ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

FORMATION OF EPOXY FATTY ACIDS DURING PHOTOOXIDATION

M. Sc. THESIS

Melek DEMİRCİ

Department of Food Engineering

Food Engineering Programme

DECEMBER 2015

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

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To my family,

FOREWORD

The practical work of this thesis was carried out at the Department of Food Safety and Food Quality in Ghent University, located in Ghent, Belgium.
Hereby, I would like to thank to my promoter Prof. dr. ir. Bruno De Meulenaer for accepting me as a thesis student in the laboratory.
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ABBREVIATIONS

PUFAs : Polyunsaturated fatty acids
PV : Peroxide value
CD : Conjugated dienes
CT : Conjugated triens
SPME : Solid Phase Microextraction
IDF : International Dairy Federation
***p*-AV** : *p*-anisidine value
SPE : Solid Phase Extraction
ES : Epoxystearate
EOL : Epoxylinolenate
EO : Epoxyoleate
SLO : Stripped linseed oil
SSO : Stripped sunflower oil
SVOO : Stripped virgin olive oil
VOO : Virgin olive oil
LOD : Limit of detection
LOQ : Limit of quantification
EFA : Epoxy fatty acid
FAME : Fatty acid methyl ester
GC : Gas chromatography

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FORMATION OF EPOXY FATTY ACIDS DURING PHOTOOXIDATION

SUMMARY

Lipid oxidation which adversely affects the human health, the quality and nutritional value of food products, also causes economic losses, occurs in series of reactions. Toxic products are formed during oxidation and make it a health issue. Lipid oxidation can be divided into categories such as photooxidation, autoxidation and thermoxidation depending on the factors triggering the oxidation including oxygen, heat, light and metal ion concentration. Photooxidation is one of the oxidation types which occurs in the presence of light and photosensitizers like chlorophyll and riboflavin. Photosensitizers cause the formation of singlet oxygen from ground state by energy transfer from light. Singlet oxygen leads to the formation of hydroperoxides from unsaturated fatty acids. In this study, photooxidation of three oil types (linseed, sunflower and olive oil) were investigated in the presence of chlorophyll. Fatty acid methyl esters (FAMEs) and epoxy fatty acid content of the oil samples were monitored by gas chromatograph with a flame ionization detector (GC-FID). Samples were kept under fluorescent light for two months. Virgin olive oil used as a chlorophyll source and its chlorophyll stripped counterpart (stripped virgin olive oil) were added to the samples to compare the oxidation levels. Primary oxidation products were also determined by peroxide value (PV), conjugated dienes (CD) and conjugated trienes (CT) analyses. Linseed oil samples had around 38% linolenic acid (C18:3) as the primary component, while sunflower oil samples had around 45% linoleic acid (C18:2). On the other hand, olive oil samples showed around 62% oleic acid (C18:1) as the primary component. Linseed oil samples were found to have the highest oxidation rate due to the high degree of unsaturation of linolenic acid. The amount of CD was higher than CT in all samples. Total epoxy fatty acid amount of all oil blends with chlorophyll were higher than oil blends without chlorophyll in the beginning of the incubation. Linseed oil blend with chlorophyll was found to have the highest amount of total epoxy (82.42μg g^{-1}) in the beginning of the incubation. Sunflower oil blend with chlorophyll and olive oil blend with chlorophyll were determined as 62.97μg g^{-1} and 61.77μg g^{-1}, respectively. In the end of incubation, linseed oil blend without chlorophyll was found to have the highest amount of total epoxy fatty acid (10866.28μg g^{-1}) compared to the other oil blends. In the beginning of incubation, *cis* configuration was dominated in all oil blends with chlorophyll, but *trans* domination was seen in the oil blends without chlorophyll in the end of incubation.

