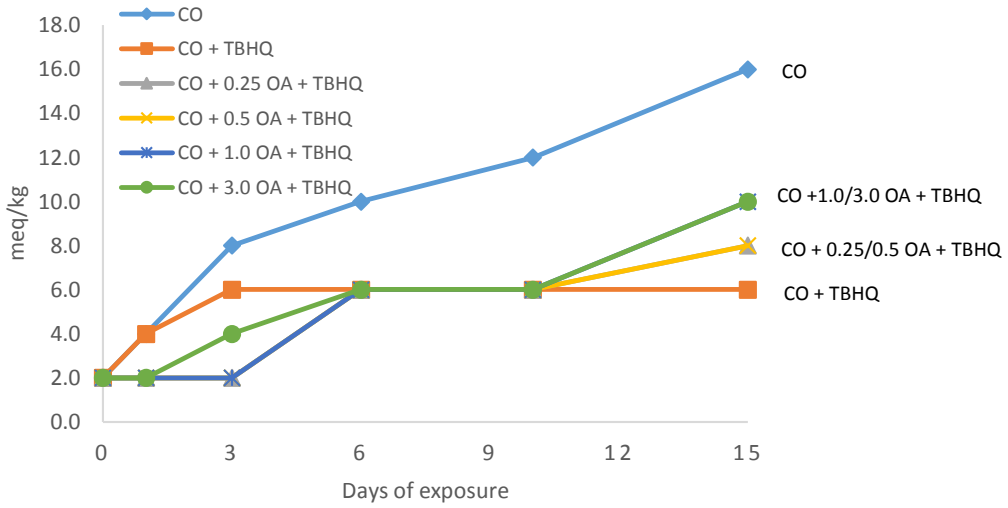


Figure 6.35: Effect of OA concentration on the PV of CO in the presence of TBHQ

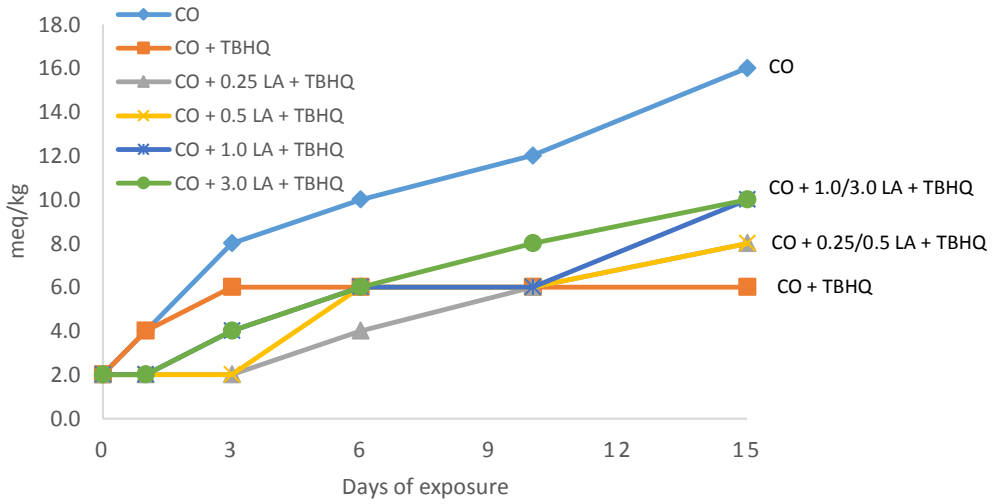


Figure 6.36: Effect of LA concentration on the PV of CO in the presence of TBHQ

Studies have shown that fatty acids reduce TBHQ performance when added into CO mixture of any concentrations. OA shows to have the least effect on TBHQ performance when added at any concentration. All fatty acid at 1 and 3% has shown to reduce TBHQ performance where the peroxide value results on the 15th days are

slightly higher (10 meq/kg) compare to the ones with lower concentration of other fatty acids (8 meq/kg). Overall studies revealed that, TBHQ performs without the addition of any fatty acid in preventing both TAG hydrolyses and peroxide formation. The presence of OA does not shows no significant effect on TBHQ performance on delaying CO degradation but for peroxide formation OA exhibit pro-oxidant on the 15th day of heat treatment.

6.4.4 Performance of PG in inhibiting Oxidation of Canola Oil in the Presence of Fatty Acids

On the TAG Decomposition:

The performance of PG in hindering TAG Hß hydrolyses in the presence of PA, SA, OA and LA are shown in Figure 6.37 to Figure 6.40 respectively. Total acid number of samples mixture with propyl gallate slightly higher than other oil samples. This is due to the acid nature of propylgallate and also formation of gallic acid. Findings shows that the addition of all fatty acids manage to improve propyl gallate performance. The best fatty acid which gave the highest synergistic effect is PA while the worst is OA. Even though synergistic effect was seen, the total acid number is still higher than CO alone. Just like previous studies, the higher the amount of fatty acid presence in the oil mixture the lower the performance of propyl gallate in preventing Hß TAG hydrolyses. The performance of fatty acid in hindering TAG hydrolyses from good to worst are as follows: PA > SA >LA > OA

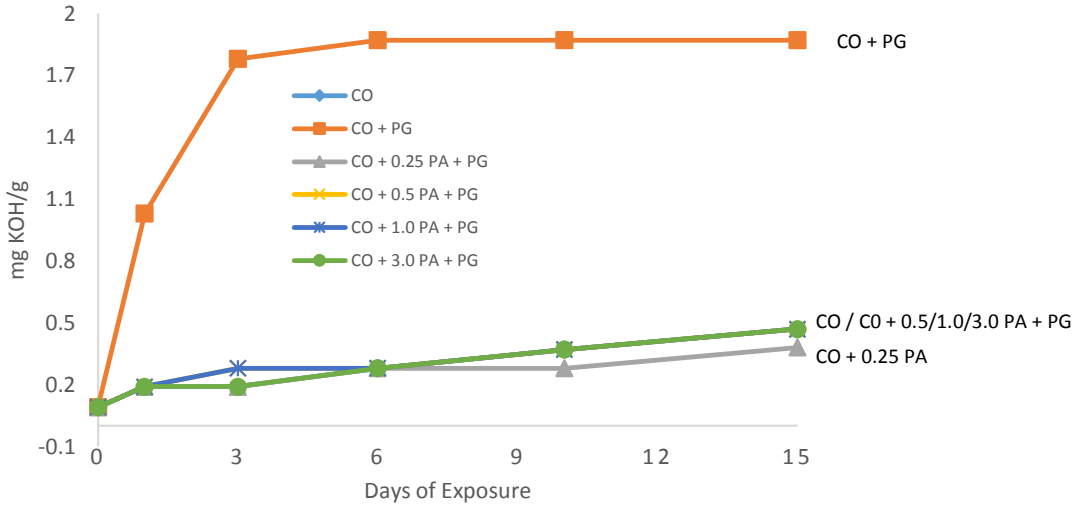


Figure 6.37: Effect of PA concentration on the TAN of CO in the presence of PG

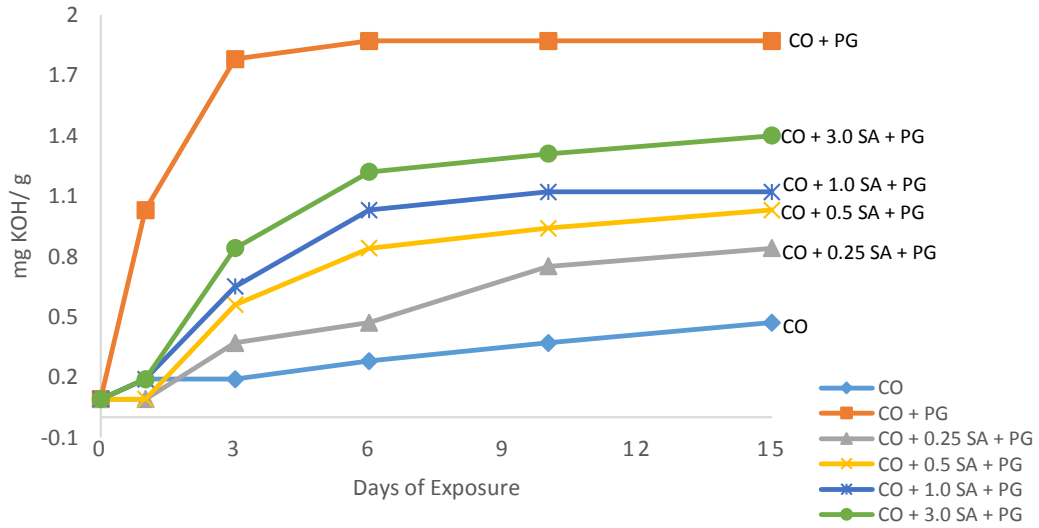


Figure 6.38: Effect of SA concentration on the TAN of CO in the presence of PG

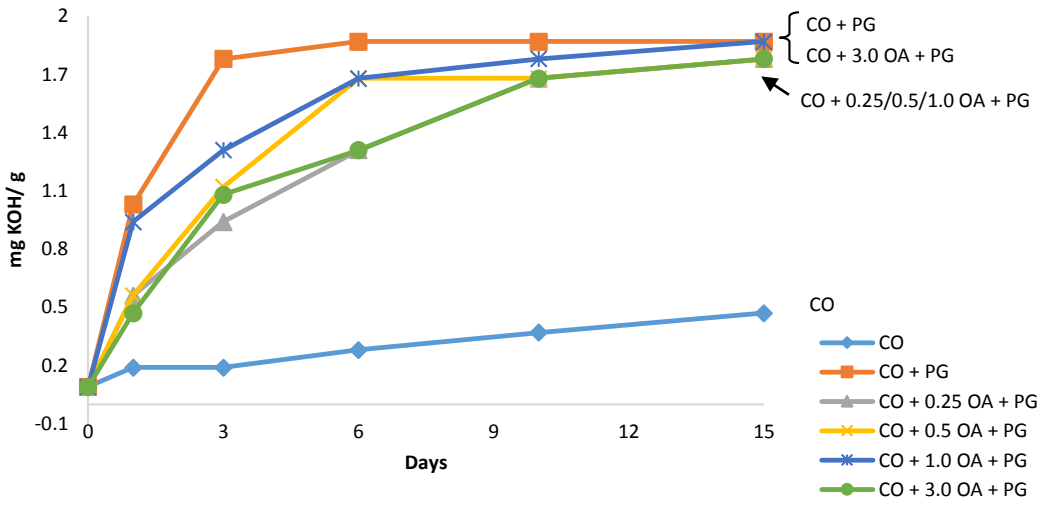


Figure 6.39: Effect of OA concentration on the TAN of CO in the presence of PG

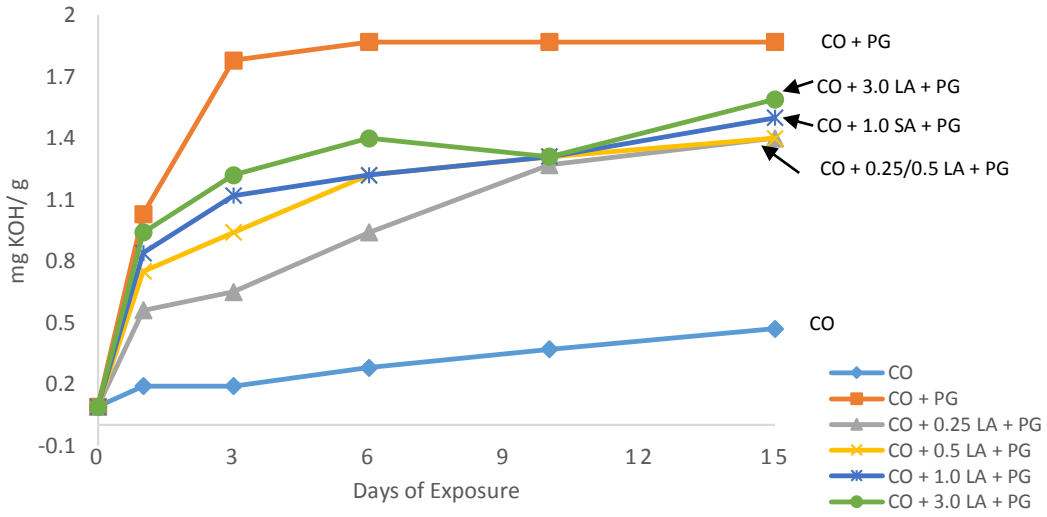


Figure 6.40: Effect of LA concentration on the TAN of CO in the presence of PG

On the Peroxide Formation:

PV results of CO with PG in the presence of PA, SA, OA and LA are presented in Figure 6.41 to 6.44 respectively.

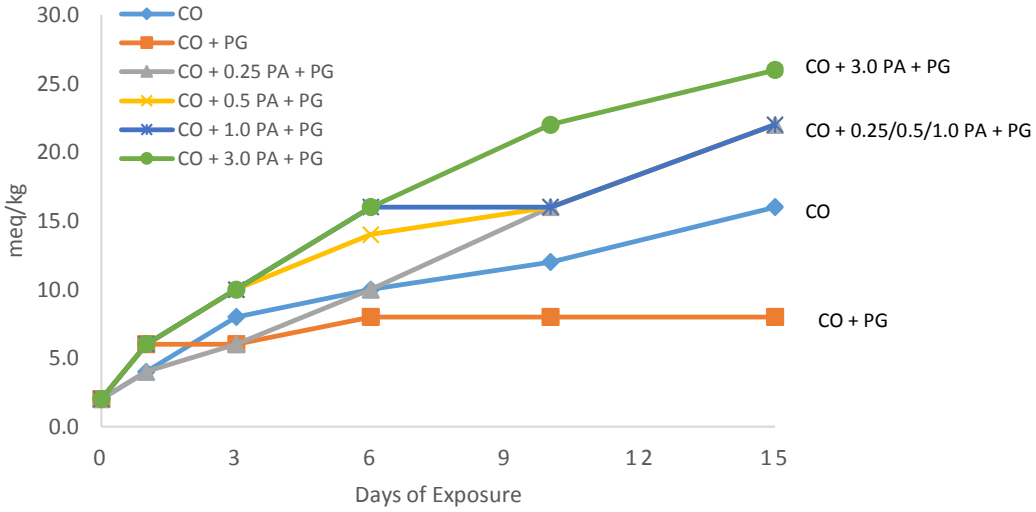


Figure 6.41: Effect of PA concentration on the PV of CO in the presence of PG

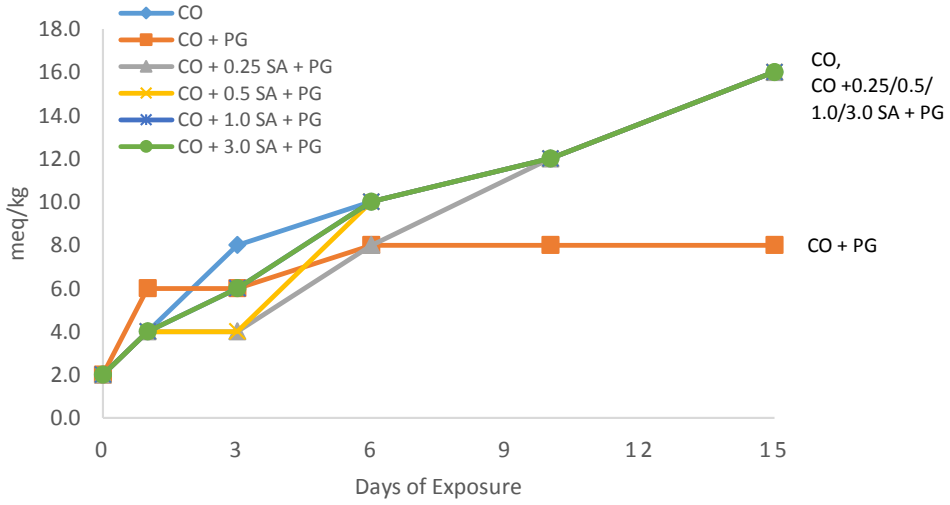


Figure 6.42: Effect of SA concentration on the PV of CO in the presence of PG

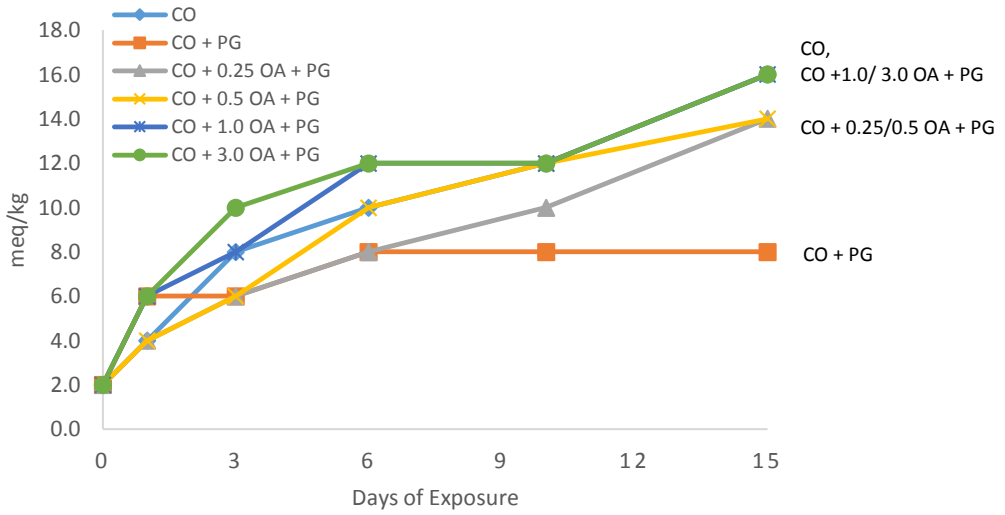


Figure 6.43: Effect of OA concentration on the PV of CO in the presence of PG

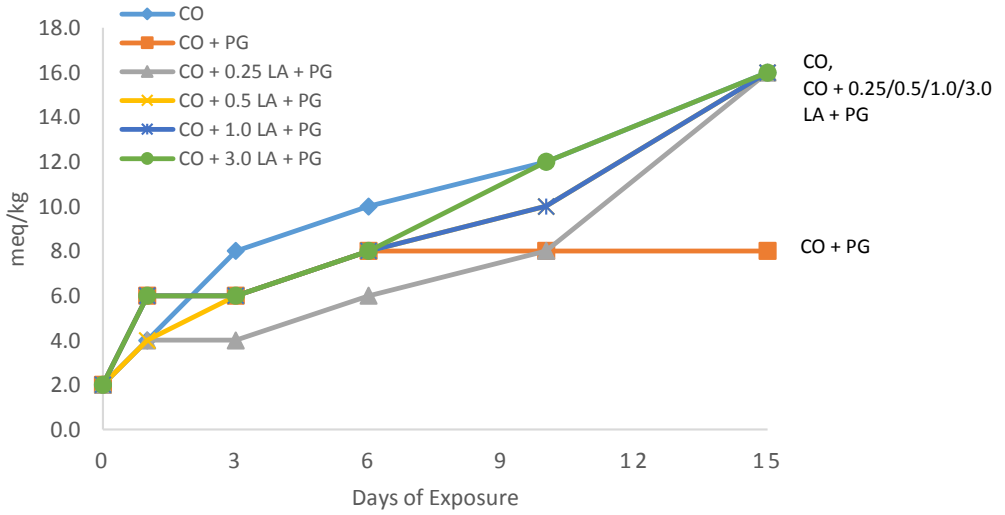


Figure 6.44: Effect of LA concentration on the PV of CO in the presence of PG

Analyses on PV reveal negative effects were shown for all sample mixtures. Addition of fatty acid in samples resulted in the termination antioxidant properties of propyl gallate. Addition of PA shows to worsen the peroxide formation directly resulted to the increment in peroxide value were it is higher than the CO alone and

other CO with PG mixture (Figure 6.41). OA at lower amount (0.25-0.5%) manages to slow the peroxide formation even though the combination shows an antagonistic interaction. Overall, the sequence of fatty acids that affect PG performance in delaying peroxide formation from good to worse are as follows: OA > LA ≈ SA > PA.

In conclusion, PG proved to be an effective antioxidant without the addition of fatty acids. The presence of fatty acid resulted to the depletion of propyl gallate ability in delaying peroxide formation. PG is not suitable for delaying the $H_{ß}$ TAG hydrolyses but the presence of fatty acids manage to improve the PG performance.

6.5 Summary

Exposing CO to heat treatment (60˚C) for15 days resulted to the degradation of the oil quality. This was proven from the analyses that were done where the peroxide value of the samples rose from 2.0 meq/kg to 16.0 meq/kg. Total acid number of the oil also increased from 0.09 mg KOH/ g up to 0.47 mg KOH/g. This shows that the oil will degrade in time at this temperature and in line with the results obtain from previous studies (Krevaitis *et al*, 2013; Catel, 2012; Yildiz *et al.,* 2001; Yoshida *et al*., 1992;Vaisey-Genser and Ylimaki, 1985).

The addition of antioxidant manage to delay the peroxide formation and $H_{ß}$ TAG hydrolyses. Studies revealed that the antioxidant manage to delay the peroxide formation about 50-60% while $H_{ß}$ TAG hydrolyses was successfully reduced about 20-30%. The best antioxidant in delaying CO degradation is TBHQ. Other three antioxidants tested have shown the same performance towards CO.

Combination of CO with fatty acids studies shows unsaturated fatty acids manage to reduce total acid number of the samples about 50-60% while the saturated acid had shown reverse effects on CO when added at 1 and 3%. Saturated acid at lower levels has shown no effect on CO $H_{ß}$ TAG hydrolyses. Oxidative stability studies revealed that SA, OA and LA manage to delay the peroxide formation on CO. The ability of these fatty acids acting as antioxidants towards CO have been the highlight of this research findings. Only PA shows an undesirable effect on CO. PA had shown pro-oxidant activity towards CO. It can be suggested that any vegetable oil that contains high composition of PAs is not suitable to be blend with CO.

In tri-species system for oxidation, deterioration of the quality in the oil was due to the tri-species oxidation of PA which highly promotes the formation of peroxide. The presences of PA were seen to reduce all antioxidant performance (BHA, BHT, TBHQ, and PG).

Different trend was seen on antioxidant performance on delaying $H_{ß}$ TAG hydrolyses in the presence of fatty acids. PA has shown no effect on BHT performance but reduce TBHQ and BHA performance. Synergistic effect can only be seen between PA and PG. SA has shown to reduce BHA, BHT and TBHQ performance. OA has shown no effect towards BHT and TBHQ performance. However, OA has shown to improve with BHA and PG. LA has shown to improve all antioxidant performance. Combination of fatty acids with PG manage to lower the TAG degradation, but still, PG is not suitable to be used as antioxidant due to high acid formation.

In a nut shell, the combination of LA with BHT has shown the best result in delaying both peroxide formation and $H_{ß}$ TAG hydrolyses. OA which is believed to be the most FFA occurred in CO has shown no reducing or improving effect on BHT. So, the appearance of OA in CO will not affect BHT performance in delaying oil degradation.

CHAPTER 7

RESULTS AND DISCUSSION ON SAFFLOWER OIL

7.1 Oxidation of Safflower Oil

Safflower oil (SO) exhibits the highest level of LA of any commercially available oil. Physical attributes this oil is pale yellow colour. As an edible oil, the high level of unsaturation creates problem in controlling oxidation of the oil and SO is also known to have a relatively short shelf life which means the oil should be kept cool after the bottle is opened to maintain it freshness.

TAN analyses were done on SO samples that were exposed to heat for 15 days at 60˚C. Samples of SO were taken at every 0 day and after 1, 3, 6, 10 and 15 days for analyses. Results of the analyses are shown in Figure 7.1.

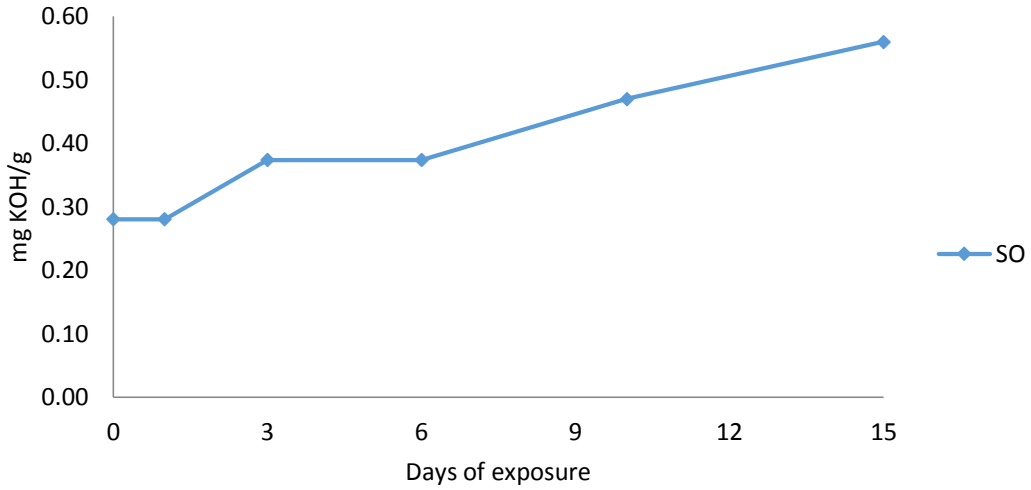


Figure 7.1: Total acid number (TAN) development of SO treated at 60˚C

Figure 6.1 shows acid development in SO during heat treatment at 60˚C. The TAN began to increase after the first day of heat treatment and maintain on the 3rd day until the 6th day. After that, the TAN increment continues until day 15. The initial TAN for SO before the heat treatment (0.28 mg KOH/g) is slightly higher than PO (0.09 mg KOH/g) and CO (0.09 mg KOH/g). The increment of TAN due to hydrolyses process are in accordance to the results obtained by Kakde and Chavan., 2012)

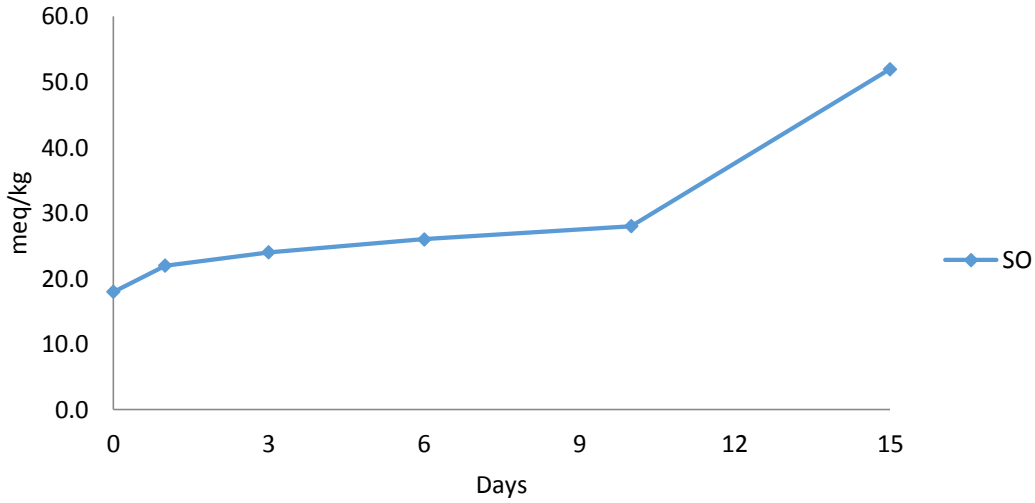


Figure 7.2: Peroxide value (PV) development of SO treated at 60˚C

Peroxide formation of SO during heat treatment was analysed using PV. Figure 7.2 display the development of PV during the heat treatment on SO. Results shows that PV increase gradually with time. The increment of PV shows that the oils are able to oxidize at 60˚C which reflects its storage temperature (Frankel, 1993). To attain a highly stable vegetable oil during storage, it is necessary to take antioxidant to help delay the oxidation process thus protecting oil for a longer period from degradation.

According to previous studies on SO, the high content of linoleic acid (LA) makes the oil susceptible to oxidation rancidity (Sharon *et al*., 1969). This was also the main reason of the high PV of the SO compared to PO and CO where the PV on the 1st to 15th days of heat exposure of SOs are, 18-52 meq/kg compared to PO and CO which is only around 2-22 meq/kg.

7.2 Oxidation Safflower Oil in the Presence of Antioxidants

The performances of antioxidants on SO were studied .Antioxidants were used to monitor the development of acids and peroxide formation. The antioxidants used are similar to those of previous studies on PO and CO which is chain breaking radical scavenger antioxidant (BHA, BHT, TBHQ and PG). The results of these studies were compared with SO samples without any antioxidant addition (control).

7.2.1 TAGs Decomposition

The development of total acid number analyses of SO in the presence of antioxidants during heat treatment is shown in Figure 6.3. The three antioxidants used, TBHQ, BHA and BHT exhibit the ability in reducing the TAG decompositions of SO.

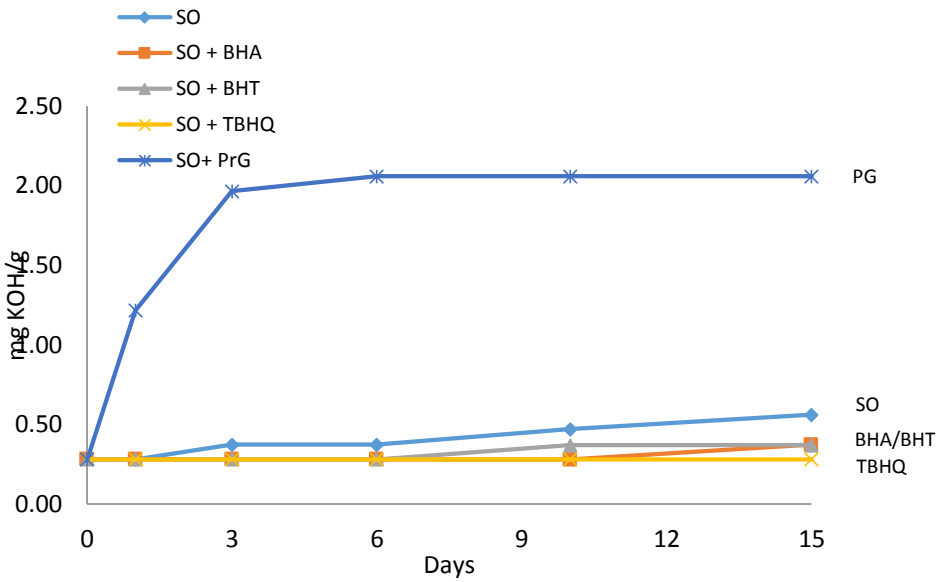


Figure 7.3: Total acid number of SO samples with four different types of antioxidants: BHA, BHT, TBHQ and PG

Results (Figure 7.3) show that TBHQ managed to prevent $H_{ß}$ TAG hydrolyses from the first day of heat treatment until the final day of heating process. BHA and BHT shows similar total acid number on the final day but BHA manage to delay $H_{ß}$ TAG hydrolyses better than BHT. These can be seen when TAN of samples containing BHT increased earlier than samples containing BHA.

Experimental results on PG comes last compared to other antioxidant where PG has shown to increase the total acid number of the samples mixture since the 1st day of heat treatment and this was believed to be due to the self-decomposition of PG. The order of the antioxidants performance are as follows TBHQ ≈ BHA≈ BHT. This supports the theoretical order of the antioxidants based on the interaction energy between the antioxidants and $H_{β}$ of TAG trilinoleic which is as follows: PG

(-39.05 kJ/mol) > TBHQ ≈ (-20.39 kJ/mol) ≈ BHA (-20.28 kJ/mol) ≈ BHT (-22.66 kJ/mol).

7.2.2 Peroxide Formation

PV analyses data (Figure 7.4) show important oxidative changes in SO samples treated without antioxidant (control), with BHA, BHT, TBHQ and PG. Oxidations of SO in the presence of antioxidant are lower compared to the control. The peroxide formation was successfully reduced almost 50% to 60%.

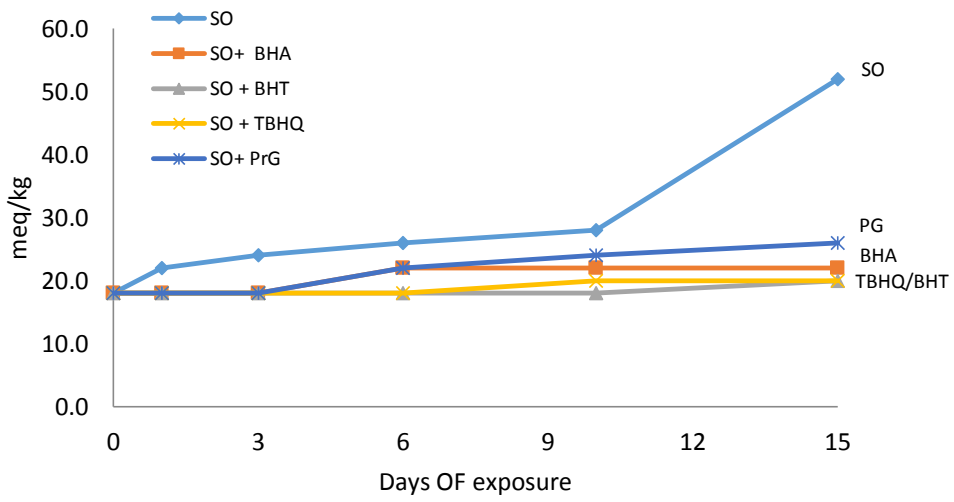


Figure 7.4: Peroxide value of SO samples with four different types of antioxidants: BHA, BHT, TBHQ and PG

Based on the interaction energy value, BHT, TBHQ and BHA shares similar interaction energy value (16 to 23 kJ/mol) and can be related to the experimental

results (PV) which were also similar to one another. However, on the end of the 15th day, BHT exhibit similar performance as TBHQ. However, upon further analysis done, it was shown that BHT has the highest inhibition period than TBHQ. These phenomena are in parallel with the energy interaction theoretical results where the interaction between TAG trilinoleic $C_9OO•$ radical and antioxidant as follows: TBHQ (23 kJ/mol)≈ BHA (-23 kJ/mol) > BHT (-17 kJ/mol) PG (-10 kJ/mol).

Overall studies on the effect of antioxidant on peroxide formation shows that all four antioxidants tested managed to reduce the production/formation of peroxide. Antioxidant performances are ranked as follows: BHT≈ TBHQ ≈ BHA > PG.

7.3 Oxidation of Safflower Oil in the Presence of Fatty Acids

The major component of vegetable oil is TAG. At certain temperature and environment, TAG will hydrolyse to form mono or diglycerides and free fatty acids. Since the major fatty acid composition of SO is linoleic acid, the expected fatty acid that will be released through $H_ß$ TAG hydrolyses will mostly be linoleic fatty acids. Therefore, the present studies are to observe the effect of linoleic and the other three selected fatty acid on the oxidative stability of SO.

7.3.1 TAGs Decomposition

The overall analysis revealed the addition of FAs at low concentration (0.5%) shows no significant effect towards the decomposition of TAGs. However at higher FAs concentration which is more than 1% were seen to contribute to the defects of the SO due to the presence of fatty acids. Experiments revealed that the order are similar compared to the order of the energy interaction value, for instance PA,OA,LA possess the similar interaction energy value while SA possess the highest interaction energy value. The order of the lowest to the highest interaction energy is as follows: LA (I=-27.12 kJ/mol) ≈ OA (I=-27.18 kJ/mol) ≈ PA (I =-27.19 kJ/mol) < SA (I=-29.46 kJ/mol). The lowest interaction energy allows the rearrangement of TAGs takes place leading to the formation of FAs. Detail analyses further elaborated in the next paragraph.

TAN for all samples before treatment is 0.28 mg KOH/g which was similar as TAN of the control. Addition of PA and LA (Figure 7.5 and Figure 7.8) shows similar trend where the addition of these acids at 1% and 3% (w/w) worsen the TAG hydrolyses while at 0.5% (w/w), no effect were seen on SO. At lower loading (0.25%, w/w), both PA and LA manage to delay the TAG hydrolyses. Addition of OA (Figure 7.7) also shows to cause deterioration of the SO H_{β} TAG hydrolyses when added at high concentration (1% and 3%, w/w) but no effect were seen when OA when added at 0.25% and 0.5% (w/w) concentration. The addition of SA (Figure 7.6) shows no effect on SO when added at 0.25, 0.5 and 1 % (w/w) but at

3% (w/w), the addition of SA shows small increment on TAN results when compared to the control samples.

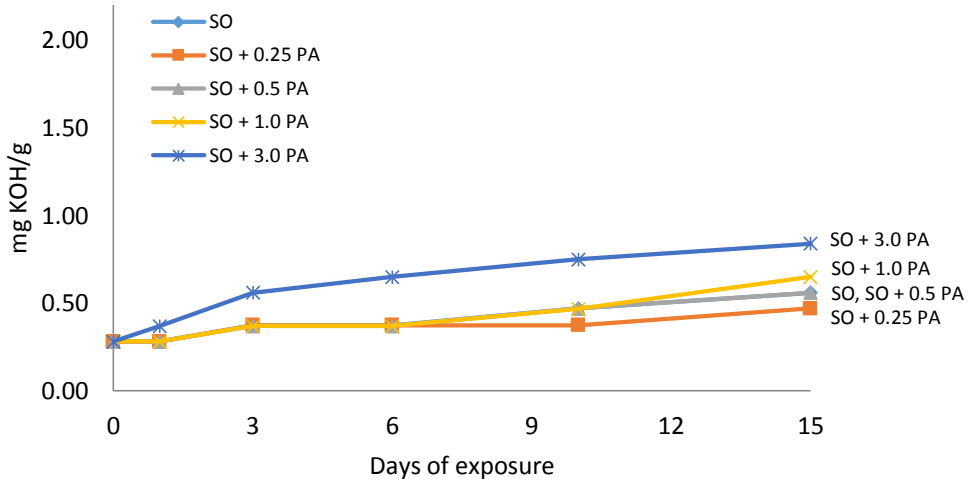


Figure 7.5: Total acid number (TAN) of SO samples with PA at four different concentrations: 0.25, 05, 1, and 3% (w/w).

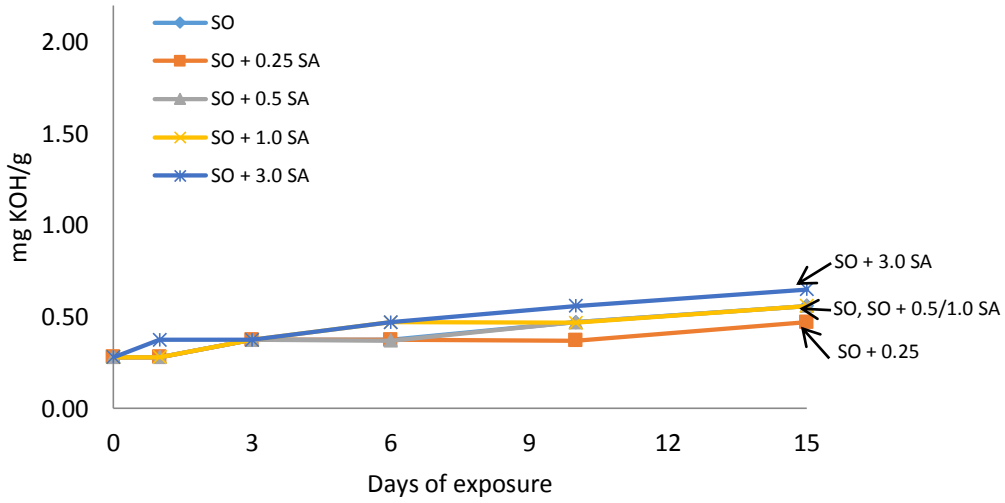


Figure 7.6: Total acid number (TAN) of SO samples with SA at four different concentrations: 0.25, 05, 1, and 3% (w/w).

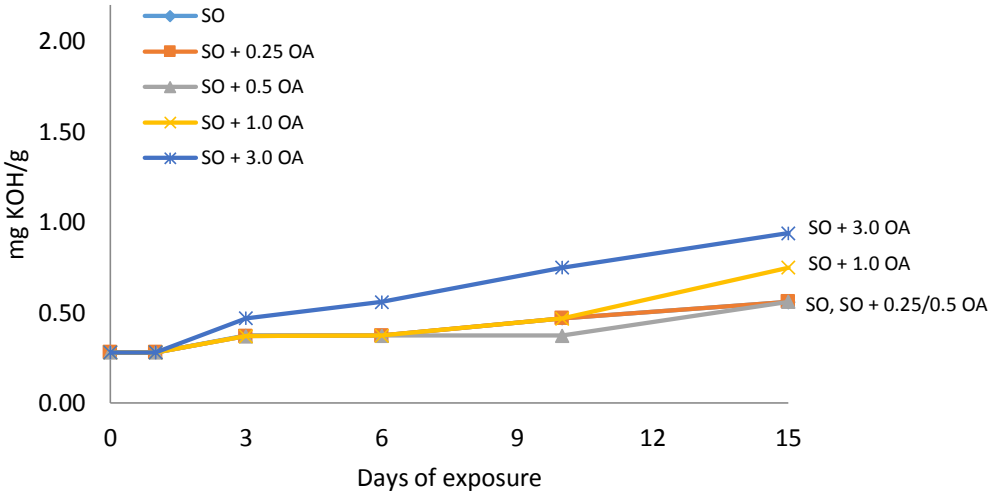


Figure 7.7: Total acid number (TAN) of SO samples with OA at four different concentrations: 0.25, 05, 1, and 3% (w/w).

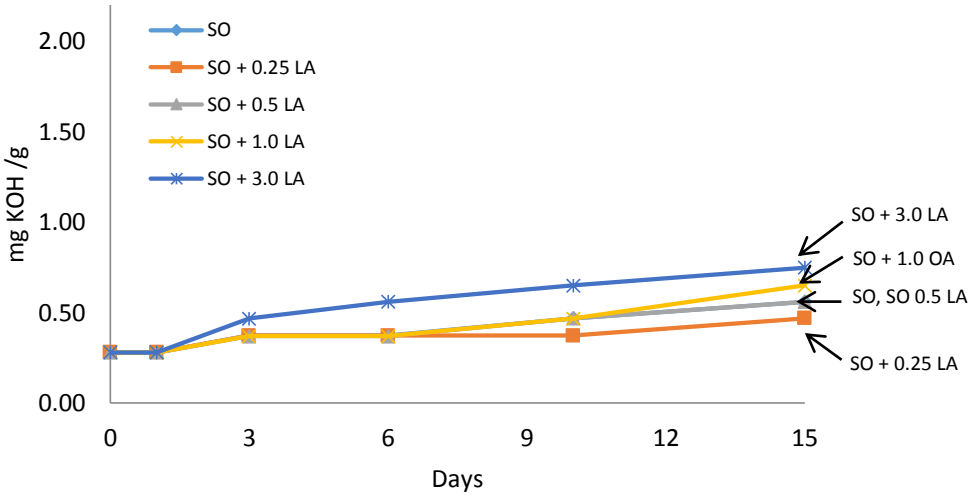


Figure 7.8: Total acid number (TAN) of SO samples with LA at four different concentrations: 0.25, 05, 1, and 3% (w/w).

7.3.2 Peroxide Formation

PV results of SO mixture with PA, SA, OA and LA were tabulated in Figure 7.9, 7.10, 7.11 and 7.12 respectively. An Experimental study has shown that, the addition of FAs (SA, PA, OA and LA) at any concentration (0.25%-3%) will promote oxidation. Theoretically, SA possess the weakest interaction energy with TAG trioleic and $C_9OO\bullet$ radical which contributes high oxidation activity causing severity in the damage of the SO. The other three FAs; PA, OA and LA also cause almost similar damaging effect. The analysis further concluded that the order of the fatty acid in promoting oxidation from worst to least is as follows: SA> LA≈ OA ≈ PA. The accuracy of the order were proven when further compared to the order of the interaction energy: OA (-25.98 kJ/mol) ≈ PA (-23.32 kJ/mol) ≈ LA (-23.07 kJ/mol) > SA (-22.54 kJ/mol).

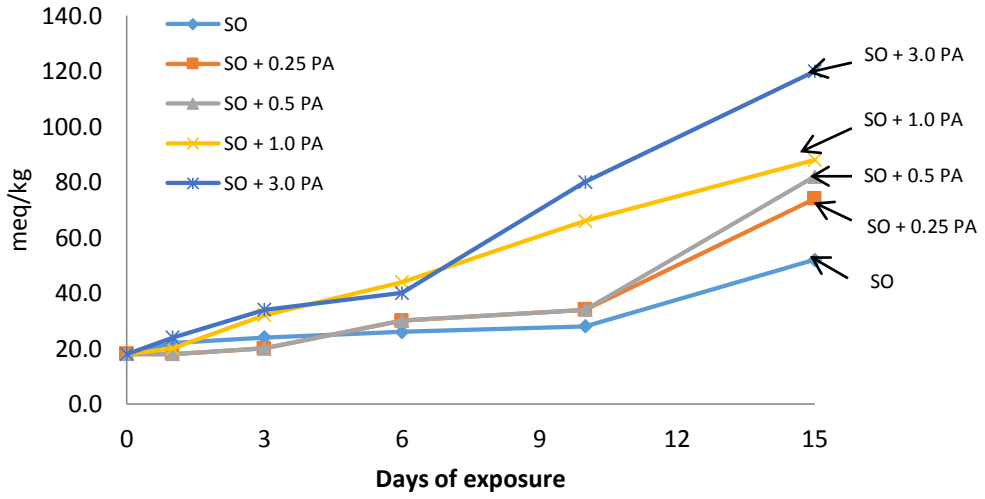


Figure 7.9: Peroxide value (PV) of SO samples with PA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

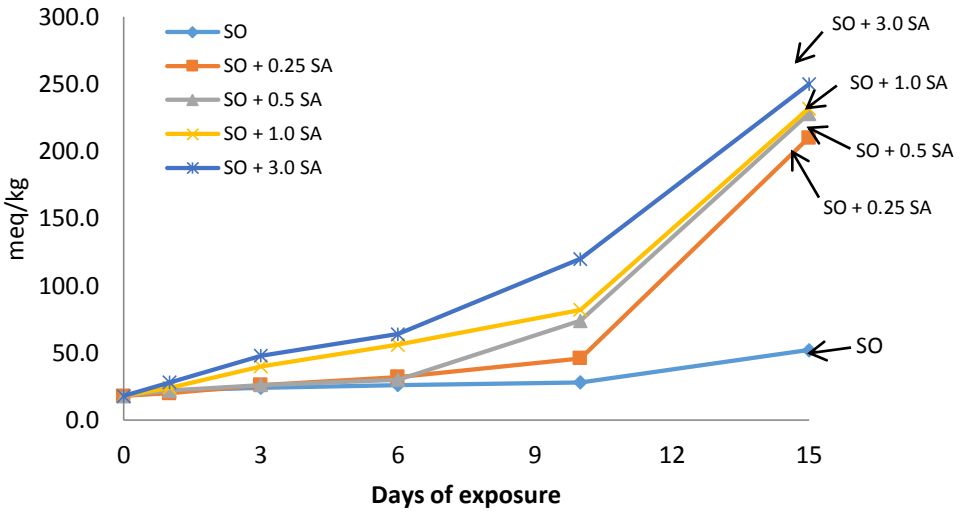


Figure 7.10: Peroxide value (PV) of SO samples with SA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

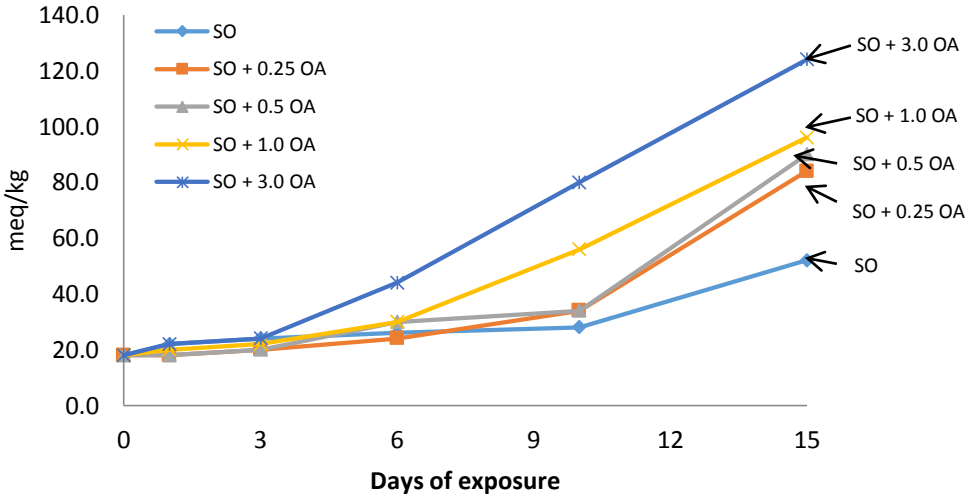


Figure 7.11: Peroxide value (PV) of SO samples with OA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

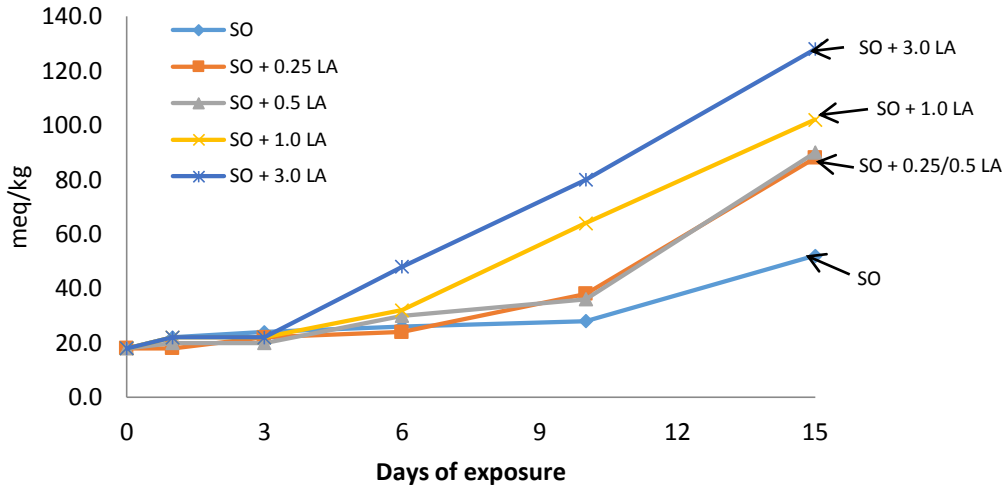


Figure 7.12: Peroxide value (PV) of SO samples with LA at four different concentrations: 0.25, 0.5, 1, and 3% (w/w).