FOTOOKSİDASYON SIRASINDA OLUŞAN EPOKSİ YAĞ ASİTLERİNİN İNCELENMESİ

ÖZET

Gıdaların kalite ve besin değerini olumsuz etkileyerek insan sağlığını tehdit eden ayrıca ekonomik kayıplara da neden olan yağ oksidasyonu, bir dizi kimyasal reaksiyonla gerçekleşir. Yağ oksidasyonu sonucu toksik maddeler oluşmakta ve yağ oksidasyonunu bir sağlık sorunu haline getirmektedir. Gıdalarda oksidasyon sonucu acımsı tat ve aroma oluşumu, klorofil gibi pigmentlerin parçalanması sonucu renk kaybı kaliteyi olumsuz etkilemekte, linoleik asit gibi bazı esansiyel yağ asitlerinin reaksiyonu sonucu ise besin değerinde azalma meydana gelmektedir.

Doymamış yağ asitleri, bir ya da daha fazla çift bağ içeren uzun karbon zincirleridir. En bilinen örnekleri 18 karbon içeren oleik, linoleik ve linolenik asittir. Oleik asit bir, linoleik asit iki ve linolenik asit üç çift bağ içermektedir. Linolenik asit, ilk çift bağının karbon zincirindeki üçüncü karbonda bulunması nedeniyle aynı zamanda ω-3 yağ asiti olarak adlandırılmaktadır. Aynı şekilde linoleik asit ω-6 ve oleik asit ω-9 yağ asiti olarak adlandırılır.

Yağ oksidasyonu, oksijen, ısı, ışık, metal iyon konsantrasyonu gibi oksidasyonu tetikleyen faktörlere bağlı olarak otoksidasyon, termoksidasyon, fotooksidasyon ve enzimatik oksidasyon şeklinde dörde ayrılmaktadır. Otoksidasyon, oksijen varlığında serbest radikallerin zincir reaksiyonları sonucu stabil ürünlerin oluştuğu bir oksidasyon türüdür. Otoksidasyon, başlangıç, gelişme ve sonlanma olarak üç aşamada gerçekleşir. Başlangıç aşamasında, doymamış yağ asitlerinden hidrojen koparılmasıyla radikal oluşumu gerçekleşir. Daha sonra oluşan alkil radikalleri oksijen ile reaksiyona girerek gelişme aşamasında peroksil radikalleri oluştururlar (ROO•). Bu peroksil radikaller başka bir yağ asit zinciri ile reaksiyona girerek oksidasyonun birincil ürünleri olan hidroperoksitleri oluştururlar. Sonlanma aşamasında ise, iki serbest radikal arasındaki reaksiyon sonucu daha stabil ve radikal olmayan alkol, aldehit, keton ve epoksi asitler gibi ikincil ürünler oluşur. Termoksidasyon reaksiyonları otoksidasyona benzer şekilde gerçekleşen fakat hidroperoksitlerin daha hızlı parçalandığı, ısıyla katalizlenen oksidasyon türüdür. Enzimatik oksidasyonda ise esas etken lipaz, fosfolipaz ve lipoksijenaz gibi enzimlerdir. Bu çalışmada sadece fotooksidasyon üzerinde durulmuştur.

Fotooksidasyon ise, ışık ve klorofil, riboflavin gibi fotosensitizer denilen ışığa duyarlı maddelerin varlığında gerçekleşen bir oksidasyon türüdür. Sensitizerler ışıktan aldıkları enerji ile uyarılmış duruma geçerler. Uyarılmış sensitizer, enerjisini moleküler oksijene aktararak singlet oksijeni oluşturur. Doymamış yağ asitleri ise singlet oksijen ile reaksiyona girerek hidroperoksitlerin oluşumuna neden olur. Fotooksidasyon reaksiyonları oldukça reaktif olan singlet oksijen yüzünden başlangıç aşaması olmadan, bu yüzden de otoksidasyondan daha hızlı olarak gerçekleşir.

Epoksi yağ asitleri, peroksitlerin parçalanması sonucu oluşan ikincil oksidasyon ürünleridir. İnsanlar tarafından metabolize edildiğinde toksine dönüşebilen potansiyel toksik maddeler olarak bilinmektedir. Yağ oksidasyonu üzerine etkileri ve oluşum rotalarının tam olarak bilinmemesi nedeniyle varlıklarına gerekli önem verilmemektedir. Bu çalışma ile epoksi yağ asitlerinin fotooksidasyon sonucu oluşumları hakkında daha fazla bilgi edinilmesi amaçlanmıştır.