As a conclusion, the addition of any fatty acids (PA, SA, OA and LA) at any concentration will promote peroxide formation toward SO.

7.4 Effects of Fatty Acids on the Performance of Antioxidants

Previous discussion observed that antioxidants used in this study were able to delay the SO oxidation. Results further revealed that fatty acids promote TAG hydrolyses and oxidation towards SO which causes a drastic increment in PV and TAN. This subtopic will discuss the performance of antioxidants towards oxidative stability and TAG hydrolyses of SO in the presence of fatty acids.

On the TAGs decomposition:

The overall antioxidants performance BHA, BHT and TBHQ were negatively influenced by the saturated fatty acid, PA and SA, However, there were no significant effect by unsaturated FAs (OA and LA). It is worth mentioning that fatty acids tested gave synergistic effects on PG where the combinations were able to lower the decomposition process of TAGs of SO. Further detailed analysis on PG revealed that the presence of unsaturated fatty acids (OA and LA) have bigger synergistic effects compared to the saturated FAs (PA and SA).

Comparatively, the effects of FAs on all three antioxidants (BHA, BHT and TBHQ), the least significant effects are on BHT followed by BHA and TBHQ.

SO experimental results agrees with the theoretical evaluation of the interaction energy where PG has the highest interaction energy with H_{β} TAG trilinoleic that is -39.05 kJ/mol as compared to other antioxidants (I_{TBHQ}= -20.39, I_{BHA} = -20.28, I_{BHT} = -22.66); as well as compared to other FAs (I_{SA}= -29.46, I_{LA} = -27.12, I_{PA}= -27.19, I_{OA}-27.18). Antioxidants with high interaction energy are the main factors in reducing the interference from/of any FAs. The interaction energies between $H_{ß}$ of TAG trilinoleic and antioxidants can be referred to Table 4.4 while interaction energies between $H_{ß}$ of TAG trilinoleic with fatty acids can be referred to Table 4.8

On the Peroxide Formation:

Conclusively, the addition of fatty acids to SO doesn't improve the antioxidant performance used. In contrast with PG as the addition of FAs were seen to give synergistic effect increasing the effectiveness of PG in preventing the production/development of the peroxide group. Other antioxidants BHT and TBHQ seems not affected by the presence of the FAs. Comparisons done on all four antioxidants, the addition of FAs were seen to have the most evident effect on BHA. The strength in preventing FAs effect is written as follows: PG > BHT> TBHQ> BHA. Detailed analyses on the interference of FAs on antioxidants found that OA gave the most interference and PA the least interference and the order as written as follows: OA> SA > LA > PA.

Experimental findings suggest the FAs interference in the antioxidant performance agrees with the theoretical calculations of TAG trilinoleic $C_9OO\bullet$ +TBHQ+FAs where OA possess the highest percentage of reduction in interaction energy which is about 21.85% while other fatty acids only shows about 7 to 12 % of reduction in interaction energy.

7.4.1 Performance of BHA in Inhibiting Oxidation in the Presence of Selected Fatty Acids

On the TAGs Decomposition:

Findings from the study shows that PA (Figure 7.13) and SA (Figure 7.14) promote H_{β} TAG hydrolyse. LA (Figure 7.16) promotes H_{β} TAG hydrolyse when added at 0.5, 1.0 and 3.0% (w/w) concentration. No effect were seen when LA added at 0.25% (w/w) concentration. OA (Figure 7.15) shows no effect on BHA performance when added at 0.25 %, 0.5 % and 1 %. OA promotes H_{β} TAG hydrolyse when added at 3% (w/w) concentration.

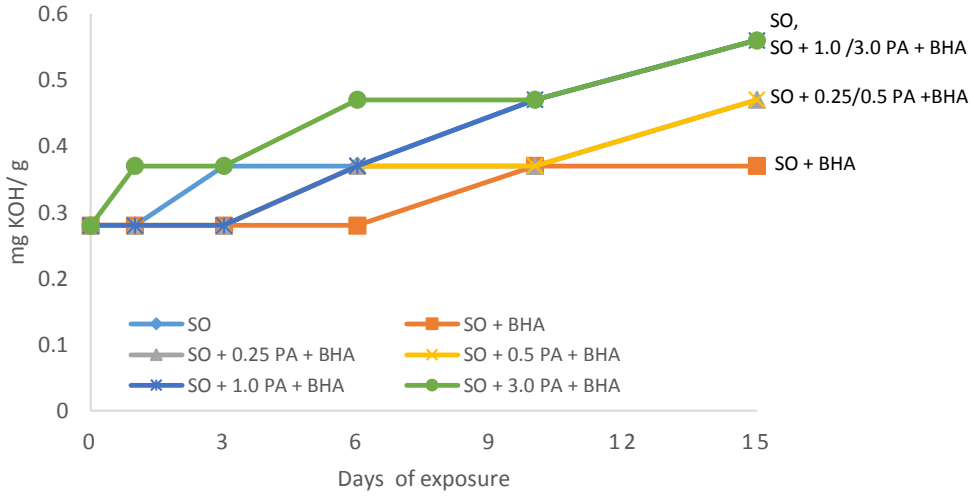


Figure 7.13: Effect of PA concentration on the TAN of SO in the presence of BHA

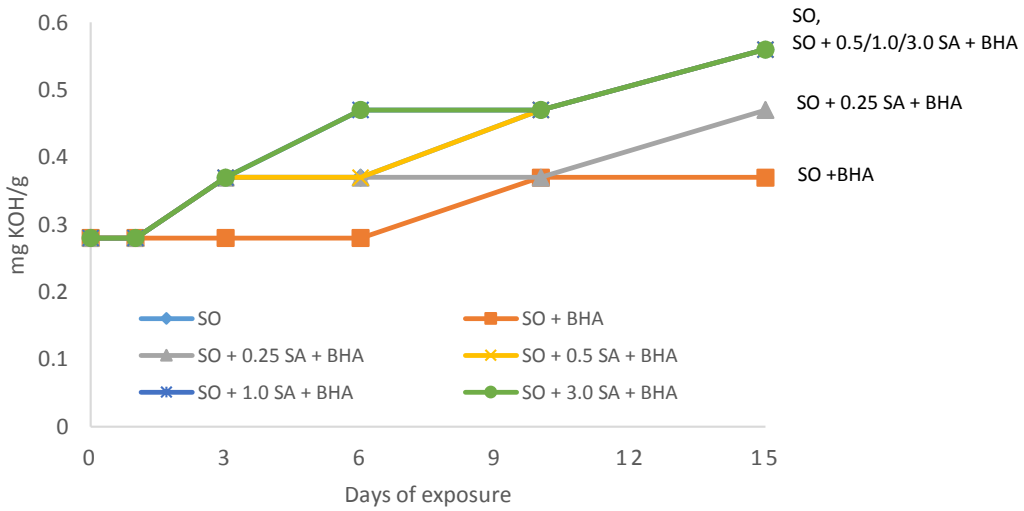


Figure 7.14: Effect of SA concentration on the TAN of SO in the presence of BHA

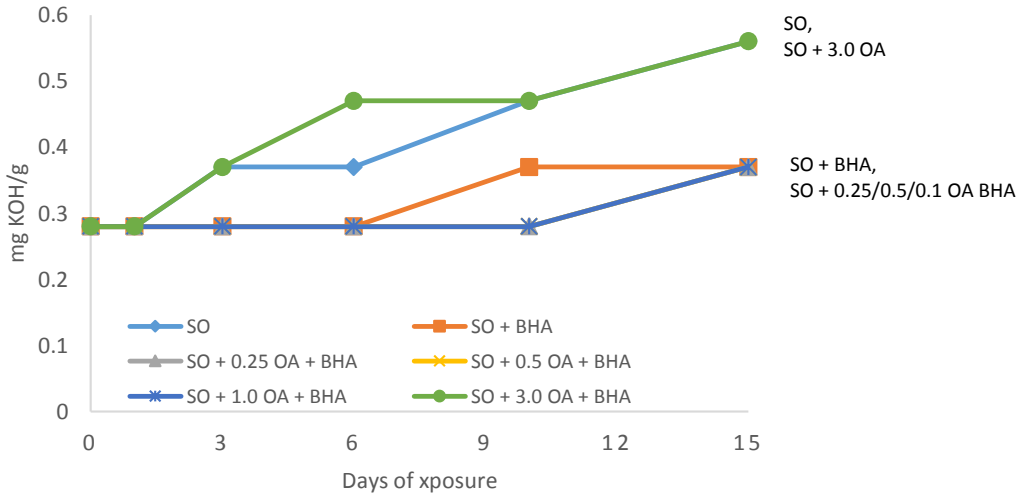


Figure 7.15: Effect of OA concentration on the TAN of SO in the presence of BHA

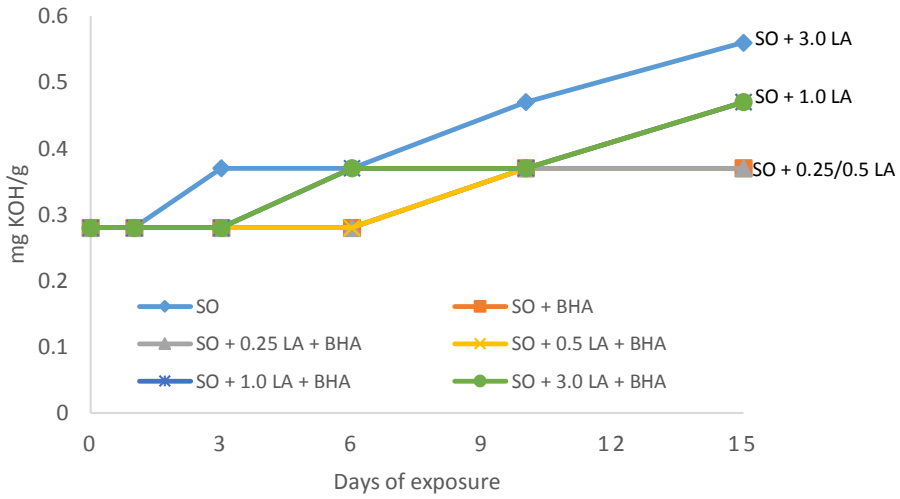


Figure 7.16: Effect of LA concentration on the TAN of SO in the presence of BHA

On the Peroxide Formation

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PA, SA and LA shows to reduced BHA performance in preventing peroxide formation. The existence of these FAs has shown to increase peroxide value compare with the samples which contain BHA only. However, OA has shown to have no effect on BHA performance at any concentration added.

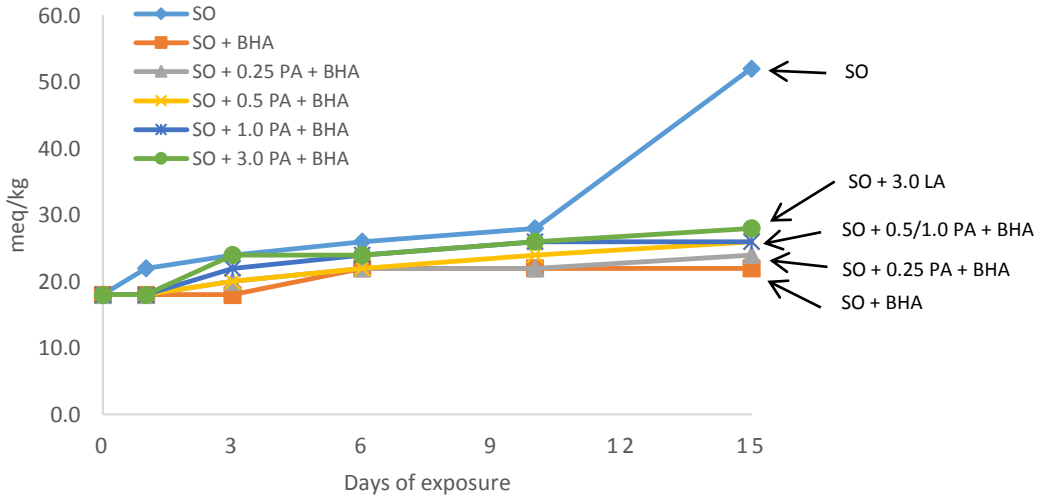

Figure 7.17: Effect of PA concentration on the PV of SO in the presence of BHA

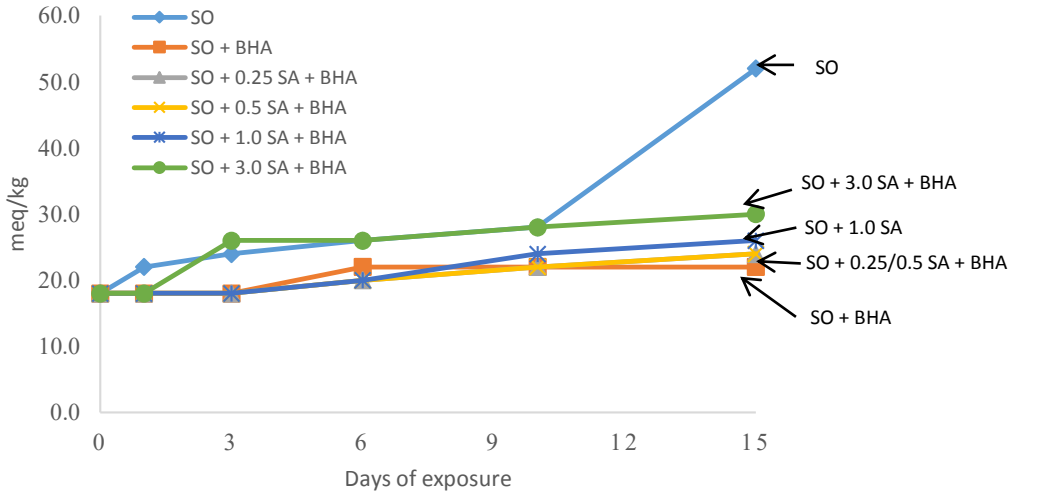

Figure 7.18: Effect of SA concentration on the PV of SO in the presence of BHA

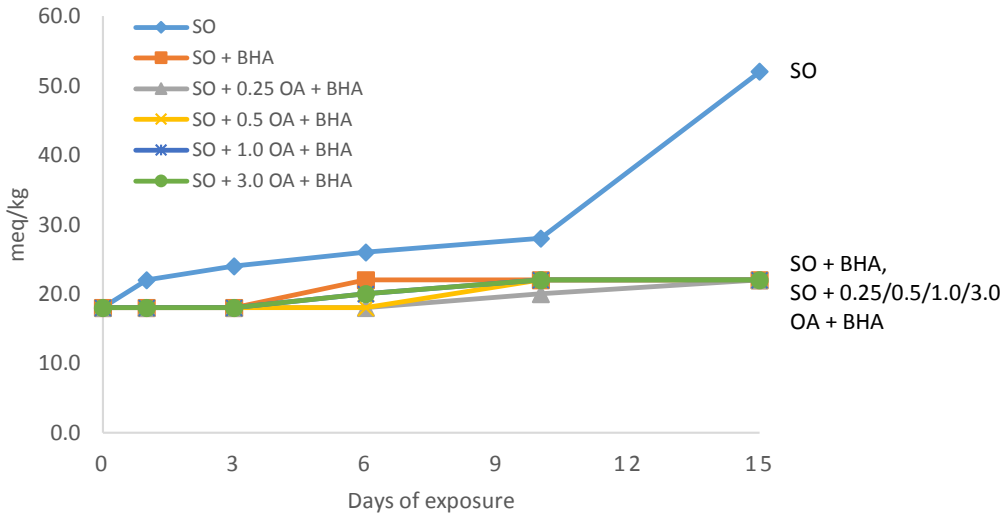

Figure 7.19: Effect of OA concentration on the PV of SO in the presence of BHA

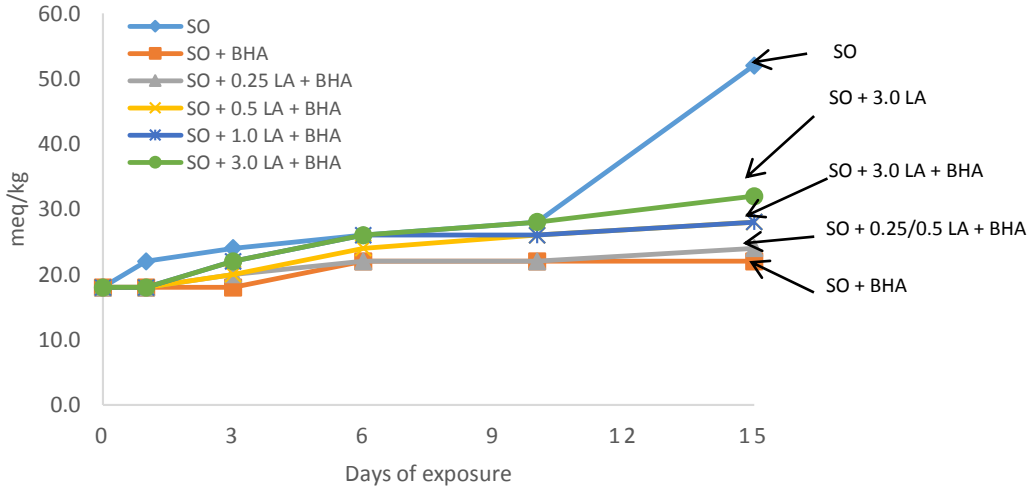

Figure 7.20: Effect of LA concentration on the PV of SO in the presence of BHA

Overall, experiments show that oleic acid shows no significant effect on BHA performance in reducing TAG hydrolyses and peroxide formation while other fatty acid reduce the BHA performance both in delaying TAG hydrolyses and oxidation. The antioxidant performance in the presence of fatty acid ranging from

good to poor in delaying $H_{ß}$ TAG hydrolyses are expressed in an ordinal form as follow: OA > LA > PA > SA. While based on peroxide value, the sequence is as follows: OA > SA > PA > LA.

7.4.2 Performance of BHT in Inhibiting Oxidation in the Presence of Selected Fatty Acids

On the TAGs Decomposition:

Previous results (subtopic 7.2) revealed that BHT exhibited the best antioxidant performance due to its ability in preventing oil oxidation. However, BHT failed in giving out optimum performance in preventing TAG hydrolyses which leads to the formation of FFA. Under this subtopic, discussion will focused on the effect of fatty acids added towards the performance of BHT in protecting SO from oxidation and $H_{ß}$ TAG degradation.

The performance of BHT in hindering TAG $H_{ß}$ hydrolyses in the presence of PA, SA, OA and LA are shown in Figure 7.21, 7.22, 7.23, and 7.24 respectively.

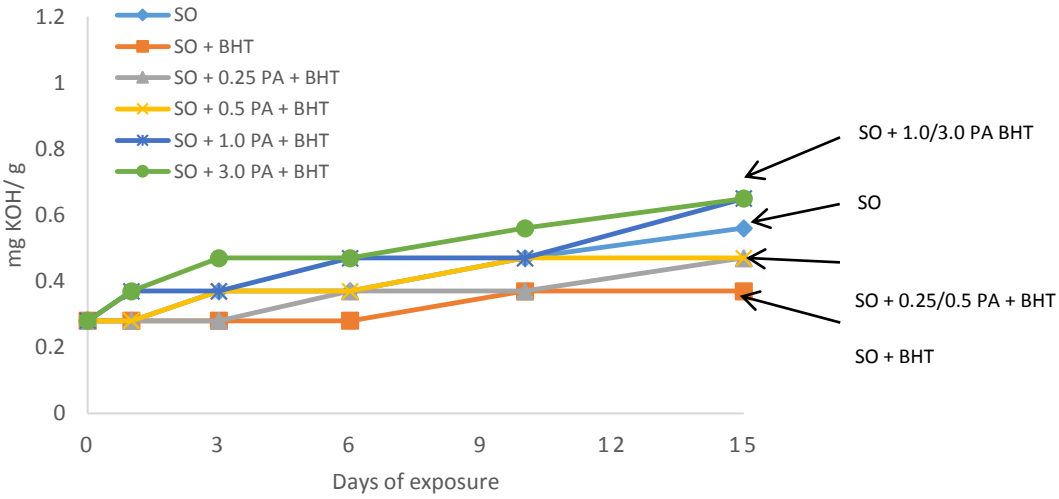

Figure 7.21: Effect of PA concentration on the TAN of SO in the presence of BHT

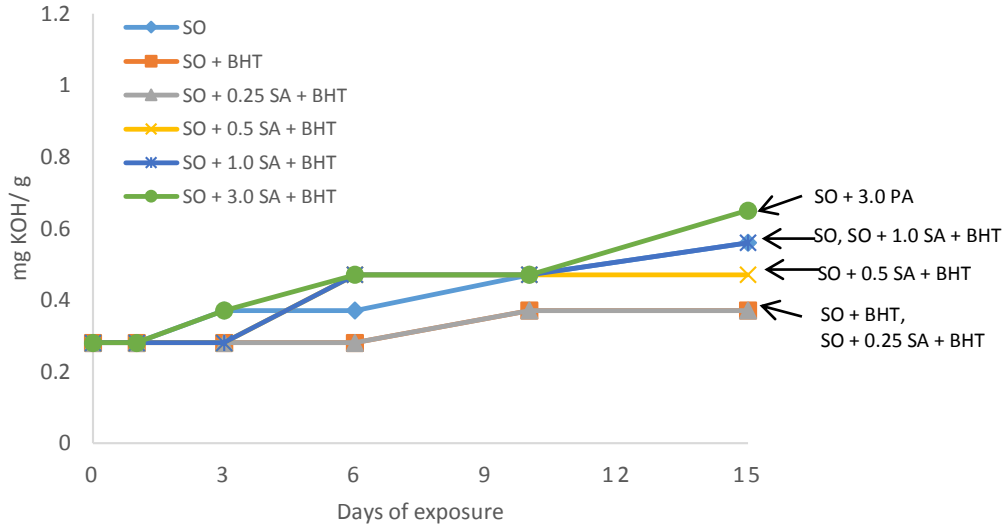

Figure 7.22: Effect of SA concentration on the TAN of SO in the presence of BHT

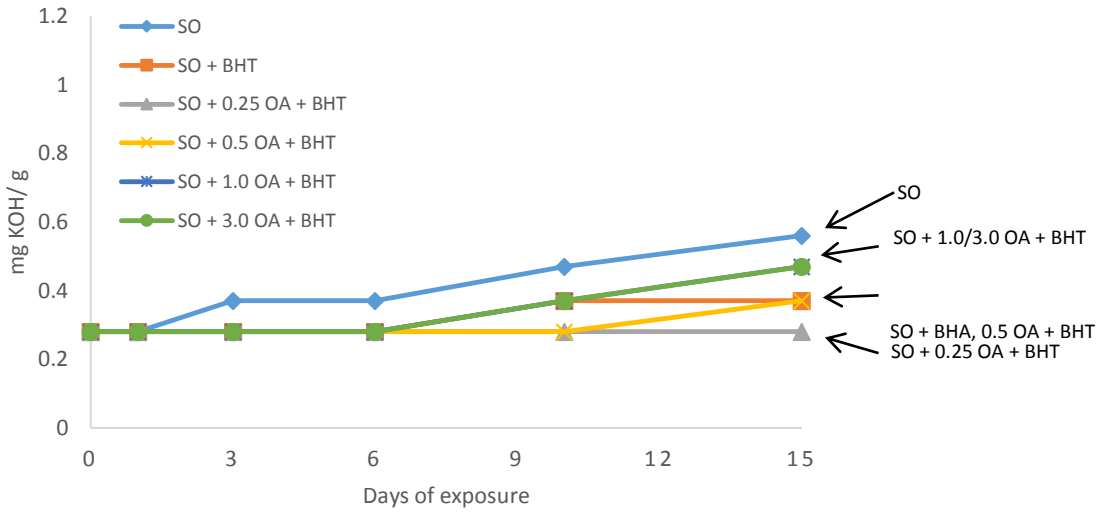

Figure 7.23: Effect of OA concentration on the TAN of SO in the presence of BHT

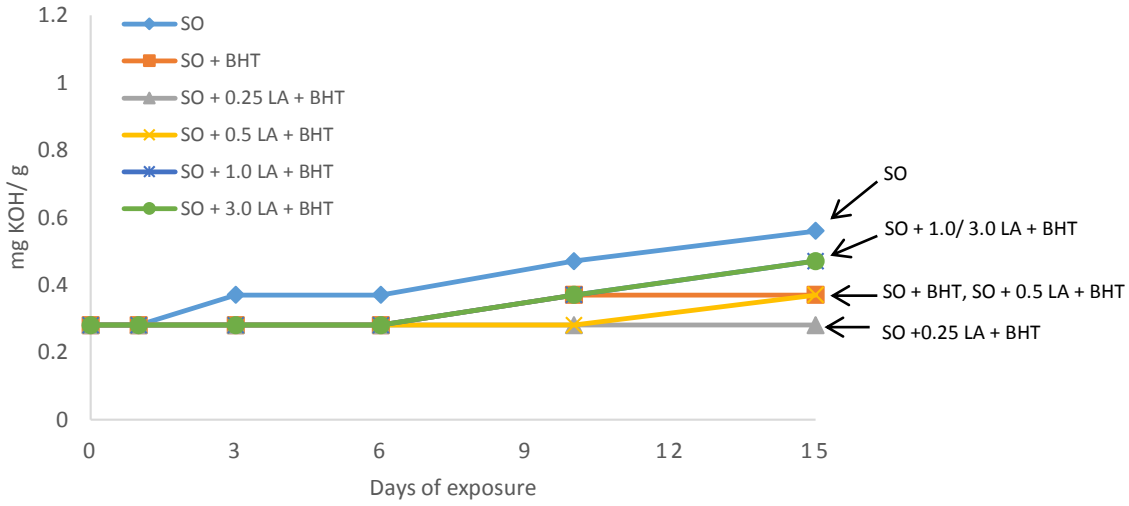

Figure 7.24: Effect of LA concentration on the TAN of SO in the presence of BHT

Experimental results show that PA reduce the performance of BHT when added at any four selected concentration. The higher the concentration of PA, resulted in to the inefficiency of BHT performance in delaying TAG hydrolyses. SA shows to reduce BHT performance when added with more than 0.25 %. At 0.25%, were seen to have no effect on

BHT performance. The unsaturated has shown similar effect towards BHT performance in delaying TAG hydrolyses. At low concentration (0.25%), both OA and LA has shown to improve BHT performance in delaying $H_{ß}$ TAG hydrolyses. However, no effect were seen when these fatty acids were added at 0.5%. BHT performance deteriorates upon the addition unsaturated at 1 and 3% w/w concentration.