Bu amaçla, üç farklı yağ türünün (keten tohumu, ayçiçek ve zeytinyağı) klorofil varlığında fotooksidasyonu incelenmiştir. Yağların hepsi sıyırma işleminden geçirilerek bir kısmı 2:1 oranında sıyrılma işlemi yapılmamış rafine zeytinyağı ile karıştırılmış diğer kısmı ise kontrol grubu olarak 2:1 oranında sıyrılma işleminden geçirilmiş rafine zeytinyağı ile karıştırılarak oksidasyon derecesinin karşılaştırılması amaçlanmıştır. Rafine zeytinyağı klorofil kaynağı olarak kullanılmış, klorofil içermeyen sıyırma işleminden geçirilmiş zeytinyağı örneklerin oksidasyon seviyelerinin karşılaştırılması için kontrol grubunda kullanılmıştır. Örnekler saydam tüplere konularak pamukla kapatılmış ve iki ay boyunca, floresan ışık altında, 6 ±1°C soğuk odada muhafaza edilmiştir.

Örneklerdeki klorofil miktarı, IUPAC standart methotlarına göre, klorofilin görünür ışığı absorbe ettiği 650, 670 ve 710 nm'lerdeki absorbansın ölçüldüğü spektrofotometre kullanılarak belirlenmiştir. Sıyırma işleminden geçirilen zeytinyağı eklenmiş örneklerde klorofilin varlığına rastlanmamıştır. Sıyırma işleminden geçirilmemiş zeytinyağı eklenen örneklerde ise 56 günlük inkübasyon sonunda klorofil miktarının azaldığı gözlenmiştir. Bununla birlikte, yağ örneklerinin renklerinin zamanla açıldığı ve örneklerin daha viskoz bir yapı aldığı görülmüştür.

Yağ asitlerinin metil esterleri gaz kromatografisi (GC) kullanılarak standart yağ asitlerinin alıkonma süresi ile örneklerdeki yağ asitlerinin karşılaştırılması sonucu belirlenmiştir. Keten tohumu yağı içeren örneklerde yaklaşık % 38 linolenik asit (C18:3), ayçiçeği yağı içeren örneklerde yaklaşık % 45 linoleik asit (C18:2) ve sadece zeytinyağı içeren örneklerde ise yaklaşık % 62 oleik asit (C18:1) baskın olarak bulunmuştur. Sıyırma işleminden geçirilmiş zeytinyağı içeren örneklerin diğerlerine göre daha az miktarda bileşik içerdiği görülmüştür.

Peroksit değeri (PV), konjuge dien (CD) ve trien (CT) analizleri ile örneklerin birincil oksidasyon ürünleri belirlenmiştir. PV analizi, farklı bir çok yöntem arasından en güvenilir olan IDF methotu kullanılarak yapılmıştır. Peroksitler stabil olmayan, kolayca parçalanabilen bilekşikler oldukları için PV en yüksek değerine ulaştıktan sonra azalma göstermektedir. Bu yüzden de düşük peroksit değerine sahip bir yağın kalitesinin iyi olduğu sonucuna varılamamaktadır. PV, genellikle CD ve CT ile beraber değerlendirilir. CD, serbest radikallerin metilen grubundaki hidrojenlerle reaksiyona girmesi ve çift bağların yer değiştirmesi sonucu oluşur. Analizler sonucu, ketentohumu yağı içeren örnekler yüksek oranda doymamış linolenik asite sahip olması sebebiyle diğer örneklerden daha yüksek PV değeri göstermiştir. Ayrıca CT değeri fotooksidasyon boyunca keten tohumu yağı içeren örnekler dışındaki örneklerde fazla değişiklik göstermemiştir. Hem başlangıçta hem de oksidasyon boyunca CD değeri bütün örneklerde CT'den daha yüksek bulunmuştur.

Örneklerdeki epoksi yağ asiti miktarı, oda sıcaklığında sodyum metoksit ile baz katalizli transmetilasyon yöntemi, katı faz ekstraksiyonu (SPE) ve GC analizi yöntemleri kullanılarak üç aşama ile belirlenmiştir. Transmetilasyon ile, sodyum metoksitin kataliz olarak kullanılmasıyla yağ asitlerinin transesterifikasyonu sonucu metil esterleri oluşumu sağlanmıştır. SPE ile takip eden GC analizi için metil esterler, polar olmayan fraksiyonlardan ve diğer safsızlıklardan ayrılmıştır. GC analizinde örnek izooktanda çözülerek kolona enjekte edilmiş, hesaplamalar sonucu örneklerdeki epoksi yağ asidi miktarı $\mu g\ g^{-1}$ yağ olarak ifade edilmiştir.

Klorofil içermeyen (sıyırma işleminden geçirilmiş zeytinyağı içeren) keten tohumu yağı örneklerindeki toplam epoksi yağ asiti miktarı 56 günlük inkübasyon süresi sonunda (10866.28 $\mu g\ g^{-1}$ yağ) klorofil içeren keten tohumu yağı örneklerinin (6200.16 $\mu g\ g^{-1}$ yağ) yaklaşık iki katı bulunmuştur. Bununla birlikte, klorofil içeren keten tohumu yağı örneklerinin başlangıç toplam epoksi yağ asidi miktarının en fazla olduğu görülmüştür (82.42 $\mu g\ g^{-1}$ yağ). Klorofil içeren ayçiçeği yağı ve zeytinyağı örneklerinin epoksi miktarları sırasıyla 62.97 $\mu g\ g^{-1}$ yağ ve 61.77 $\mu g\ g^{-1}$ yağ olarak belirlenmiştir. Klorofilin fotosensitizer etkisinden dolayı sıyırma işleminden geçirilmemiş zeytinyağı içeren keten tohumu yağı örneklerinin daha yüksek miktarda epoksi oluşumu göstermesi beklenirken klorofil içermeyen keten tohumu yağı örneklerindeki toplam epoksi miktarı daha fazla bulunmuştur. Klorofil içermeyen keten tohumu yağı örneklerinin daha fazla miktarda epoksi oluşumu göstermesinin, sıyırma işlemi sırasında bazı antioksidan maddelerin de ayrılmış olmasından kaynaklandığı düşünülmektedir. Fotooksidasyon boyunca bütün örneklerde oluşan toplam epoksi yağ asiti miktarları karşılaştırıldığında ağırlıklı olarak linolenik asit içeren keten tohumu örneklerinin en yüksek reaksiyon hızına sahip olduğu görülmüştür.

İnkübasyon başlangıcında bütün yağ örneklerindeki epoksi yağ asitlerinin *cis* konfigürasyonu gösterdiği görülmüştür. Bunun nedeni *cis* izomerlerinin doğal olarak oluşan izomerler olması ve çifli bağ içeren çoğu yağ asidinin *cis* configürasyonuna sahip olmasıdır. Klorofil içeren ayçiçek yağı ve zeytinyağı örneklerinde inkübasyon boyunca *cis* konfigürasyonu ağırlıklı olarak bulunurken klorofil içeren keten tohumu yağında bir aylık inkübasyon süresinden sonra *trans* izomerleri baskın duruma gelmiştir. Klorofil içermeyen bütün yağ örneklerinde *trans* konfigürasyonu baskın olarak bulunmuştur.

İnkübasyon sonunda örneklerdeki epoksi yağ asidi miktarının PV ile karşılaştırıldığında çok daha fazla olduğu görülmüştür. Bu da, peroksit değerinin yağ kalitesini belirlemede tek başına yeterli olmadığını göstermektedir.