On the Peroxide Formation

Variation of PVs of PO samples against the days of exposure for PA, SA, OA and LA with BHT are illustrated in Figure 7.25 to 7.28.

BHT has shown the optimum performance in delaying oxidation when added alone into SO. All four different concentration (0.25-3.0 % w/w) of PA were added into SO has shown no effects on BHT performance. SA and LA has shown similar effect on BHT performance where both fatty acids has shown to improve BHT performance when added at 0.25- 0.5% (w/w) concentration. No effect was seen on BHT performance when SA and LA were added at 1 and 3% (w/w) concentration.

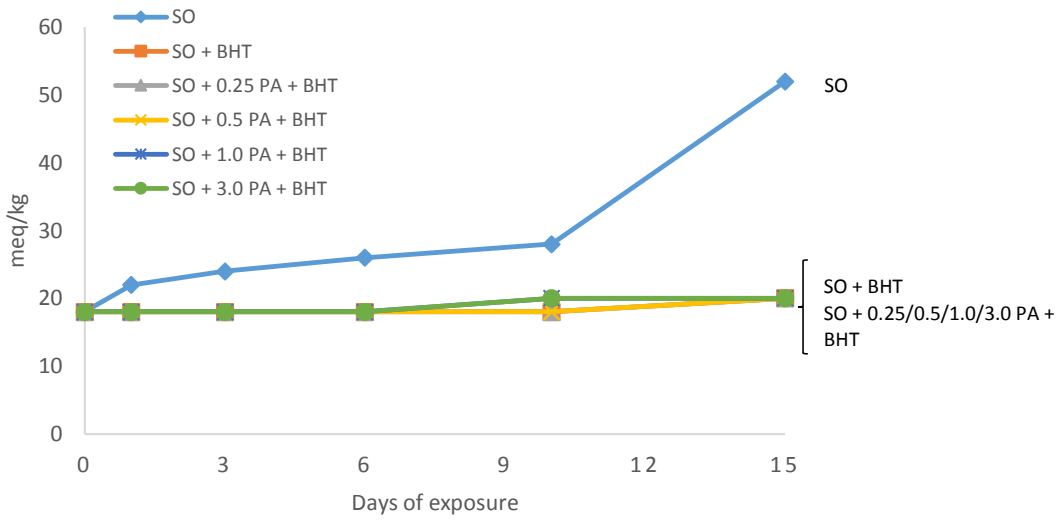

Figure 7.25: Effect of PA concentration on the PV of SO in the presence of BHT

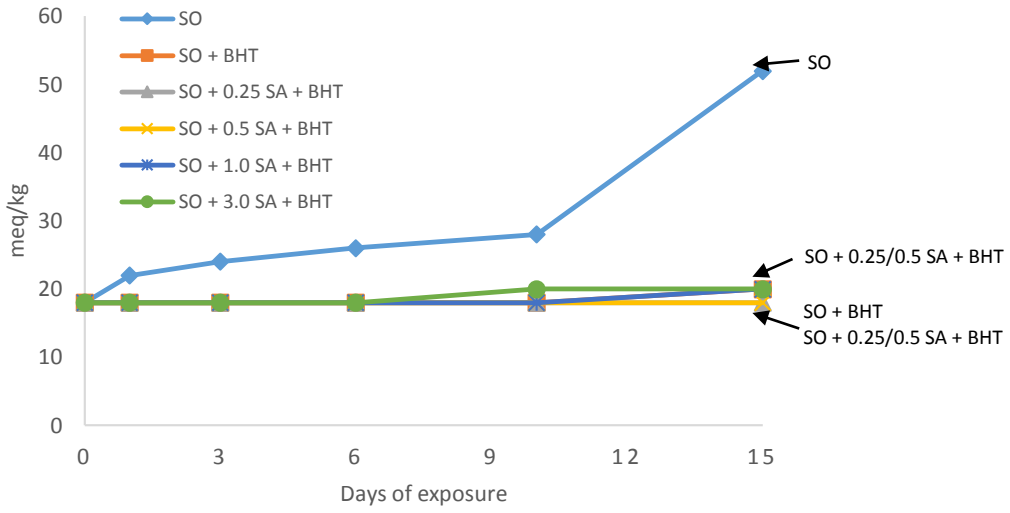

Figure 7.26: Effect of SA concentration on the PV of SO in the presence of BHT

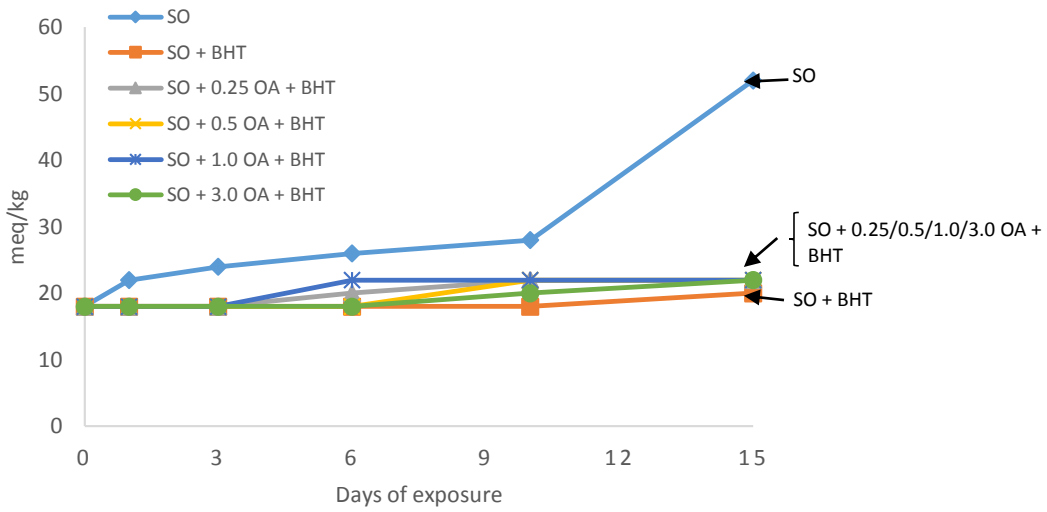

Figure 7.27: Effect of OA concentration on the PV of SO in the presence of BHT

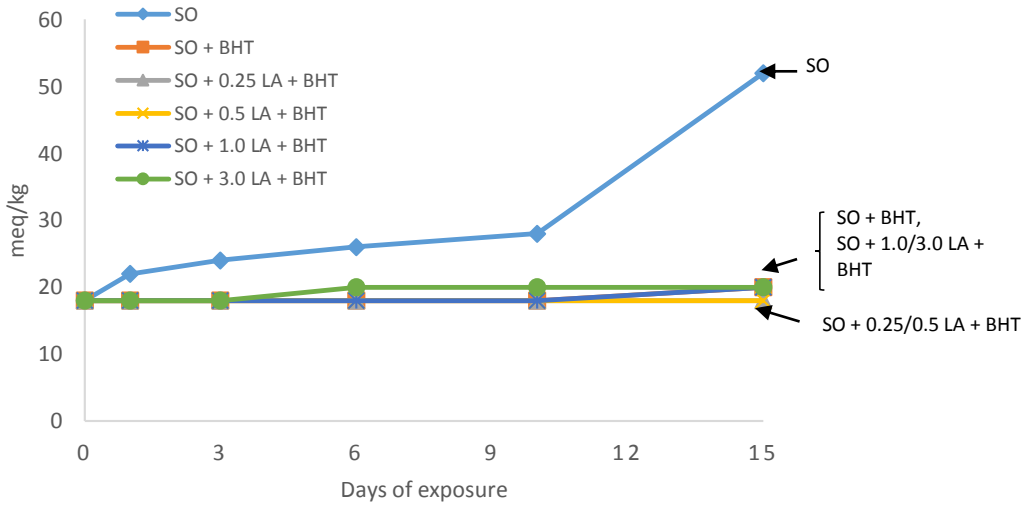

Figure 7.28: Effect of LA concentration on the PV of SO in the presence of BHT

OA has shown to reduce BHT performance in delaying SO oxidation when added into the sample at all four different concentrations. The pro-oxidant effect was observed to have begun on the sixth day of heat treatment.

Finding shows that, the presence of OA in the safflower became a contributing factor in the disruption of BHT performances especially at higher loading. While LA and SA managed to improve BHT performance when added at low concentration (0.25% w/w). Overall, the sequence of fatty acid that affect the antioxidant performance in delaying $H_{ß}$ TAG hydrolyses from good to poor are expresses in ordinal form as follow: LA > OA > SA > PA. While for peroxide value, the sequence are as follows: LA = SA > PA > OA.

7.4.3 Performance of TBHQ in Inhibiting Oxidation in the Presence of Selected Fatty Acids

On the TAGs Decomposition:

TBHQ are widely known to be best antioxidant in vegetable oil industry. In this study, TBHQ shows similar performance with BHA and BHT in delaying $H_{ß}$ TAG hydrolyses, while for oxidation, TBHQ shows similar strength with BHA and BHT in delaying oxidation process when added into SO alone. The performance of TBHQ in the presence of fatty acids (PA, SA, OA, LA) was studied. TAN results on this tri-species system are display in Figure 7.29 to 7.32.

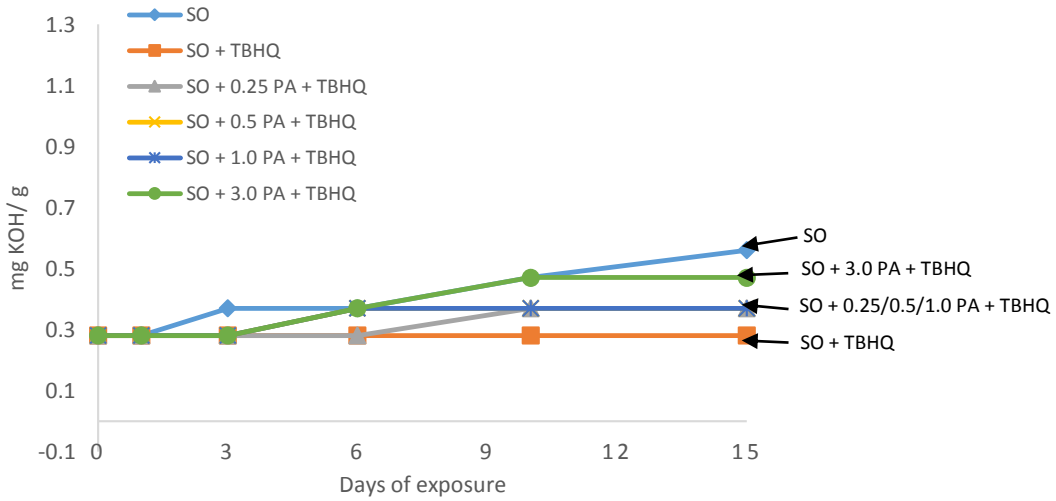

Figure 7.29: Effect of PA concentration on the TAN of SO in the presence of TBHQ

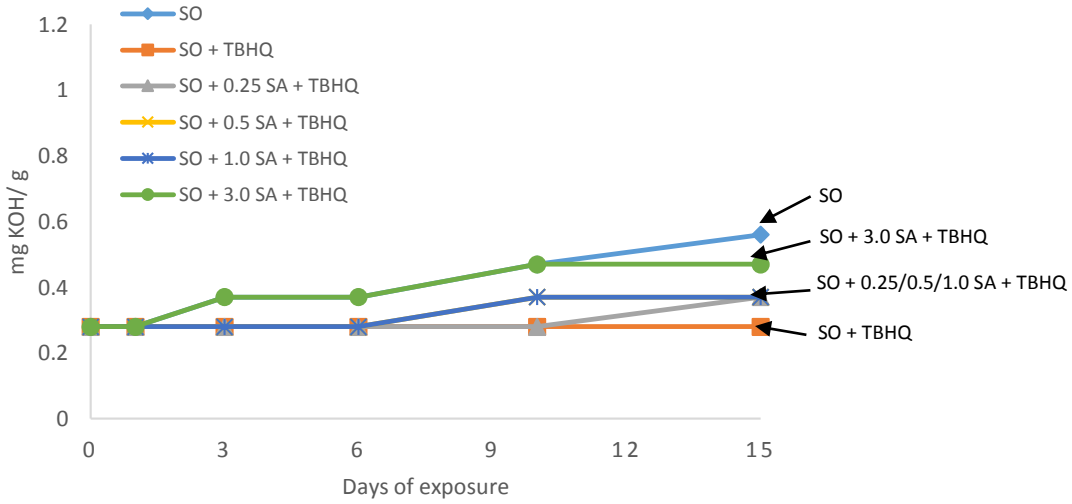

Figure 7.30: Effect of SA concentration on the TAN of SO in the presence of TBHQ

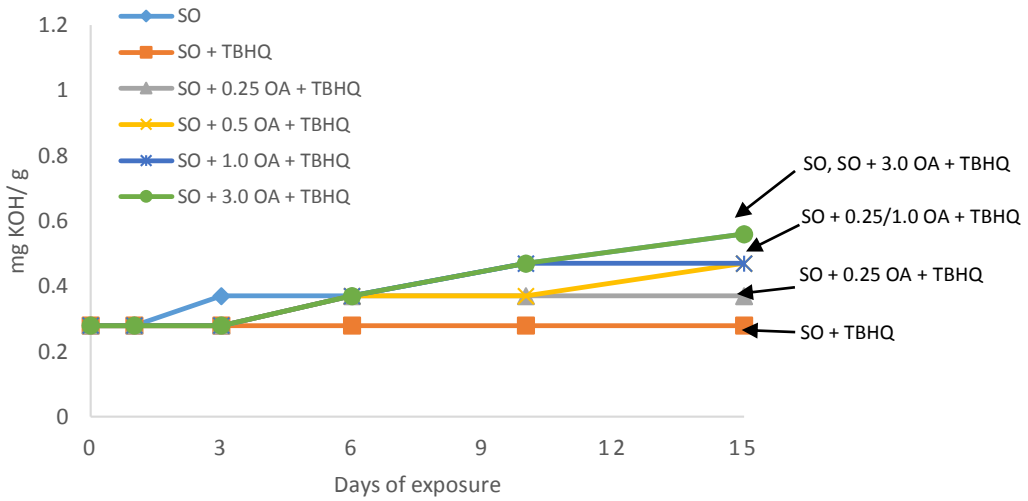

Figure 7.31: Effect of OA concentration on the TAN of SO in the presence of TBHQ

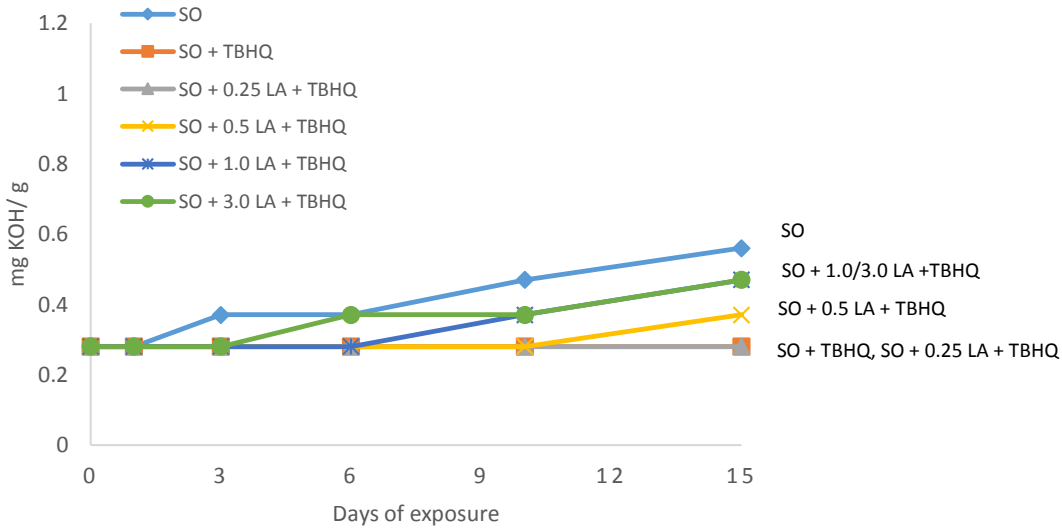

Figure 7.32: Effect of LA concentration on the TAN of SO in the presence of TBHQ

Experimental results show the presence of fatty acid reduces the performance of TBHQ in delaying $H_{ß}$ TAG. OA shows to cause major deterioration on TBHQ while SA has shown the least effect on reducing TBHQ performance in

preventing TAG hydrolyses. LA shows no effect on TBHQ performance when added at 0.25% w/w but reduces TBHQ performance when added more that 0.5%. PA seems to reduce TBHQ performance when added at all four different concentrations respectively.

On the Peroxide Formation

Peroxide development shows that most fatty acid added give only small and sometimes no effect on TBHQ performance. TBHQ also shows good antioxidant properties in preventing peroxide formation. However, all added fatty acid does not improve TBHQ performance in delaying oxidation. SA, OA and LA shows to reduced TBHQ performance when added at high concentration (1-3%) while no effect were seen when all four fatty acid added at 0.25% respectively. PA and LA has shown the least effect in reducing TBHQ performance compared to the other fatty acid. OA has shown to reduced TBHQ performance more compare to other three fatty acid (SA, PA, and LA).

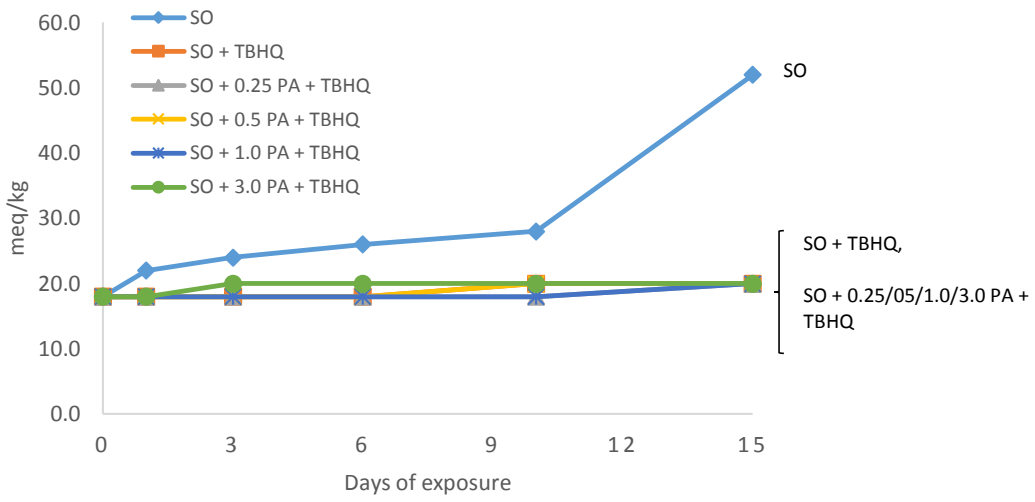

Figure 7.33: Effect of PA concentration on the PV of SO in the presence of TBHQ

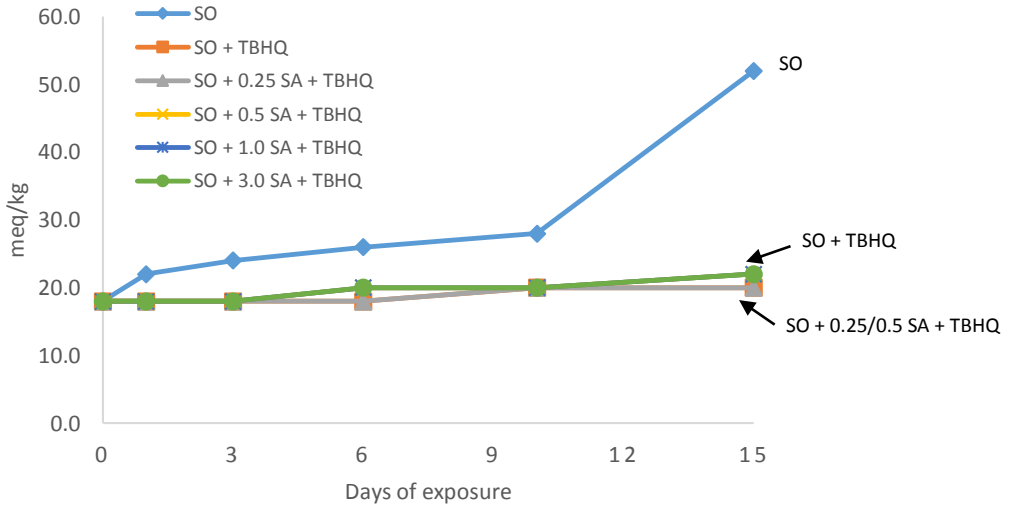

Figure 7.34: Effect of SA concentration on the PV of SO in the presence of TBHQ

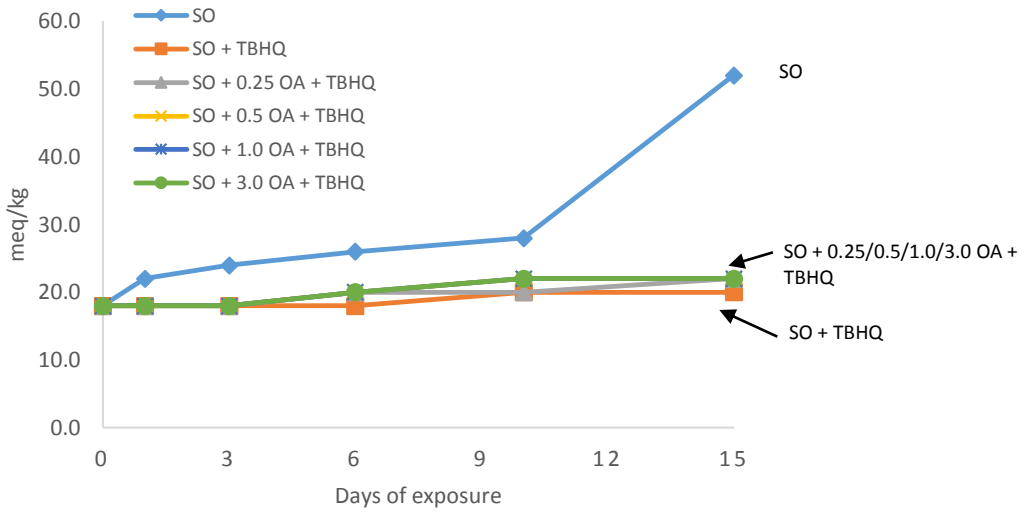

Figure 7.35: Effect of OA concentration on the PV of SO in the presence of TBHQ

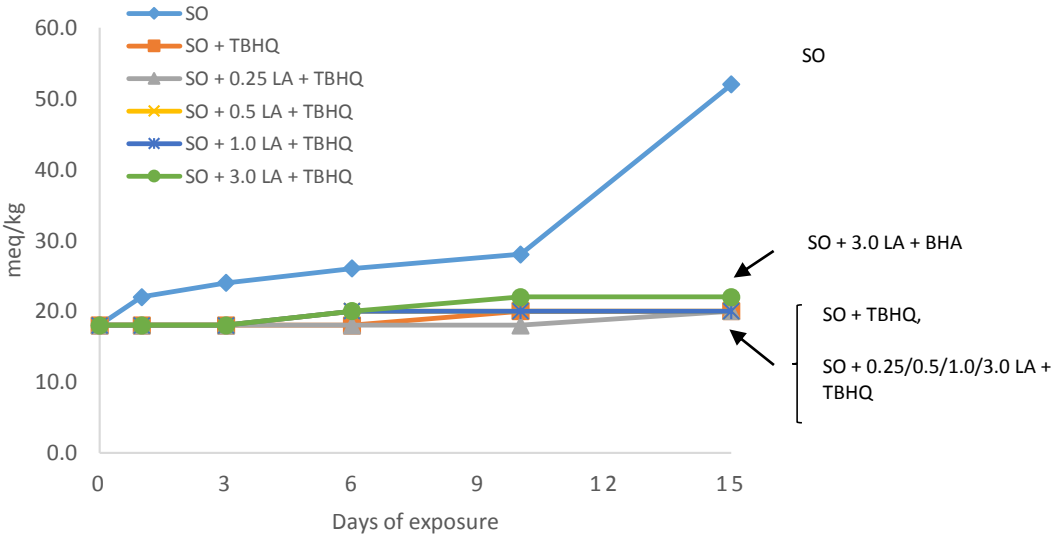

Figure 7.36: Effect of LA concentration on the PV of SO in the presence of TBHQ

Overall, fatty acids lead to the disruption of TBHQ performance on delaying $H_{ß}$ TAG hydrolyses and oxidation. LA has shown the least effect on reducing TBHQ performance for both activities. The sequence of fatty acids that affect the

TBHQ performance in delaying TAG hydrolyses from good to poor are expressed in an ordinal form as follow: SA > LA > PA > OA. While for peroxide value, the sequences are as follows: PA > LA > SA > OA.

7.4.4 Performance of PG in inhibiting Oxidation of Safflower Oil in the Presence of Selected Fatty Acids

On the TAGs Decomposition:

TAN and PV were measured for all sample mixture of SO and PG with selected fatty acid at four different concentrations. The performance of PG in hindering TAG H_{β} hydrolyses in the presence of PA, SA, OA and LA are shown in Figure 7.37 to Figure 7.40 respectively.

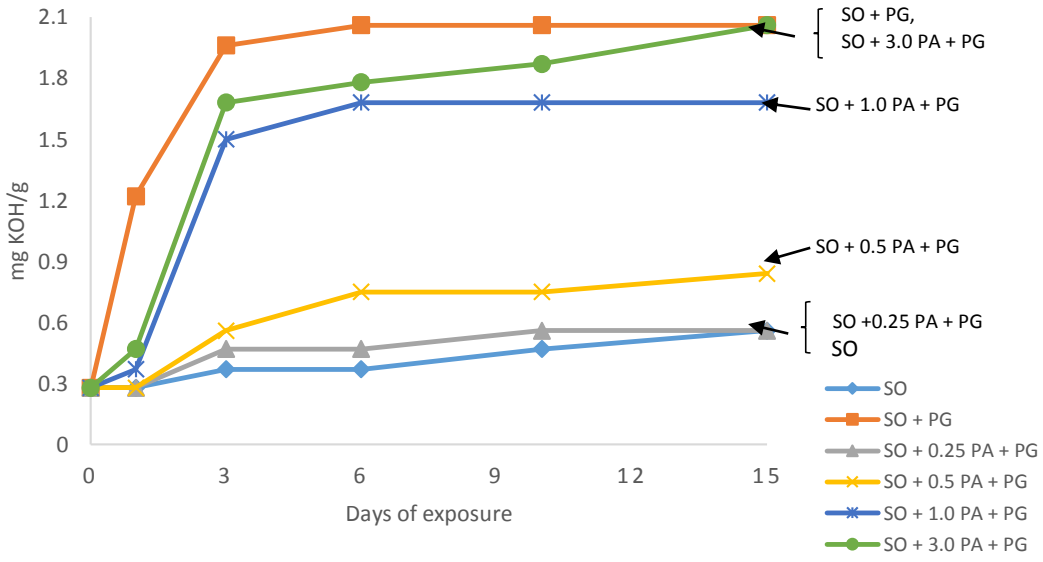

Figure 7.37: Effect of PA concentration on the TAN of SO in the presence of PG

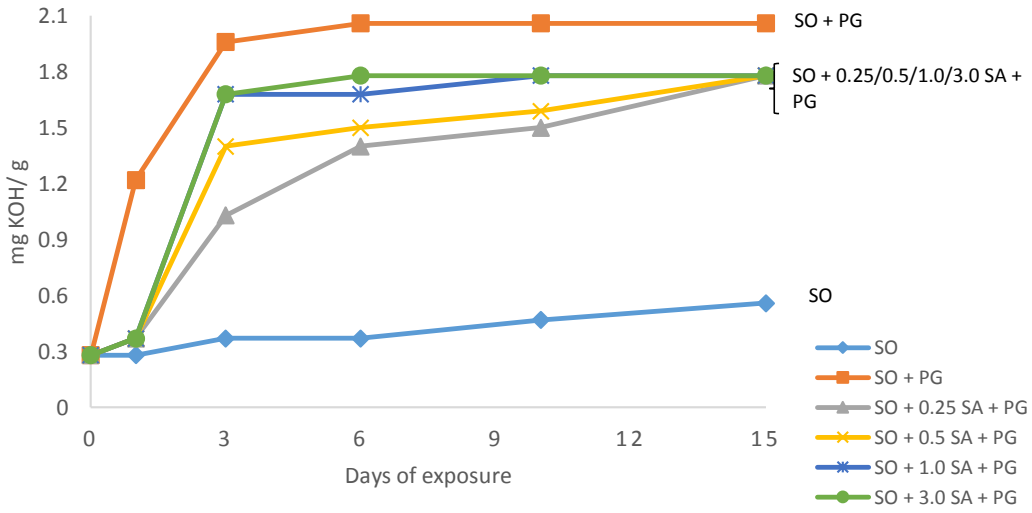

Figure 7.38: Effect of SA concentration on the TAN of SO in the presence of PG

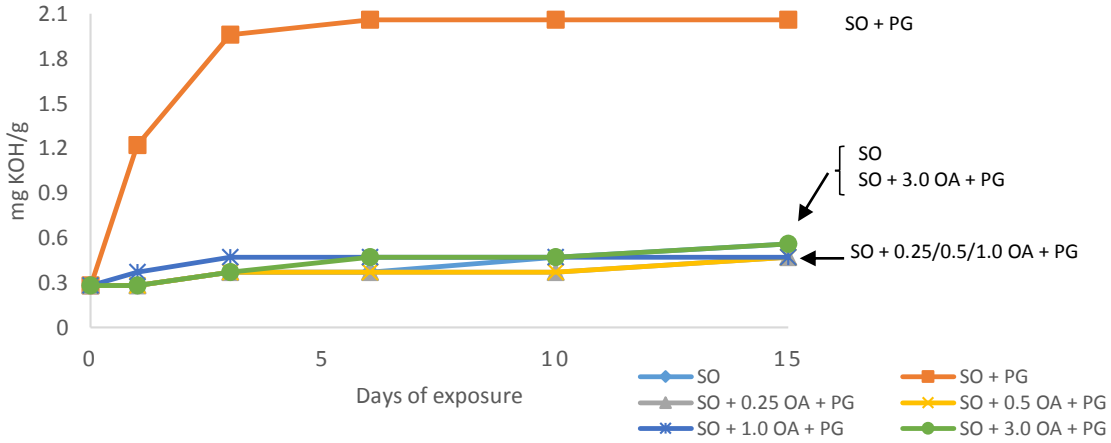

Figure 7.39: Effect of OA concentration on the TAN of SO in the presence of PG

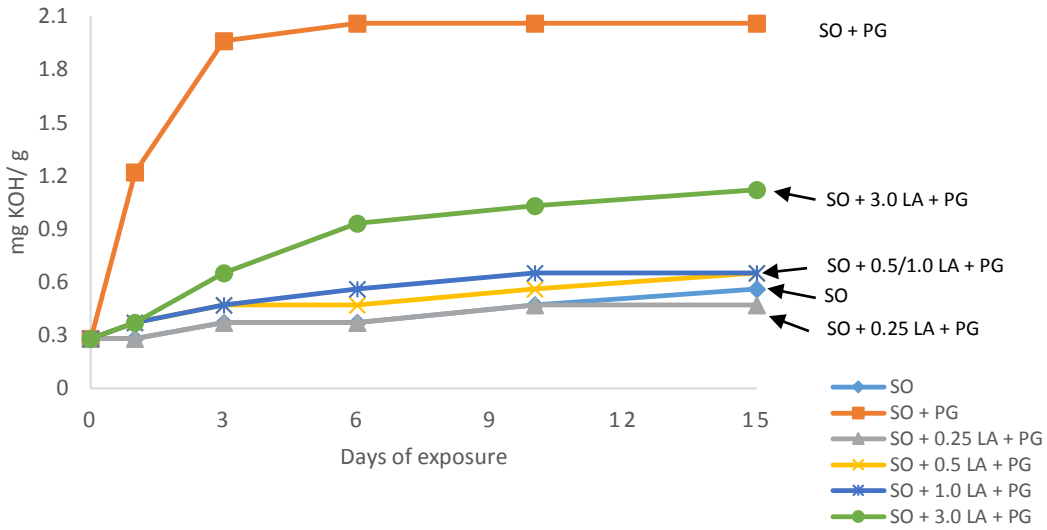

Figure 7.40: Effect of LA concentration on the TAN of SO in the presence of PG

PG has shown to improve its performance in delaying TAG hydrolyses in the presence of fatty acids. Similar trend was seen in PO and CO where the performance of PG improved when fatty acids were added to the samples. Combination of OA with PG shows the best combination where TAN development shows a very slow increment compared to other combinations. LA also proved to be a good combination. OA and LA at low concentration (0.25%) manage to reduce acid formation which were obtain from TAG hydrolyses. OA also shows good performance when added at 0.5 and 1% w/w It is also believe that at OA added, manage to prevent hydrolyses of PG. SA shows to be the worst combination even though it managed to improve PG performance.

On the Peroxide Formation:

Variation of PV's of SO samples against the days of exposure for PA, SA, OA and LA with PG are illustrated in Figure 7.41 to 7.44 respectively.

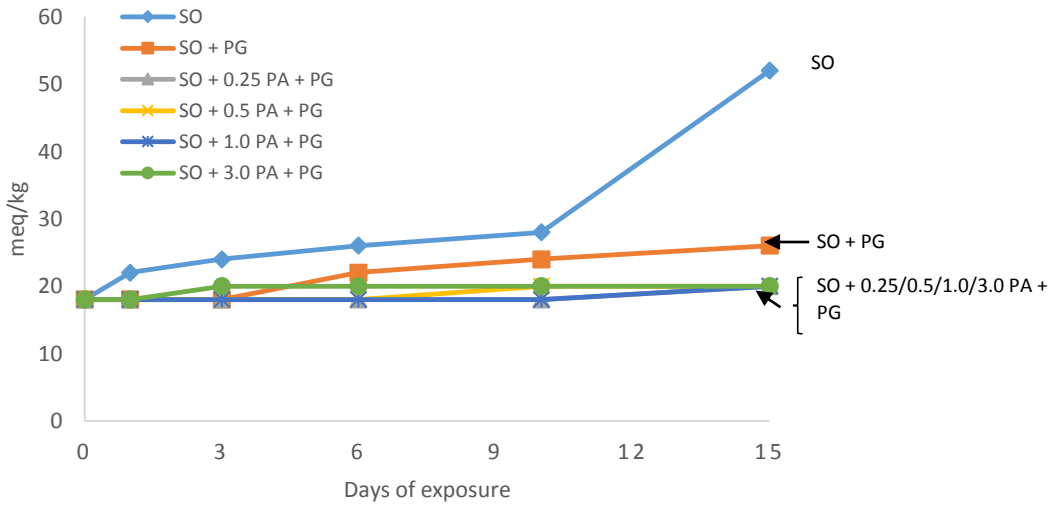

Figure 7.41: Effect of PA concentration on the PV of SO in the presence of PG

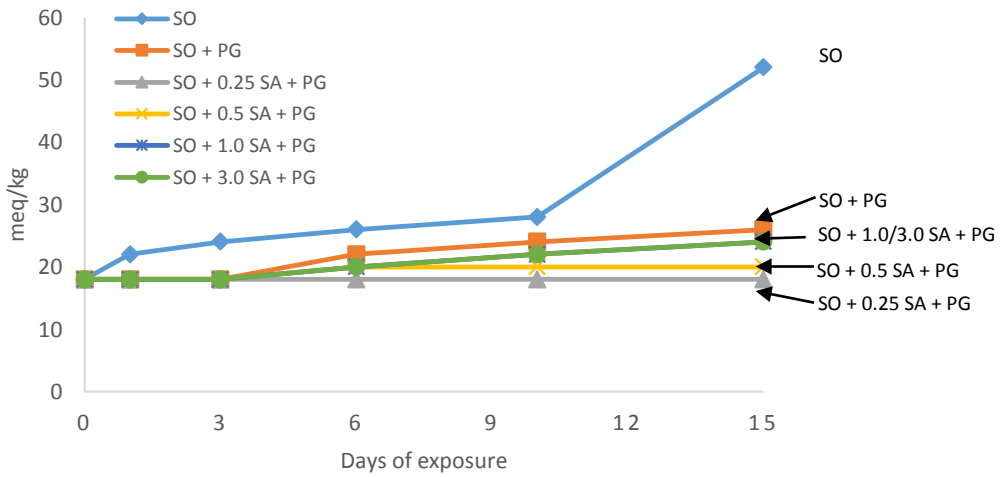

Figure 7.42: Effect of SA concentration on the PV of SO in the presence of PG

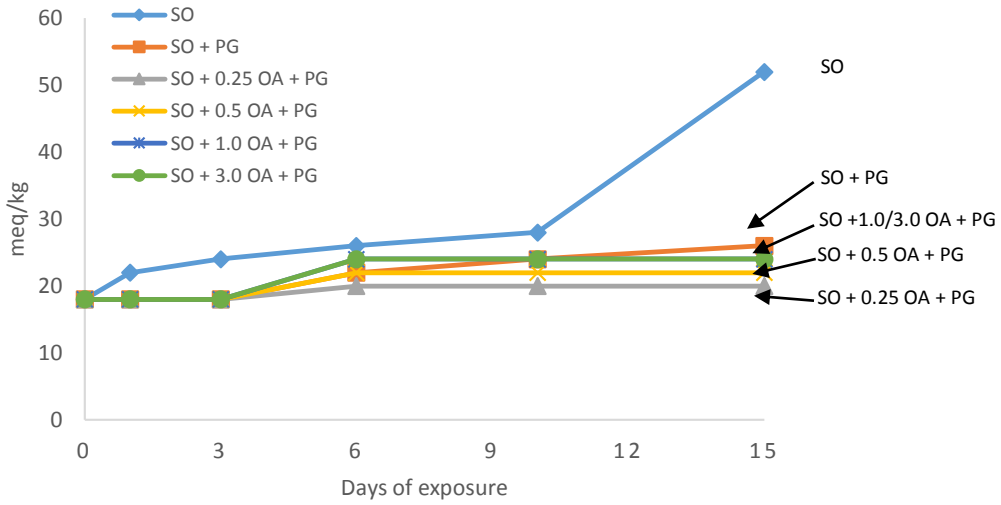

Figure 7.43: Effect of OA concentration on the PV of SO in the presence of PG

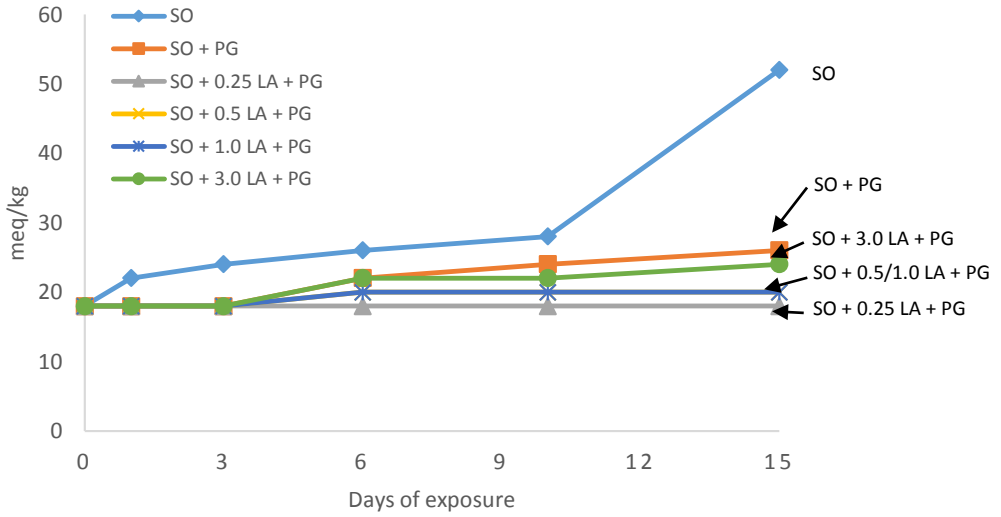

Figure 7.44: Effect of LA concentration on the PV of SO in the presence of PG

Combination of PG with any fatty acids improved SO oxidative stability. All four fatty acids manage to improve PG performance. The best fatty acid in improving PG performance is PA while OA shows the least effect in improving PG performance.

The sequence of FA that affect the PG performance in delaying TAG hydrolyses from good to poor are expressed in an ordinal form are as follows: OA > LA > PA > SA. While for peroxide value, the sequences are as follows: PA > LA > SA > OA

7.5 Summary

SO oxidized when heated at 60˚C for 15 days. The increments in PV were observed to begin on the first day of heat treatment while the increment of TAN started on the third day. The PV rose from 18 meq/kg to 52.0 meq/kg while TAN of the SO has rose from 0.28 mg KOH/g to 0.56 g KOH/g. In the presence of antioxidant, the oxidation and TAG hydrolyses of SO seems to happen slower compare to the samples without antioxidant. TBHQ, BHT and BHA are the best antioxidant in delaying peroxide formation compare to PG, while the best antioxidant in delaying SO TAG hydrolyses is TBHQ. The studies on the effect of fatty acids on SO shows that all fatty acid added promotes TAG hydrolyses when added at any concentration except SA, LA and PA at 0.25%, where it shows that the FAs mentioned managed to delay a little on TAG hydrolyses (16%). PV development shows that all fatty acids added promotes oxidation. The fatty acid have highly promoted oxidation is SA while other show similar oxidation effect. The performance of all antioxidant added in the presence of fatty acids were also studied. OA shows the least effect on BHA performance in delaying hydrolyses and oxidation while the worst fatty acids in reducing BHA performance were the

saturated fatty acids. Combination of fatty acids with BHT in delaying hydrolyses shows that the unsaturated shows the least effect on BHT performance compared to saturated. However, for oxidation development, LA has given least effect on BHT performance while OA showed the worst effect in reducing BHT performance. The presence of fatty acid on the performance of TBHQ shows that all fatty acids reduce TBHQ performance in delaying TAG hydrolyses and oxidation. LA, SA and PA gave the least effect on reducing TBHQ performance while OA shows the highest in reducing TBHQ performance. Finally, the PG performance in the presence of fatty acids shows that all fatty acids added managed to improve PG performance in delaying TAG hydrolyses and peroxide formation. The best fatty acid that improved PG performance is OA while SA shows little or no improvement of PG performance in delaying TAG hydrolyses. PA improved PG performance in delaying peroxide formation while the three other fatty acids show more or less effect on improving PG performance. Overall, studies on safflower have shown that the best antioxidant for delaying oxidation in the presence of fatty acids is BHT while the best antioxidant for delaying TAG hydrolyses is TBHQ.

CHAPTER 8

CONCLUSION AND RECOMMENDATION

8 .1 Conclusion

The research was considered a success as it met the aim and objectives of the project by evaluating the effects of FAs presence towards the performance of antioxidants in selected cooking oils. Research findings from the experiments suggest by identifying 5 factors that affect the antioxidants degree of performance with the presence of FAs which are: types of cooking oil, types of antioxidants, types of fatty acids, concentration of the fatty acids and type of oxidation consideration either TAG or peroxide formation decomposition

Evaluation performed on the effects of fatty acids on a certain antioxidant shows for all three oils, PG exhibited the best performance and the presence of any FAs gave a synergistic effects in preventing TAGs decomposition. The presence of FAs in the selected oils was shown to maximize the synergistic effects towards PG: SA for palm olein, PA for canola, OA and LA for safflower. Interesting findings which is worth further attention is that the reversal effect on the PG on its own with the presence of FAs. Evaluating the roles of FAs in all three types of oil, SA were

shown to exhibit the most evident effect in reducing/interfering with the antioxidants performance.

The presence of FAs were observed to have various effects on the performance of a certain antioxidants in preventing the formations or peroxides. Palm Olein, BHA shows to express its synergistic effect with the saturated fatty acids (PA and SA) while for safflower oil, it was observe shows that the synergistic effects of PG were seen when combined together with PA and LA. The additions of antioxidants added to canola oil were unable to prevent the formation of the peroxide group.

Thorough analysis on the direct effects on the performance, antioxidants yielded from the addition of fatty acid found that the additional unsaturated FAs (LA and OA) in palm olein and safflower oil improve the effectiveness of a certain antioxidants. However, the addition of saturated FAs (PA and SA) were shown to tremendously affect the antioxidants performance.

The selected physical parameter which is the interaction energy between species was shown to be relevant and successful in explaining almost all of the experimental phenomena. For instance, studies conducted on the effect of fatty acids on the TAG decomposition of palm olein and canola oil, both cases, the unsaturated fatty acids (OA and LA) shows to reduce the TAG decomposition due to the fact the interaction value between H_{β} and TAG trioleic and TAG Tripalmitic.

The high interaction value is able to prevent the TAG rearrangements in forming rotamer which is more vulnerable to decomposition. Furthermore, it also explained on the reasons TBHQ exhibited the best performance in preventing TAG decomposition as well as the formation of the peroxide group in all three oils; all there are believe to be linked/related to the high interaction energy value between TBHQ and H_{β} (TAG trioleic, TAG tripalmitic, TAG trilinoleic) together with TAG COO• (of TAG C_8OO• trioleic and TAG C_9OO• trilinoleic).

In conclusion, all the objectives that were outlined were successfully fulfilled and met in almost all cases, the interaction energy between species that were involves in transition state complexes were found useful in predicting antioxidants performance together with or without the presence of fatty acids.

8.2 Recommendation

Even though for this specific research interaction energy between the involving species were found useful and successful in explaining almost all the experimental phenomenon, but for a few cases, the order of performance seems to deny the theoretical findings. Such condition was expected to happen because of the TAG tri-homochains were used i.e. such as TAG of trioleic, TAG of trilinoleic and TAG of Tripalmitic. Suggestions for the upcoming researches as take into considerations in using pure/genuine TAG composition of the oils. For example,

palm olein, POO, POP and POL. However, utilization of pure components were predicted to be time consuming and suitable to be performed on a highly specific condition highly focusing on one type of oil.

Theoretical research such as this, the utilization of the best theoretical approach that is DFT, the basis set utilize could still be improved by including the diffuse function system since the system in question contains free radicals of $C_8OO•$ and $C_9OO•$. The inclusion of these functions will give more on extra orbitals for the unpaired electrons to freely move in the system understudied.