1. INTRODUCTION

Lipid oxidation is a concern due to the adverse health effects of the by-products produced. It also affects food quality thus, causing economic losses. Photooxidation is a type of oxidation which occurs in the presence of light and sensitizers. The reaction rate of photooxidation is known to be higher than autoxidation and some by-products of them also differ from each other (Kiokias et al., 2010). One of the oxidation products are epoxy fatty acids which are hard to determine by conventional methods due to their highly reactive nature (Schaich et al., 2012). Many study have shown recently that existence of epoxy fatty acids were neglected as their impact on lipid oxidation and formation route are not clearly known. In this study, epoxy fatty acids formed during photooxidation of three oil samples (olive, sunflower, linseed oil) were determined according to the method developed by Mubiru et al. (2014). The aim of this study is to contribute the knowlage of epoxy fatty acids. The samples were incubated for two months and analyzed every week to determine the peroxide value (PV), conjugated diene (CD) and conjugated triene (CT) together with the epoxy fatty acid content. Chlorophyll is known as a photosensitizer for the photooxidation reactions (Morales and Przybylski, 2013). Virgin olive oil was used as a chlorophyll source for photooxidation because it contains a considerably higher amount of chlorophyll than the other vegetable oils (Psomiadou and Tsimidou, 2002). Autoxidation and thermooxidation analyses were also performed corresponding to this study for the future comparison between different types of oxidations. However, this study will only focus on photooxidation and other type of oxidations will not be explained in detail.

2. LITERATURE OVERVIEW

2.1 Lipid Oxidation

Lipid oxidation has long been considered to be responsible for food deterioration as it causes rancidity, off-odor, undesirable flavor and loss of nutritional quality (Kolakowska, 2003). Edible oils are a source of polyunsaturated fatty acids (PUFAs) and that makes them more susceptible to lipid oxidation. There are a lot of factors having an impact on their oxidation including heat, metal ion concentration, light, oxygen, and others (Kiokias et al., 2010).

The unsaturated fatty acids are long carbon chains with one or more double bond. Typical examples are oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids which have one, two and three double bonds respectively and 18 carbon atoms. Linseed oil is a source of linolenic acid in which around 60% is present. Linolenic acid is also called a ω-3 fatty acid because the first double bond is located at the third carbon with respect to the length of the carbon chain. Similarly, oleic acid is called a ω-9 and linoleic acid a ω-6 fatty acid. Oleic acid is the main fatty acid present in olive oil and linoleic acid in sunflower oil. The average content of these fatty acids in these oils is shown in Table 2.1 (Juita et al., 2012).

Table 2.1: Selected fatty acid compositions of linseed, olive and sunflower oil (Juita et al., 2012).

	Fatty acid composition (%)		
	C18:1	C18:2	C18:3
Linseed oil	14-24	14-19	48-16
Olive oil	78	7	1
Sunflower oil	20	69	Trace

Lipid oxidation occurs in a series of reactions and mainly the products of these reactions cause nutritional and quality losses. The lipid oxidation products can be

either primary such as hydroperoxides or secondary such as aldehydes, ketones, acids, and alcohols (Morales and Przybylski, 2013).

Lipid oxidation can be divided into categories such as photooxidation, autoxidation and thermoxidation depending on the factors triggering the oxidation including light, oxygen, heat, and metal ions. Autoxidation is a free radical chain reaction affected by oxygen while photooxidation is an ene reaction between singlet oxygen and a double bond activated by light and photosensitizers. The ene reaction results in a *trans* configuration by addition of oxygen to the double bond which is then shifted to allylic position and isomerized (Knothe et al., 2007).

$$\underset{cis}{RCH{=}CHCH_2CHR' + {}^1O_2} \rightleftharpoons \underset{trans}{RCH(OOH)CH{=}CHR'} \quad \textbf{(2.1)}$$

Thermoxidation reactions are similar to the autoxidation but higher in terms of reaction kinetics in which the decomposition rate of LOOH and formation of *trans* isomers are increased at low to moderate temperatures. Moreover, thermoxidation process in frying temperatures more than 150°C causes acyl chain cleavage and radical formation (Schaich et al., 2012). Despite the lower oxygen solubility at high temperatures, thermoxidation occurs still faster that autoxidation (Marquez-Ruiz et al., 2013).

Enzymatic oxidation is another type of oxidation in which different kind of enzymes are involved including lipoxygenases, lipases, phospholipases, and lipolytic acyl hydrolases. Hydroperoxides (mainly 9-, 13- isomers) are formed by the enzymatic reaction of unsaturated fatty acids and then converted to secondary products such as aldehydes, ketones, and alcohols (Morales and Przybylski, 2013). However, in this study, only photo-oxidation will be explained in detail.

2.1.1 Lipid oxidation mechanism

Autoxidation takes place in three steps, namely initiation, propagation and termination.