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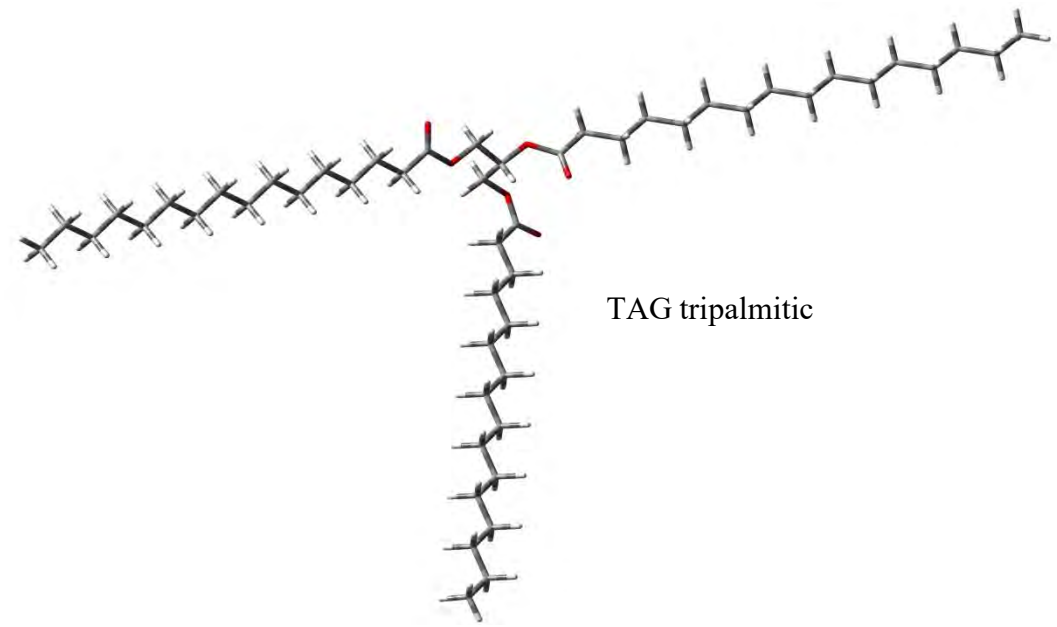
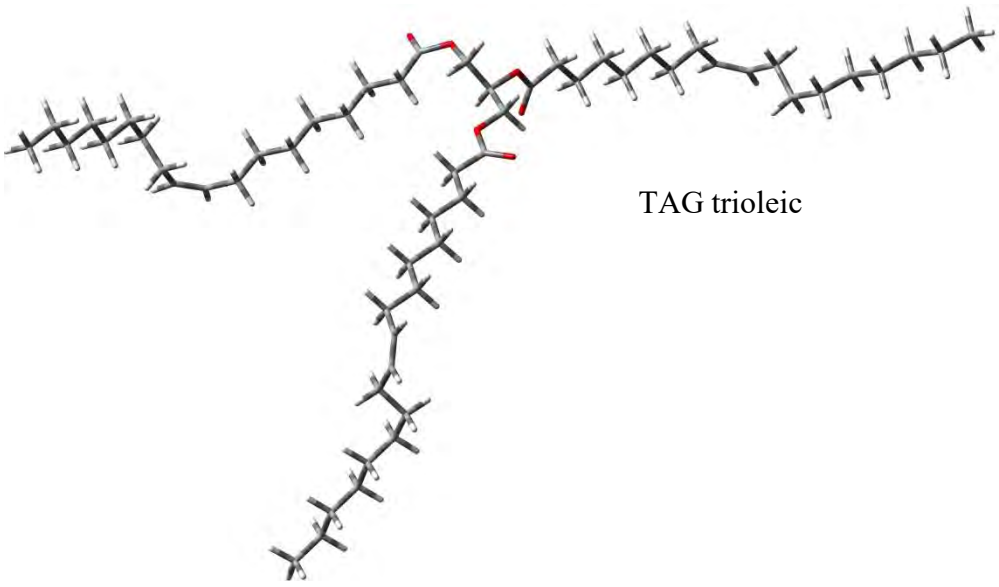
APPENDIX 1

Molecular structure for *ab initio* quantum mechanical studies

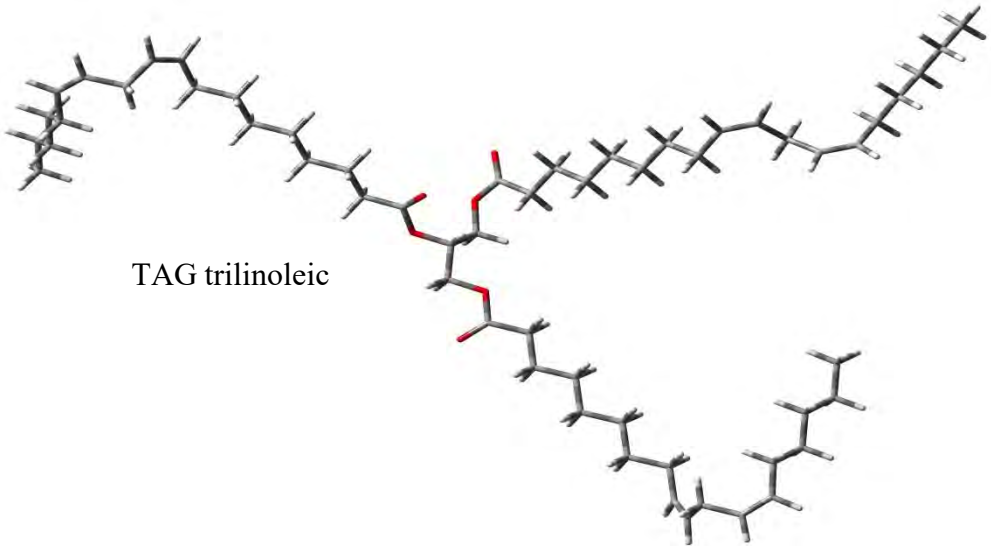


a) Molecule which represent the TAG system in palm olein

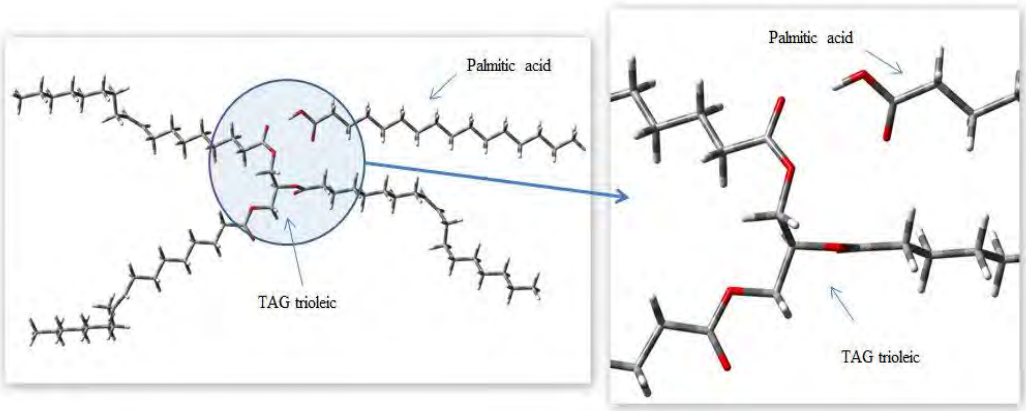


b) Molecule which represent the TAG system in canola oil

Appendix 1 (continue)

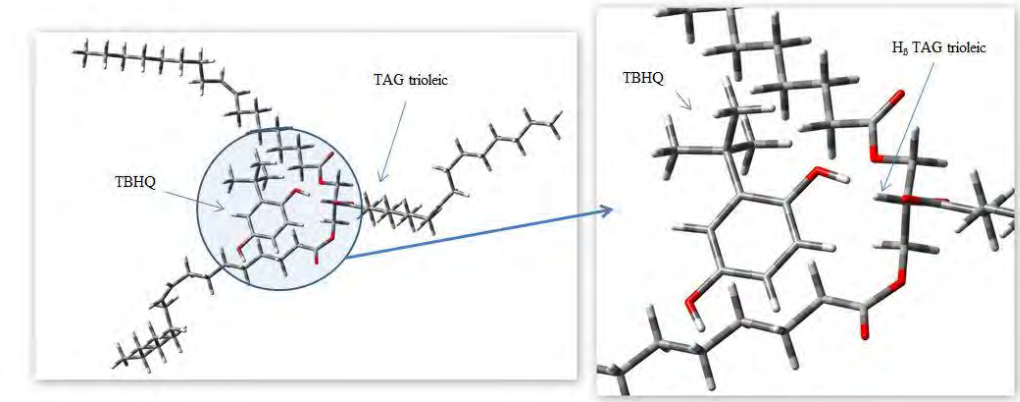


c) Molecule which represent the TAG system in safflower oil, TAG trilinoleic

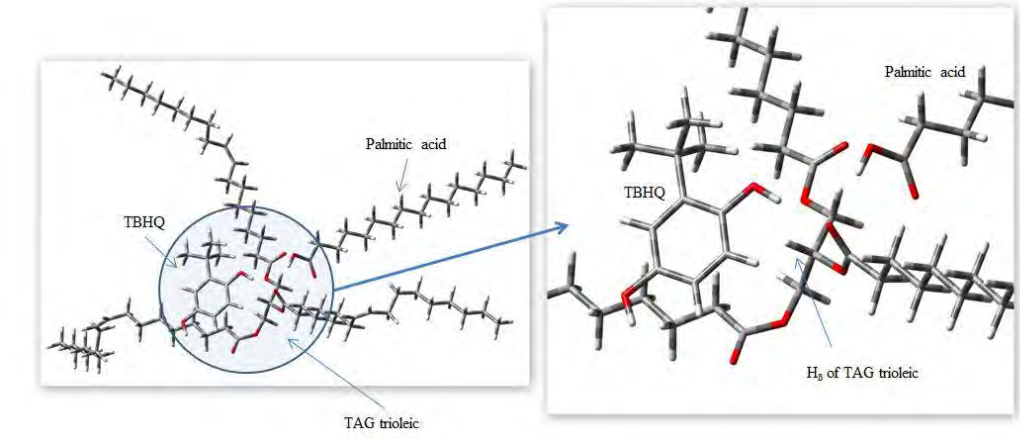


d) Interaction between H_{β} TAG trioleic with palmitic acid

Appendix 1 (continue)

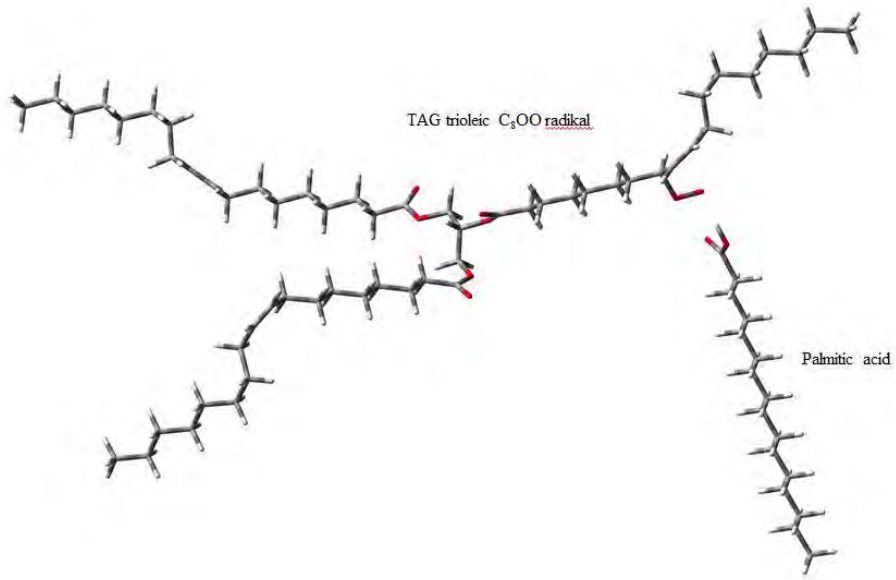


e) Interaction between Hß TAG trioleic with TBHQ

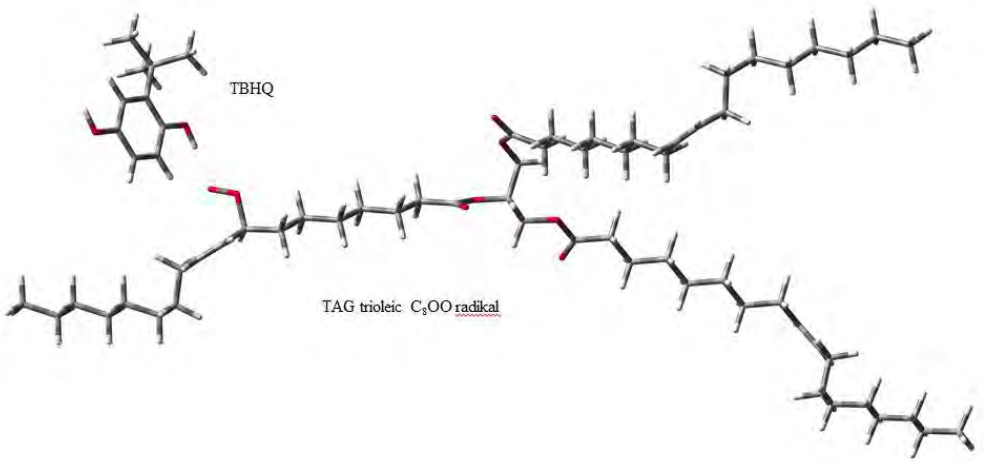


f) Interaction between Hß of TAG trioleic with TBHQ and palmitic acid

Appendix 1 (continue)

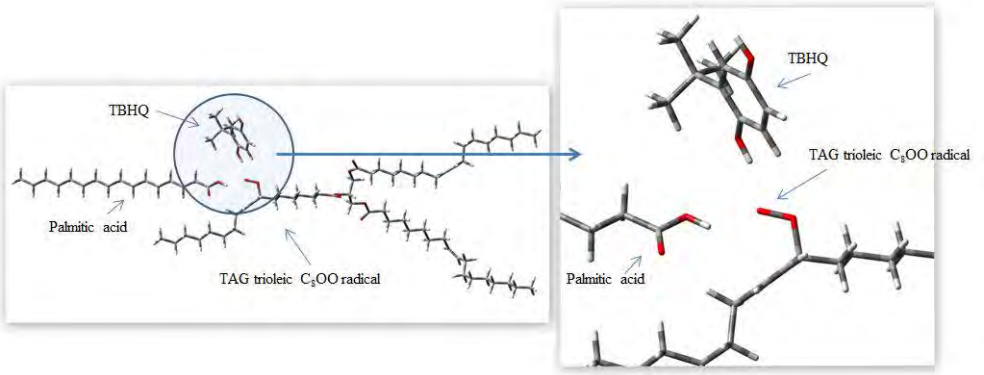


g) Interaction between TAG trioleic C_8 OO radical with palmitic acid



h) Interaction between TAG trioleic C_8 OO radical with TBHQ

Appendix 1 (continue)



i) Interaction between TAG trioleic C_8 OO radical with TBHQ and palmitic acid

APPENDIX 2

Summary of PES Scan of the hydrogenic system

Complex Systems	Original dihedral angle, DFT/6-31G (d,p)	Dihedral angle using PES scan	Comments
Tripalmitic + PA	66.40	70.00	Dihedral angle is in the range of the initial
Tripalmitic + SA	65.82	70.00	Dihedral angle is in the range of the initial
Tripalmitic + OA	65.24	70.00	Dihedral angle is in the range of the initial
Tripalmitic + LA	59.87	60.00	Dihedral angle is in the range of the initial
Trioleic + PA	153.23 (-)	210.00	Dihedral angle is in the range of the initial
Trioleic + SA	156.65 (-)	200.00	Dihedral angle is in the range of the initial
Trioleic + OA	55.46	50.00	Dihedral angle is in the range of the initial
Trioleic + LA	73.80	70.00	Dihedral angle is in the range of the initial
Trilinoleic + PA	63.75	70.00	Dihedral angle is in the range of the initial
Trilinoleic + SA	57.40	60.00	Dihedral angle is in the range of the initial
Trilinoleic + OA	60.85	60.00	Dihedral angle is in the range of the initial
Trilinoleic + LA	61.85	60.00	Dihedral angle is in the range of the initial
Tripalmitic + BHA	15.69	20.00	Dihedral angle is in the range of the initial
Tripalmitic + BHT	19.87 (-)	340.00	Dihedral angle is in the range of the initial
Tripalmitic + TBHQ	14.45	20.00	Dihedral angle is in the range of the initial

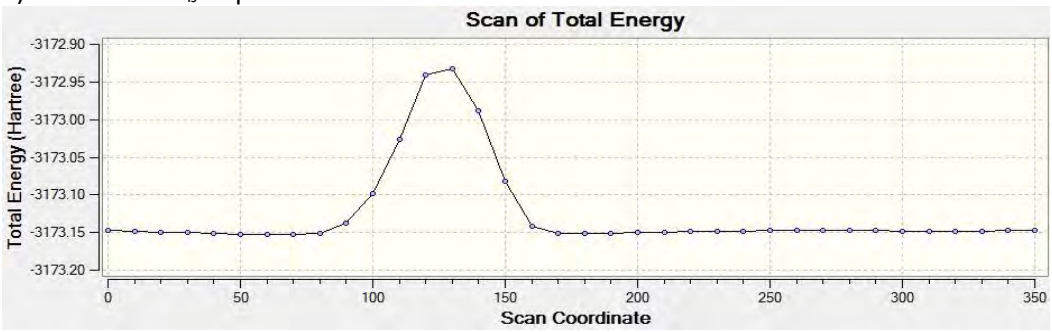
Tripalmitic + PG	39.04	46.00	Dihedral angle is in the range of the initial
Trioleic + BHA	12.55	20.00	Dihedral angle is in the range of the initial
Trioleic + BHT	54.95 (-)	310.00	Dihedral angle is in the range of the initial
Trioleic + TBHQ	9.68	20.00	Dihedral angle is in the range of the initial
Trioleic + PG	37.26	41.00	Dihedral angle is in the range of the initial
Trilinoleic + BHA	13.90	20.00	Dihedral angle is in the range of the initial
Trilinoleic + BHT	35.22 (-)	330.00	Dihedral angle is in the range of the initial
Trilinoleic + TBHQ	11.85	20.00	Dihedral angle is in the range of the initial
Trilinoleic + PG	36.55	41.00	Dihedral angle is in the range of the initial
TAG Trioleic C_8 OO radical + PA	87.29	260.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trioleic C_8 OO radical + SA	112.07	240.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trioleic C_8 OO radical + OA	95.24	250.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trioleic C_8 OO radical + LA	82.79	90.00	Dihedral angle is in the range of the initial
TAG Trioleic C_8 OO radical + BHA	0.02	0.00	Dihedral angle is in the range of the initial
TAG Trioleic C_8 OO radical + BHT	57.11 (-)	350.00	Dihedral angle is in the range of the initial
TAG Trioleic C_8 OO radical + TBHQ	1.32	0.00	Dihedral angle is in the range of the initial
TAG Trioleic C_8 OO radical + PG	1.13	0.00	Dihedral angle is in the range of the initial
TAG Trilinoleic C_9 OO radical + PA	167.71	170.00	Dihedral angle is in the range of the initial

TAG Trilinoleic C_9 OO radical + SA	48.46 (-)	80.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 OO radical + OA	37.13 (-)	80.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 OO radical + LA	57.71 (-)	80.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 OO radical + BHA	0.69	0.00	Dihedral angle is in the range of the initial
TAG Trilinoleic C_9 OO radical + BHT	92.98	0.00	Dihedral angle is in the range of the initial
TAG Trilinoleic C_9 OO radical + TBHQ	0.84	0.00	Dihedral angle is in the range of the initial
TAG Trilinoleic C_9 OO radical + PG	0.00	0.00	Dihedral angle is in the range of the initial
TAG Trioleic C_8 $OO^{\cdot}$ + TBHQ + PA	179.461 (-)	220.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trioleic C_8 $OO^{\cdot}$ + TBHQ + SA	178.569 (-)	0.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trioleic C_8 $OO^{\cdot}$ + TBHQ + OA	174.926	220.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trioleic C_8 $OO^{\cdot}$ + TBHQ + LA	176.813	220.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 $OO^{\cdot}$ + TBHQ + PA	61.87	130.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 $OO^{\cdot}$ + TBHQ + SA	79.54	150.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 $OO^{\cdot}$ + TBHQ + OA	85.91	150.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.
TAG Trilinoleic C_9 $OO^{\cdot}$ + TBHQ + LA	67.96	178.00	Reoptimazation at DFT/6-31G (d,p) produced the same results as the initial.

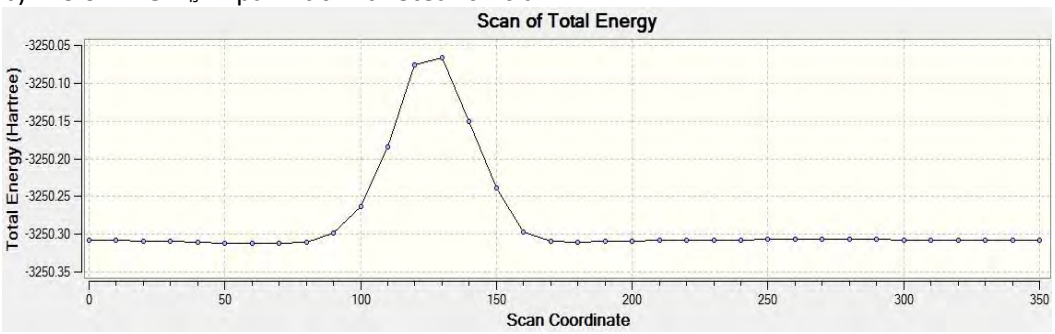
APPENDIX 3

Potential energy surface (PES) scan of TAG $H_ß$ tripalmitic and fatty acids

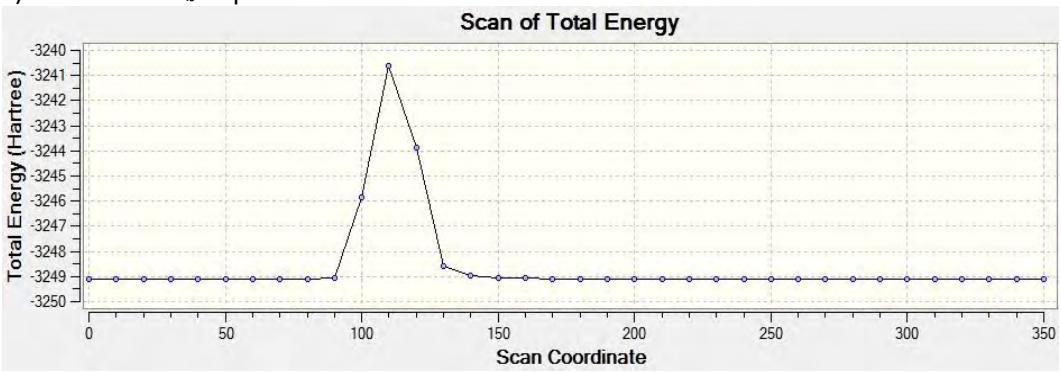
a) PES of TAG $H_ß$ Tripalmitic with Palmitic Acid



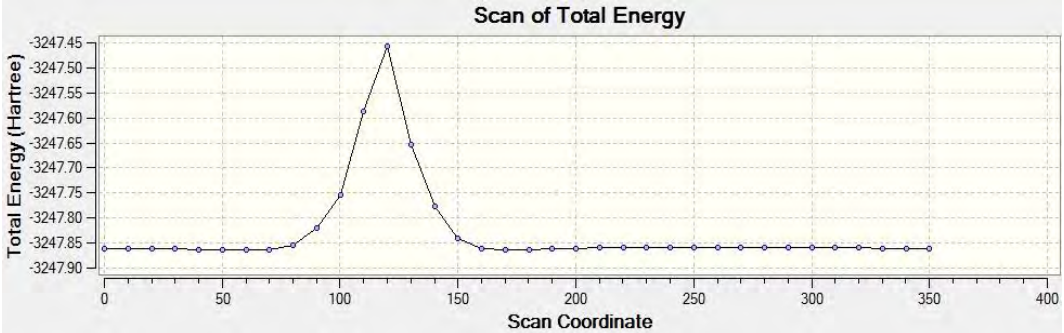
b) PES of TAG $H_ß$ Tripalmitic with Stearic Acid



c) PES of TAG $H_ß$ Tripalmitic with Oleic Acid



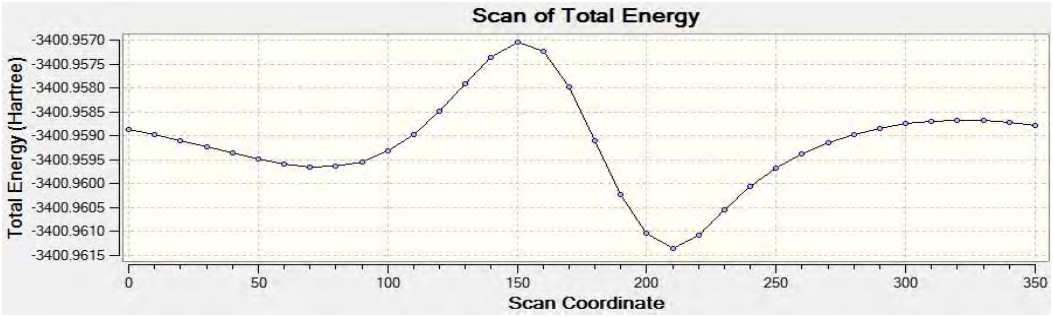
d) PES of TAG $H_ß$ Tripalmitic with Linoleic Acid



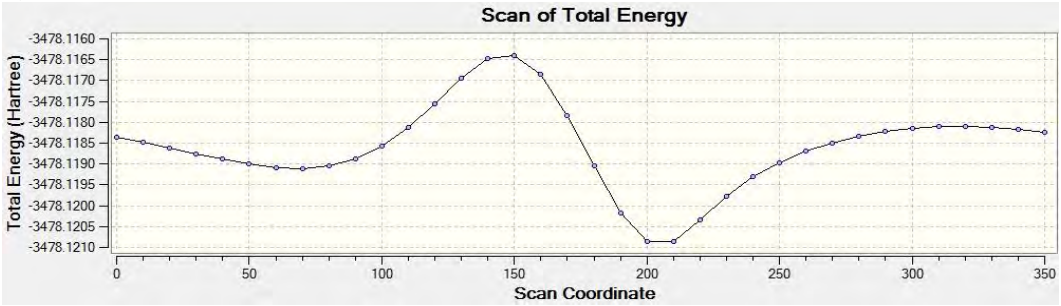
APPENDIX 4

Potential energy surface (PES) scan of TAG Hß trioleic and fatty acids

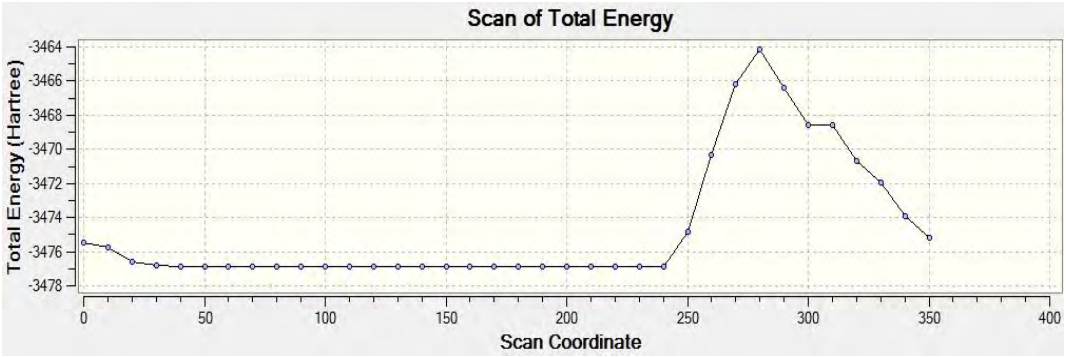
a) PES of TAG Hß Trioleic with Palmitic Acid



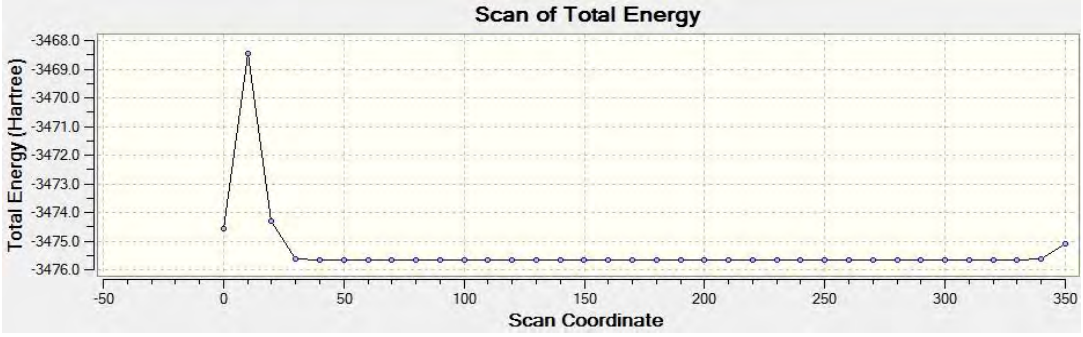
b) PES of TAG Hß Trioleic with Stearic Acid



c) PES of TAG Hß Trioleic with Oleic Acid



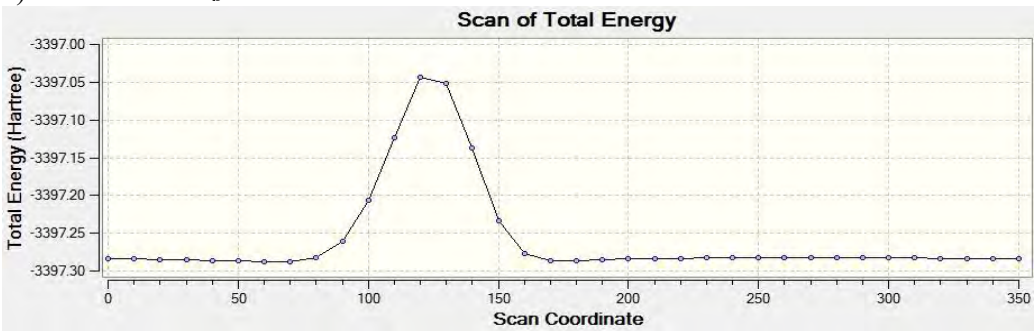
d) PES of TAG Hß Trioleic with Linoleic Acid



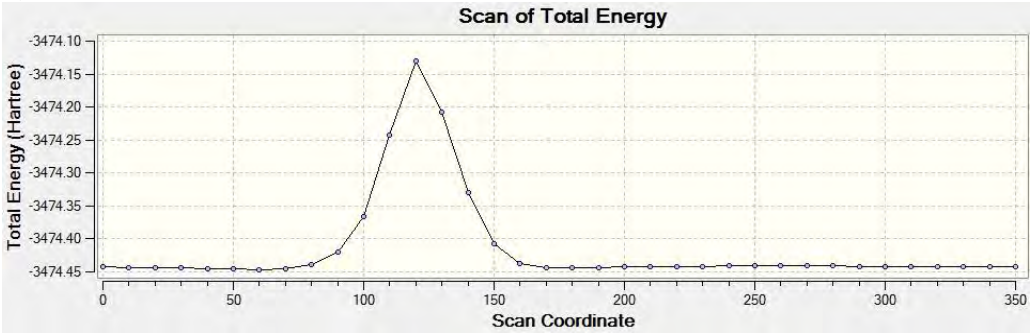
APPENDIX 5

Potential energy surface (PES) scan of TAG Hß trilinoleic and fatty acids

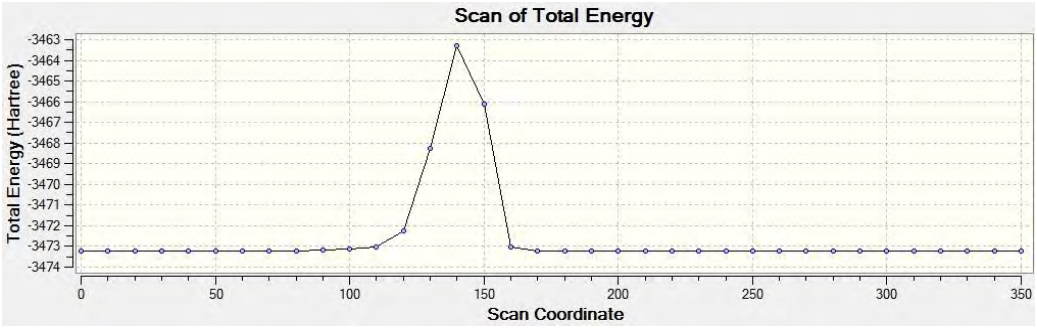
a) PES of TAG Hß Trilinoleic with Palmitic Acid



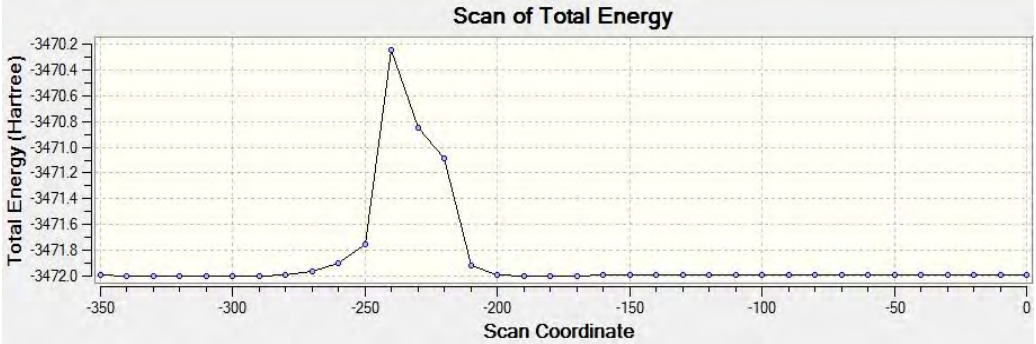
b) PES of TAG Hß Trilinoleic with Stearic Acid



c) PES of TAG Hß Trilinoleic with Oleic Acid



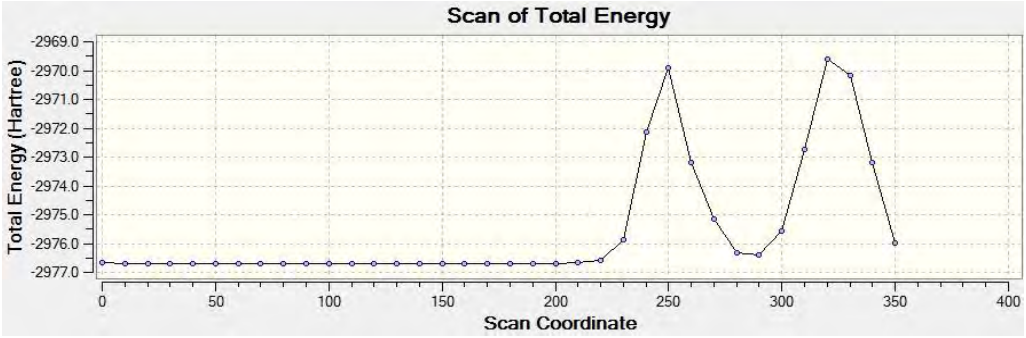
d) PES of TAG Hß Trilinoleic with Linoleic Acid



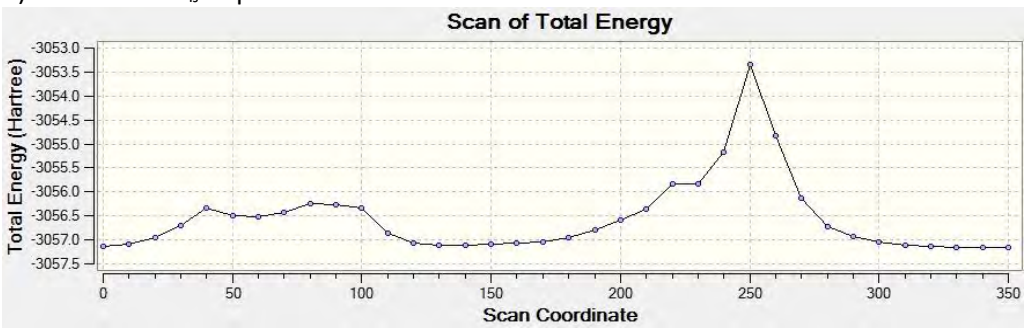
APPENDIX 6

Potential energy surface (PES) scan of TAG Hß tripalmitic and antioxidants

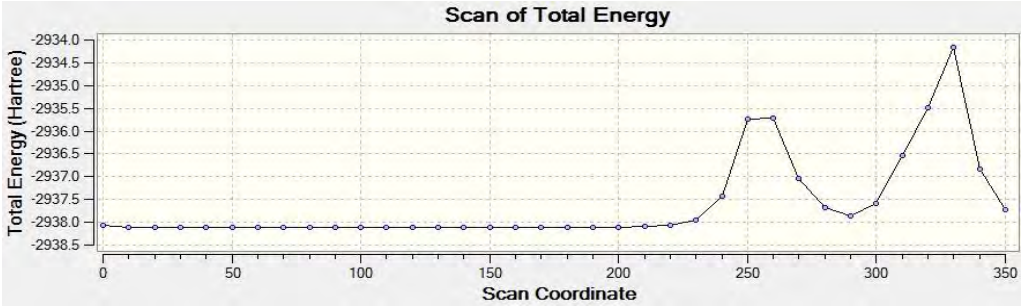
a) PES of TAG Hß tripalmitic with BHA



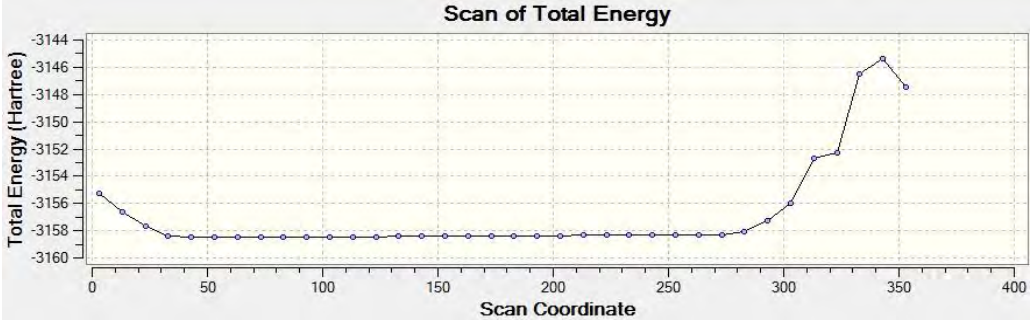
b) PES of TAG Hß Tripalmitic with BHT



c) PES of TAG Hß Tripalmitic with TBHQ



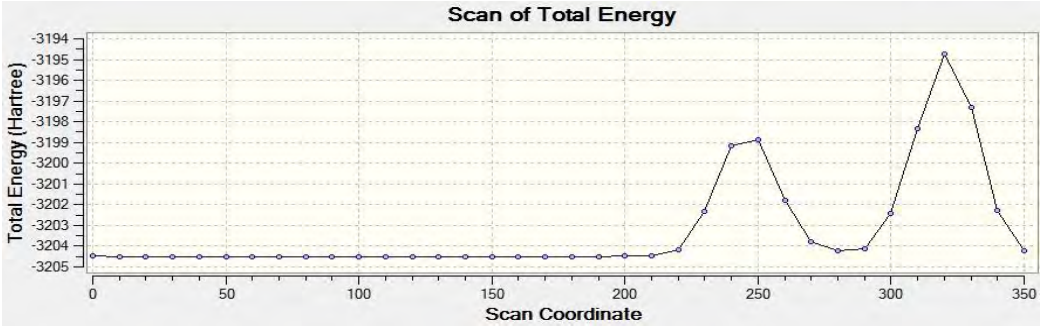
d) PES of TAG Hß Tripalmitic with PG



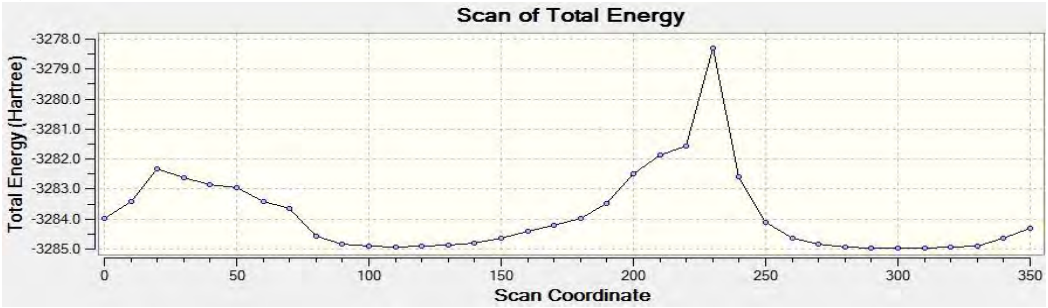
APPENDIX 7

Potential energy surface (PES) scan of TAG Hß trioleic and antioxidants

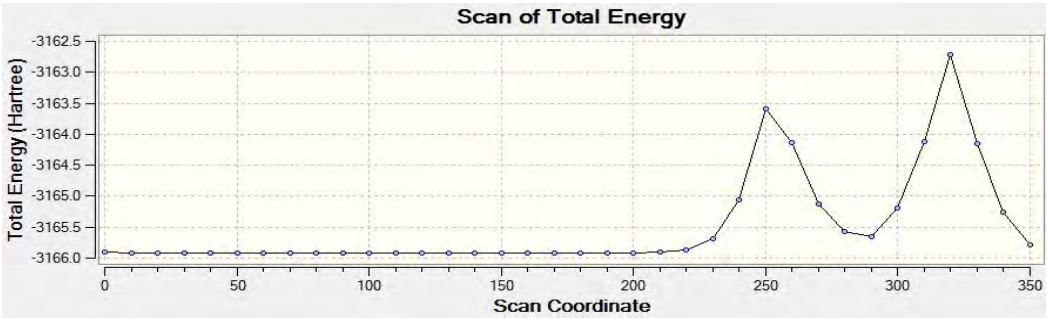
a) PES of TAG Hß Trioleic with BHA



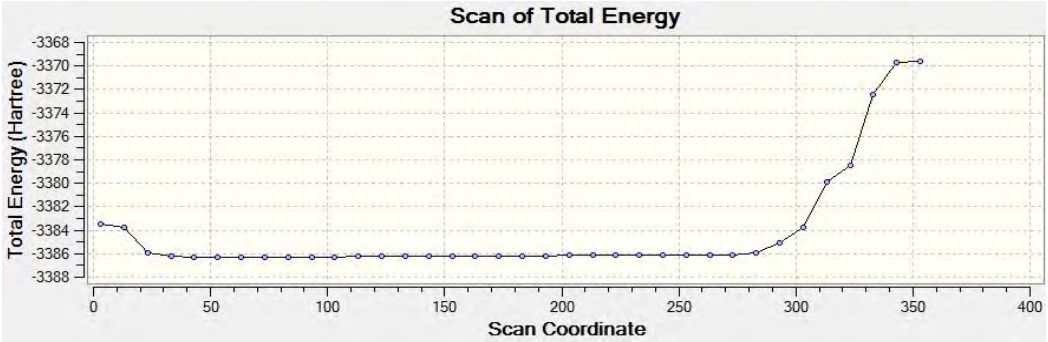
b) PES of TAG Hß Trioleic with BHT



c) PES of TAG Hß Trioleic with TBHQ



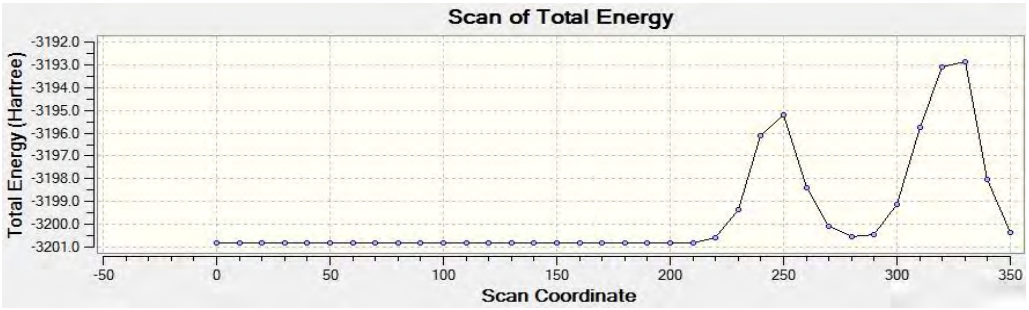
d) PES of TAG Hß Trioleic with PG



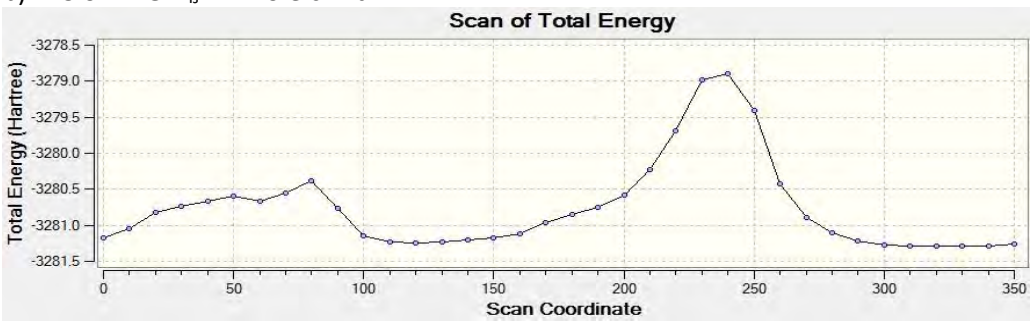
APPENDIX 8

Potential energy surface (PES) scan of TAG Hß trilinoleic and antioxidants

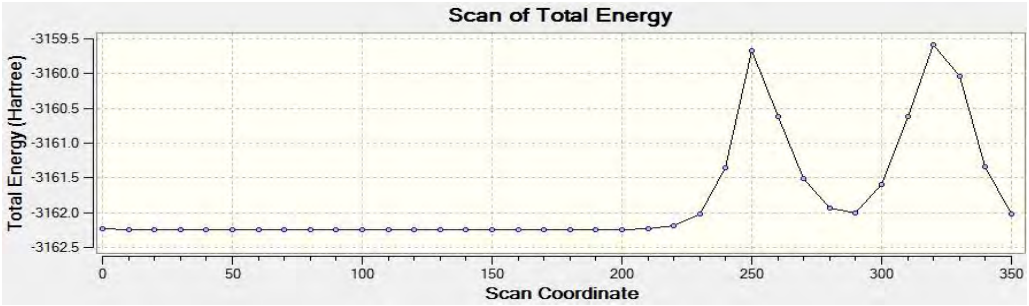
a) PES of TAG Hß Trilinoleic with BHA



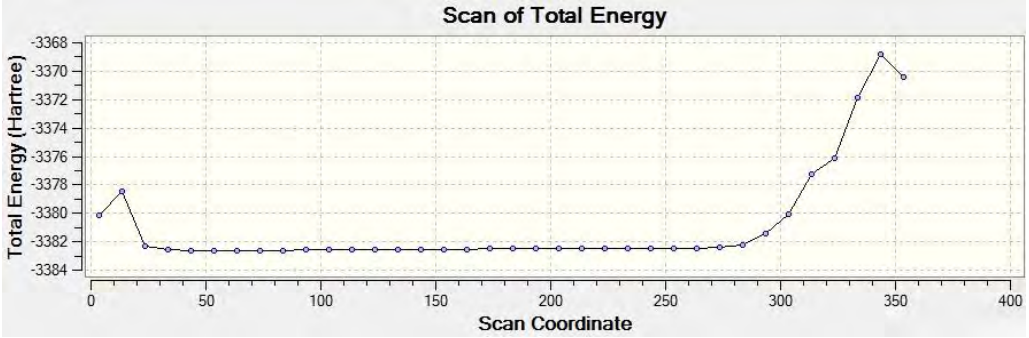
b) PES of TAG Hß Trilinoleic with BHT



c) PES of TAG Hß Trilinoleic with TBHQ



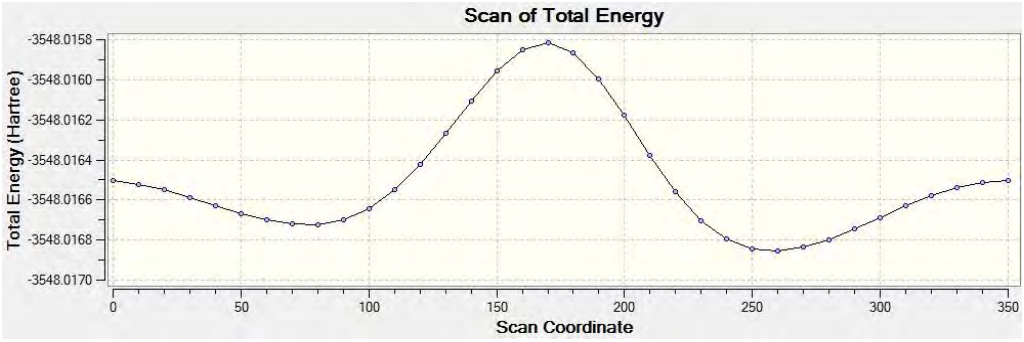
d) PES of TAG Hß Trilinoleic with PG



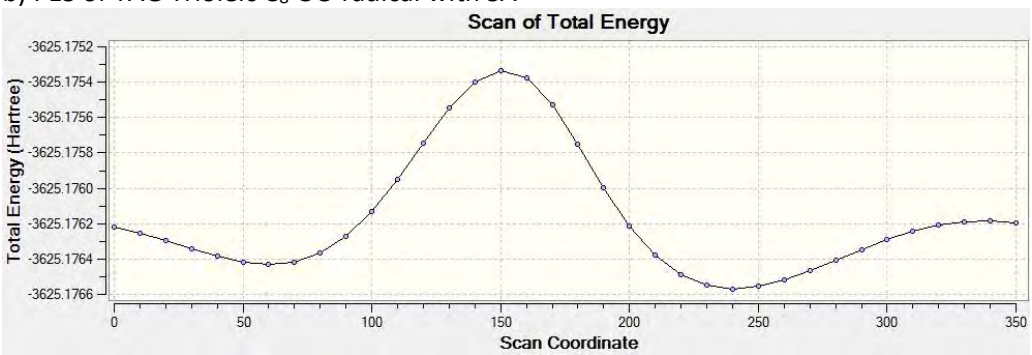
APPENDIX 9

Potential energy surface (PES) scan of TAG trioleic C_8 OO radical and fatty acids

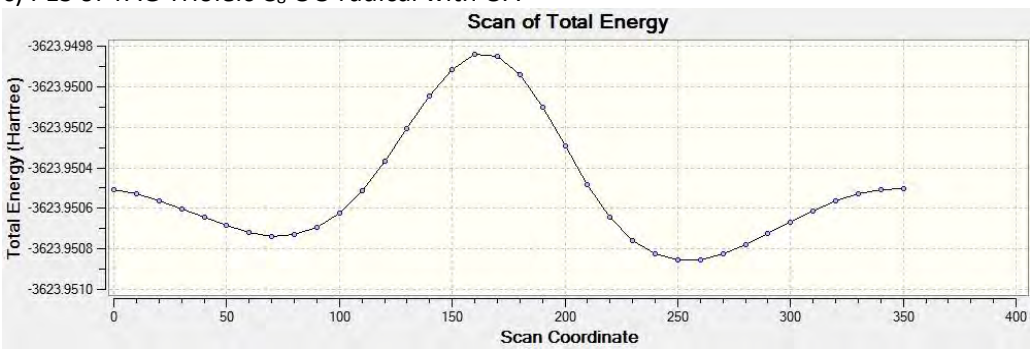
a) PES of TAG Trioleic C_8 OO radical with PA



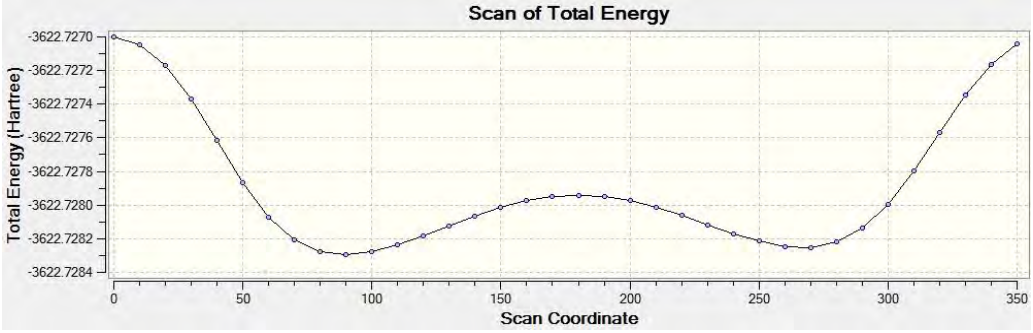
b) PES of TAG Trioleic C_8 OO radical with SA



c) PES of TAG Trioleic C_8 OO radical with OA



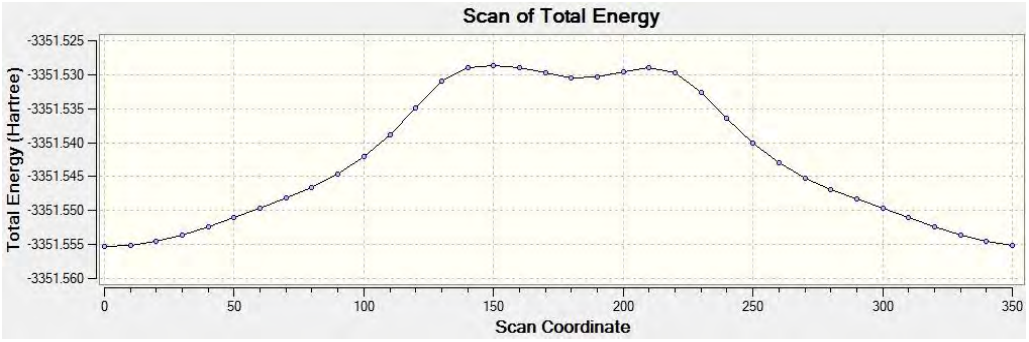
d) PES of TAG Trioleic C_8 OO radical with LA