In the initiation step, the formation of unsaturated fatty acid radicals occurs by abstracting a hydrogen atom from unsaturated lipids (Kolakowska, 2003). The reaction starts with an initiator including UV-light, heat, metal ions and proceeds in a

rate depending on the presence of antioxidants, type of oil, the number of double bonds in a chain and temperature (Kiokias et al., 2010).

$$RH \longrightarrow R\bullet + H\bullet \qquad \textbf{(2.2)}$$

In the propagation step, alkyl radicals which are formed in the initiation step react with molecular oxygen and form peroxyl radicals, ROO•. Then, hydroperoxides are formed with a reaction of peroxyl radicals and another fatty acid chain (Kiokias et al., 2010), causing the formation of a new fatty acid radical which will further react with oxygen.

$$R\bullet + O_2 \longrightarrow ROO\bullet \qquad \textbf{(2.3)}$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \qquad \textbf{(2.4)}$$

In the termination step, finally, more stable, non-radical products are formed after the reaction between two free radicals (Márquez-Ruiz et al., 2013). For example, if peroxyl radicals react with other peroxyl radicals or with alkyl radicals, peroxides are formed, ROOR.

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2 \qquad \textbf{(2.5)}$$

$$R\bullet + ROO\bullet \longrightarrow ROOR \qquad \textbf{(2.6)}$$

$$R\bullet + RO\bullet \longrightarrow ROR \qquad \textbf{(2.7)}$$

$$R\bullet + H\bullet \longrightarrow RH \qquad \textbf{(2.8)}$$

$$RO\bullet + H\bullet \longrightarrow ROH \qquad \textbf{(2.9)}$$

$$ROO\bullet + H\bullet \longrightarrow ROOH \qquad \textbf{(2.10)}$$

After decomposition of peroxides, volatiles such as alcohols, aldeyhdes and non-volatile products such as ketones and epoxides are formed, which gives off-flavor and odor to the food product (Gordon, 2004).

2.1.2 Photooxidation

Photooxidation is a type of oxidation which occurs in the presence of light and sensitizer such as chlorophyll, riboflavin, myoglobin, and hemoglobin. Unsaturated fatty acids react with highly reactive singlet oxygen and form hydroperoxides which

further decompose, similar as in the autoxidation. However, the products are different in structure from autoxidation and reaction rate depends on the degree of unsaturation (Kiokias et al., 2010; Schaich et al., 2012). Photooxidation reactions are also faster than autoxidation as the high electrophilic $^1O_2$ causes the reaction without induction period (Knothe et al., 2007). Most oils have naturally present sensitizers which are activated by transfer of energy in the presence of light (Morales and Przybylski, 2013).

The mechanism of photooxidation is showed below:

$$\text{Sensitizer}_{ground} + h\upsilon \longrightarrow \text{Sensitizer}_{excited} \quad \textbf{(2.11)}$$

$$\text{Sensitizer}_{excited} + RH \longrightarrow \text{Sensitizer}_{H} + R\bullet \quad \textbf{(2.12)}$$

$$\text{Sensitizer}_{excited} + {}^3O_2 \longrightarrow \text{Sensitizer}_{ground} + {}^1O_2 \quad \textbf{(2.13)}$$

$${}^1O_2 + RH \longrightarrow ROOH + {}^3O_2 \quad \textbf{(2.14)}$$

Sensitizers are excited by the energy from light, they react with ground state oxygen and form singlet oxygen which leads to the formation of hydroperoxides from unsaturated fatty acids (Morales and Przybylski, 2013). Triplet oxygen is the most stable state of oxygen with two unpaired electron in a magnetic field. However, singlet oxygen with paired electrons has no magnetic moment and highly reactive with unsaturated fatty acids (Kiokias et al., 2010). Excited sensitizers can also transform that energy in order to generate free radicals and act like autoxidation, called Type I, while the reaction which occurs by singlet oxygen, called Type II. Type II photooxidation significantly differs from autoxidation in terms of the formation of hydoperoxides. In photooxidation, hydroperoxides are formed both at internal and external positions while in autoxidation, just externally positioned ones are formed (Schaich et al., 2012). A model about comparison of Type I and Type II oxidations is given in Table 2.2.