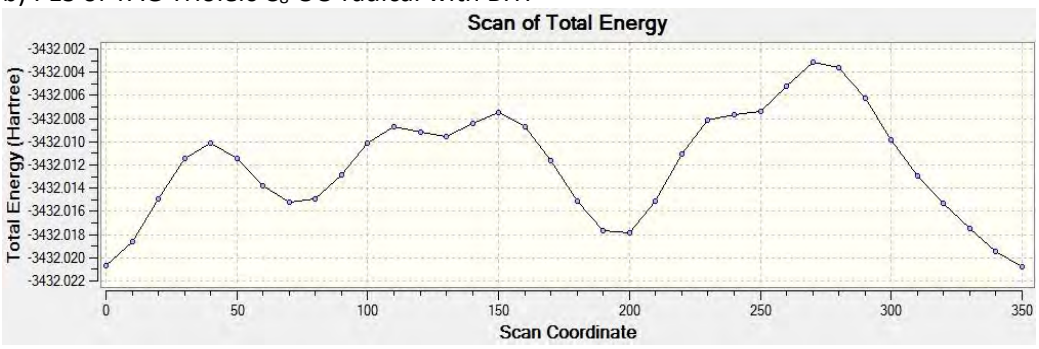
APPENDIX 10

Potential energy surface (PES) scan of TAG trioleic C_8 OO radical and antioxidants

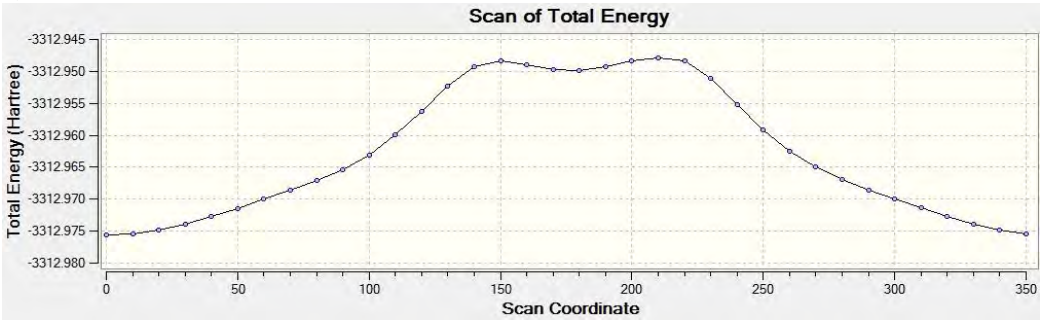
a) PES of TAG Trioleic C_8 OO radical with BHA



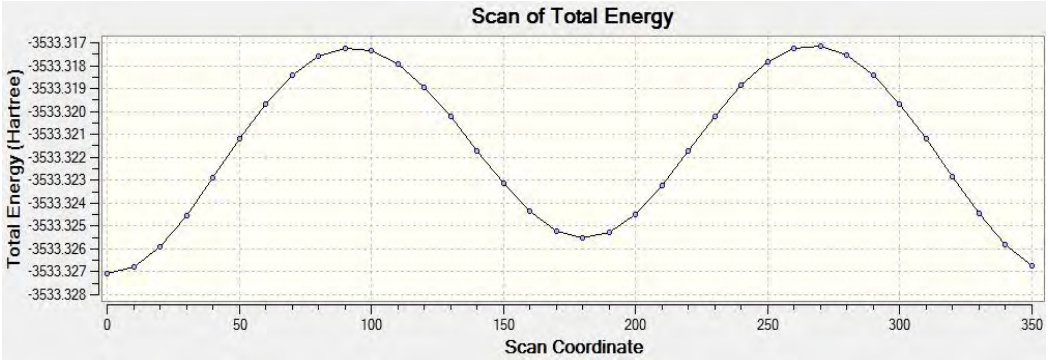
b) PES of TAG Trioleic C_8 OO radical with BHT



c) PES of TAG Trioleic C_8 OO radical with TBHQ



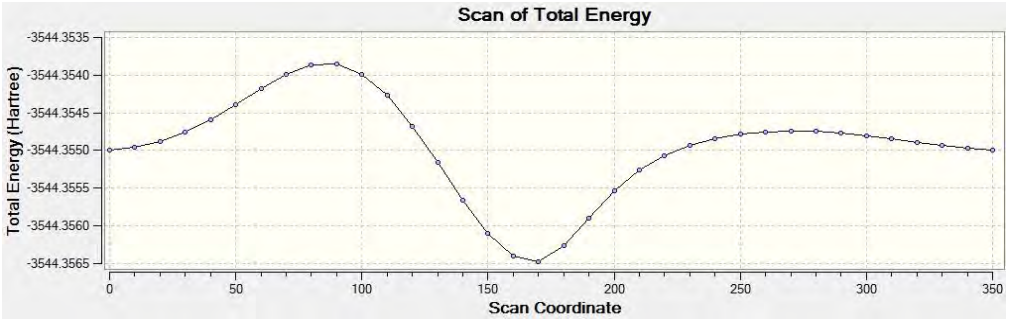
d) PES of TAG Trioleic C_8 OO radical with PG



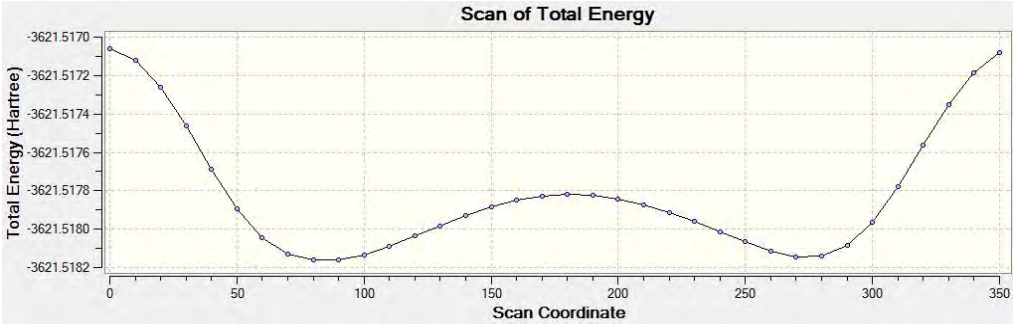
APPENDIX 11

Potential energy surface (PES) scan of TAG trioleic C_9 OO radical and fatty acids

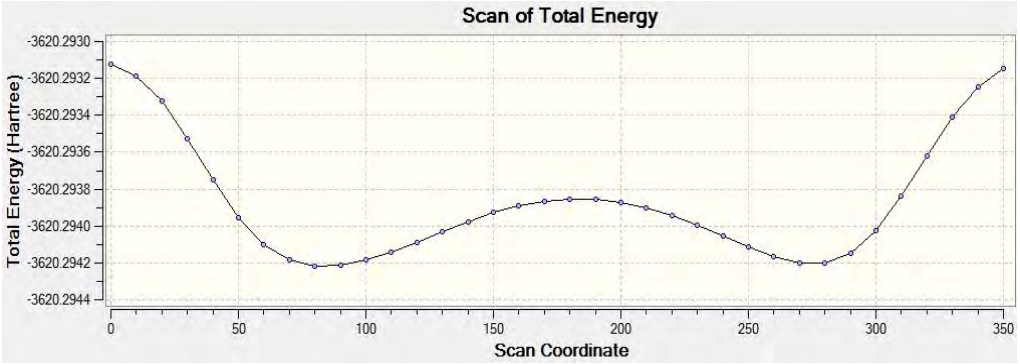
a) PES of TAG Trilinoleic C_9 OO radical with PA



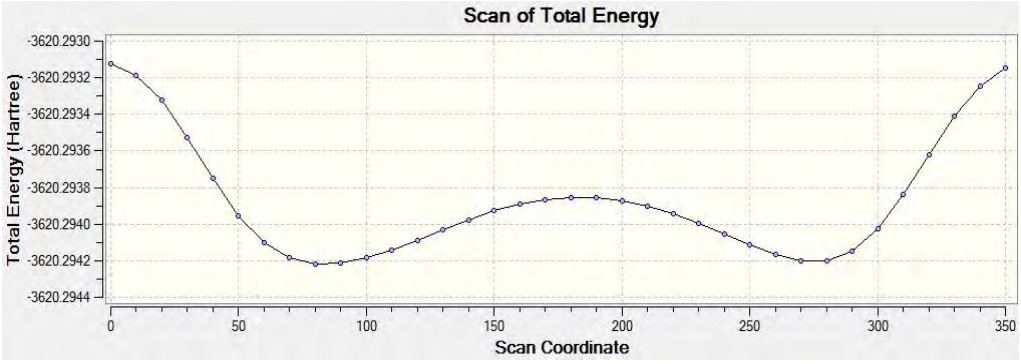
b) PES of TAG Trilinoleic C_9 OO radical with SA



c) PES of TAG Trilinoleic C_9 OO radical with OA



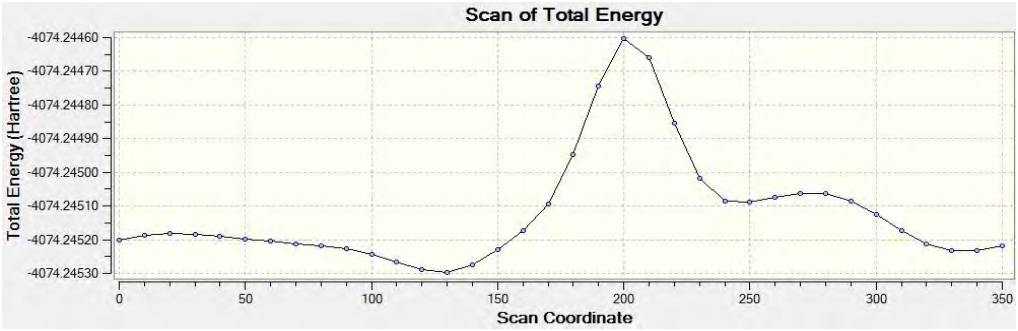
d) PES of TAG Trilinoleic C_9 OO radical with LA



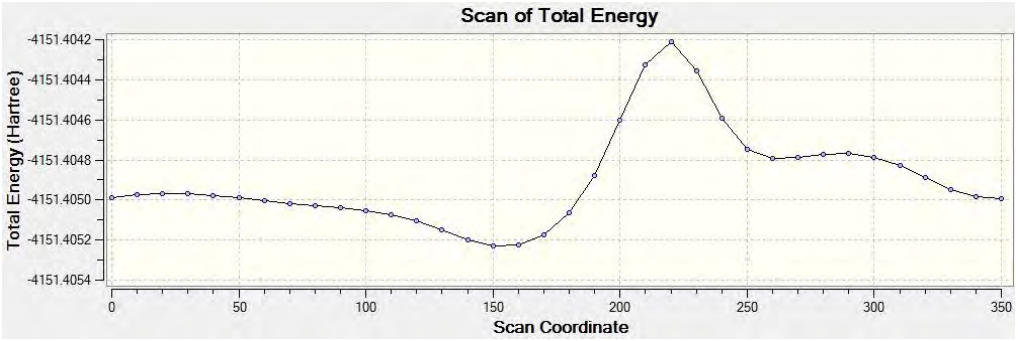
APPENDIX 12

Potential energy surface (PES) scan of TAG trioleic C_9 OO radical and fatty acids

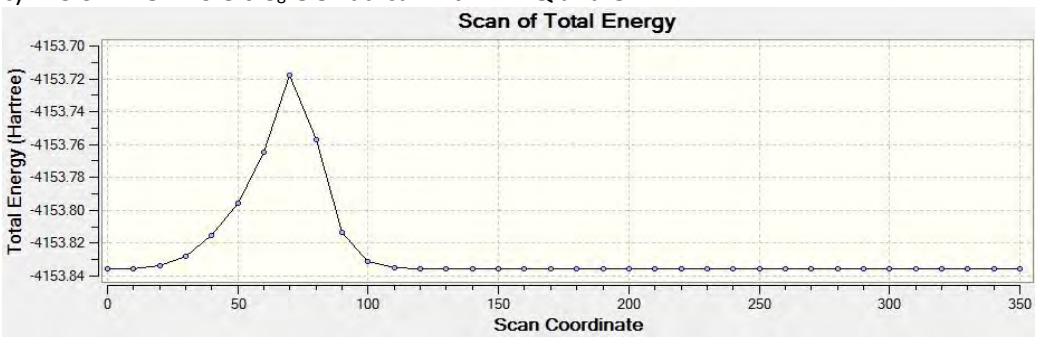
a) PES of TAG Trioleic C_8 OO radical with TBHQ and PA



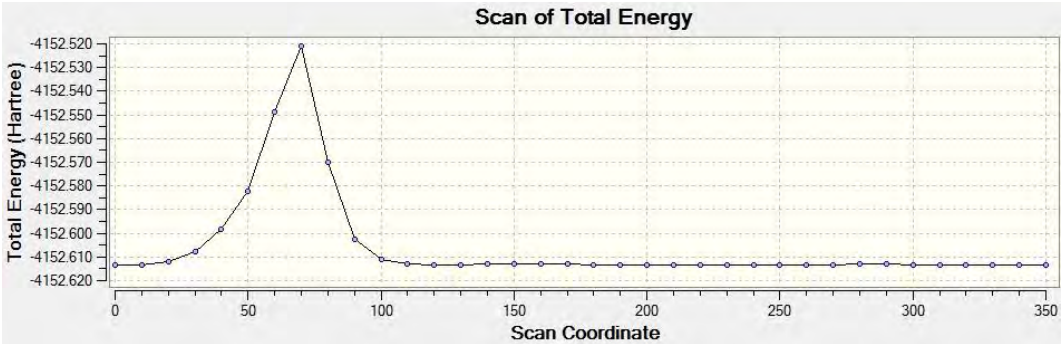
b) PES of TAG Trioleic C_8 OO radical with TBHQ and SA



c) PES of TAG Trioleic C_8 OO radical with TBHQ and OA



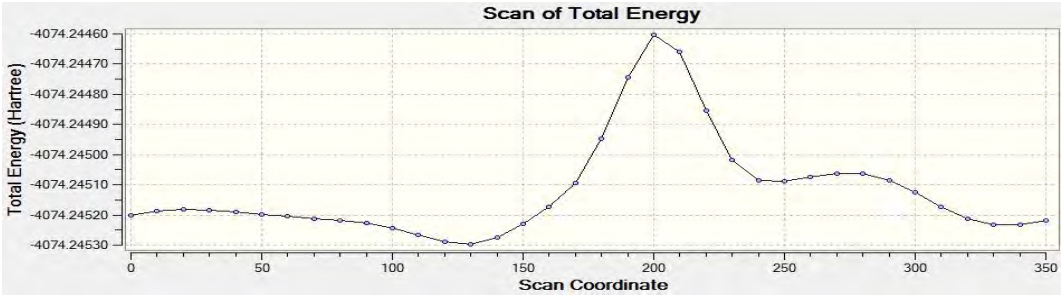
d) PES of TAG Trioleic C_8 OO radical with TBHQ and LA



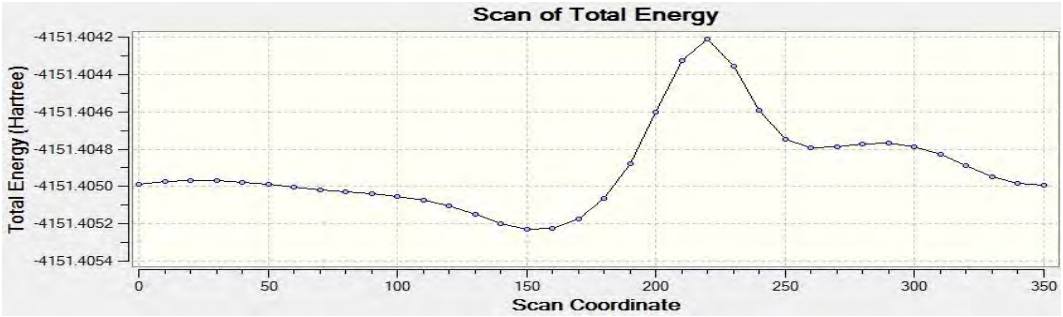
APPENDIX 13

Potential energy surface (PES) scan of TAG trioleic C_9 OO radical with TBHQ and fatty acids

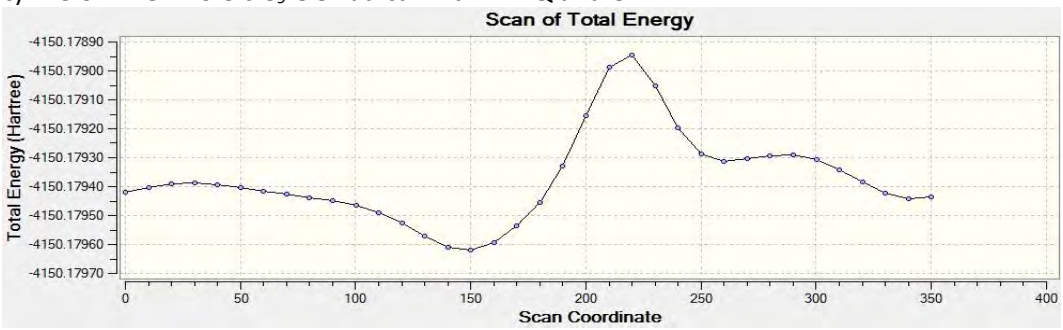
a) PES of TAG Trioleic C_9 OO radical with TBHQ and PA



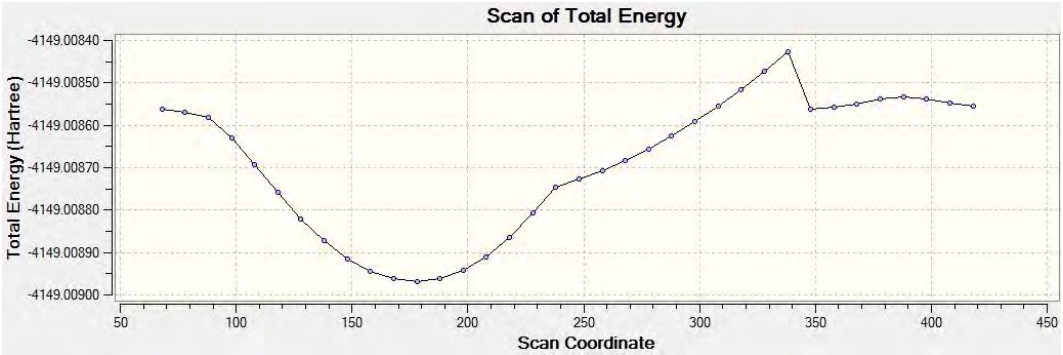
b) PES of TAG Trioleic C_9 OO radical with TBHQ and SA



c) PES of TAG Trioleic C_9 OO radical with TBHQ and OA



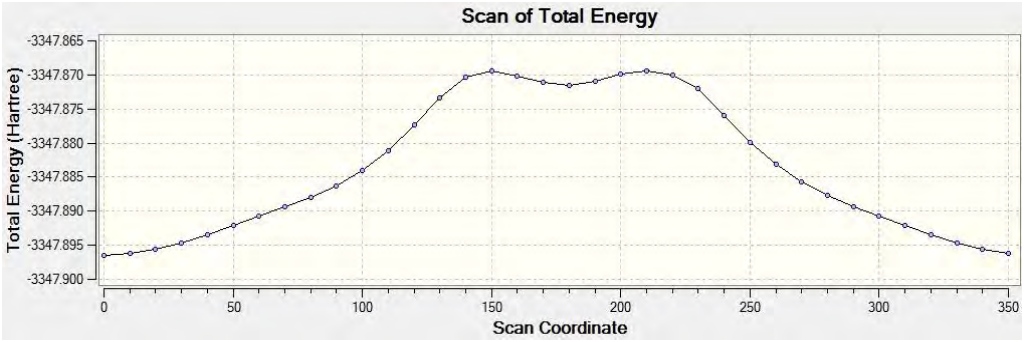
d) PES of TAG Trioleic C_9 OO radical with TBHQ and LA



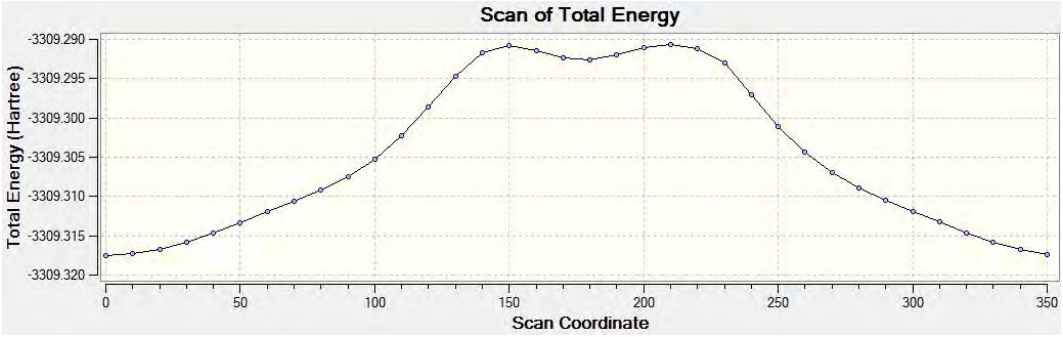
APPENDIX 14

Potential energy surface (PES) scan of TAG trioleic C_9 OO radical with antioxidants

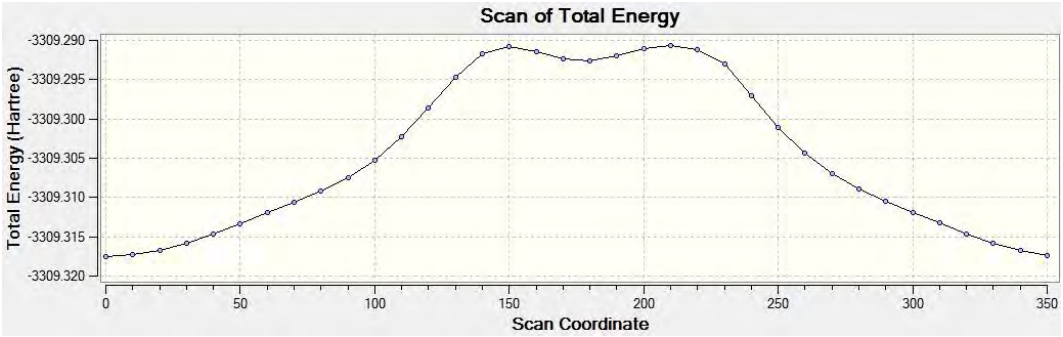
a) PES of TAG Trilinoleic C_9 OO radical with BHA



b) PES of TAG Trilinoleic C_9 OO radical with TBHQ



c) PES of TAG Trilinoleic C_9 OO radical with BHT



CURICULUM VITAE

SITI BALQIS BINTI CHE OTHMAN
(GSK 1131)

Date of Birth	:	05 December 1983
Address	:	1, Jalan P 15 H4/5, Presint 15, 62250, Putrajaya (Phone Number: 019-2121205)
Degree	:	1. Bachelor degree in Analytical and Environmental Chemistry (2006), KUSTEM 2. Master of Sciences in Chemical Sciences (2010), UMT 3. Doctor of Philosophy in Chemical Sciences (2015), UMT
Publication / Conferences	:	**Che Othman S.B**., Ku Bulat, K.H., Ariffin, J.(2009) Production of Biobased Lubricant Via Alkoxylation, Faculty of Science and Technology Universiti Malaysia Terengganu, 21030, Kuala Terengganu **Che Othman S.B**., Ku Bulat, K.H., Ariffin, J. and Wan Nik, W.N.S. (2009). Epoxidation of Palm Olein and Jatropha Oil, Graduate School Seminar, Universiti Malaysia Terengganu, 21030, Kuala Terengganu **Che Othman S.B**., Ku Bulat, K.H., Ariffin, J. and Wan Nik, W.N.S. (2009). The Effect of Hydrogen Peroxide Molar Ratio on the Epoxidation of Palm Olein and Jatropha oil, Malaysia Natural Product International Seminar (MNPIS) MS Garden, Kuantan **Che Othman S.B**., Ariffin, J., and Ku Bulat, K.H. (2010). Chemical Modification of Vegetable oil: Alkoxylation of Epoxidized Jatropha Oil, Seminar Kimia Analisis Malaysia (SKAM-23), Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia

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