Table 2.2: Comparison of Type I and Type II photooxidation (Schaich et al., 2012).

	Free Radical - Type I **$^3O_2$ Autoxidation**	**$^1O_2$ - Type II** **Photooxidation**
Mechanism	Conventional free radical chain reaction	'ene' reaction, concerted addition of $^1O_2$ to the double bonds and occurance of *trans* configuration, no free radicals involved
Kinetics	Induction period present	No induction period
	Dependent on pO_2 (oxygen partial pressure)	Independent of O_2 (when O_2 not limiting)
		Dependent on sensitizer concentration
	Rate not proportional to number of double bonds	Rate directly proportional to number of double bonds
Products	Conjugated LOOHs	Both non-conjugated and conjugated LOOHs
	External 9, 13, 16 positions dominant	LOOHs at all positions
	Scission products all from external LOOHs	High proportion of cyclics, endoperoxides, epoxides, dihydroperoxides, and trihydroperoxides at internal positions
		Scission products from internal LOOHs, altered scission position preferences

Chlorophyll is one of the photosensitizer which can oxidize unsaturated lipids both in free radical and singlet oxygen pathway (Figure 2.1). It causes the reaction of singlet oxygen with the double bonds of oleic, linoleic and linolenic fatty acids, leading to the formation of allyl hydroperoxides (Schaich et al., 2012). Chlorophyll a and b are the types of the pigment present in oil and chlorophyll b is indicated to have a higher prooxidant effect than Chlorophyll a (Giuliani et al., 2011). Many study showed that the prooxidant effect of chlorophyll in olive oil in the presence of light also depends on the amount of chlorophyll added to the oil (Morales and Przybylski, 2013). Furthermore, the chlorophyll content of extra virgin olive oil and stripped extra virgin olive oil was compared in the study of Khan and Shahidi (1999), the chlorophyll content of stripped extra virgin olive oil was found to be affected by stripping process as it was not detected after stripping process.

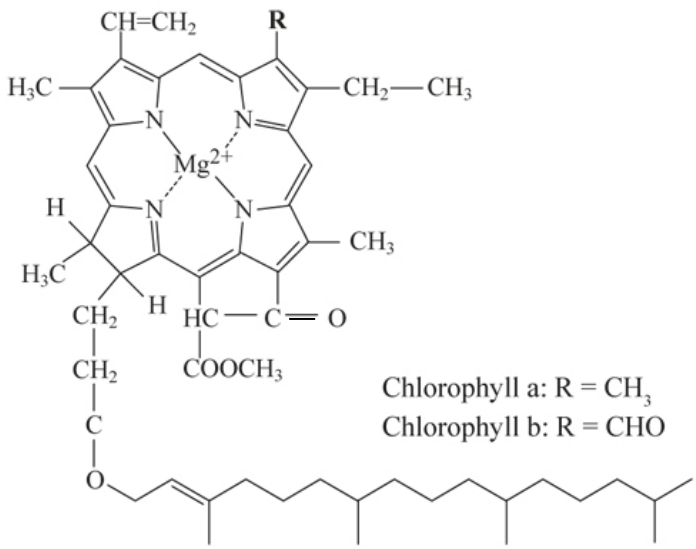

Figure 2.1: Chlorophyll structure of type a and b (Lehninger, 2008).

2.1.3 Lipid oxidation products

2.1.3.1 Primary products

Primary products of lipid oxidation are hydroperoxides, which are formed from peroxyl radicals by abstraction of hydrogen atom from fatty acids (Eldin and Pokorny, 2005). In singlet oxygen reactions, different types of hydroperoxides from the reaction are formed by direct reaction of oxygen resulting an allylic *trans* double bond. Moreover, products differ from triplet oxygen reaction from the singlet oxygen reactions which are shown in Table 2.3. *Trans*, *cis*-10-OOH and *cis*, *trans*-12-OOH

are specific to the singlet oxygen reactions as they are not cojugated like the ones formed in triplet oxygen reactions. (Frankel, 2005).

Table 2.3: Hydroperoxides formed by photooxidation reactions.

Fatty Acid	Hydroperoxide
Oleate	9-hydroperoxy-*trans*-10-octadecenoate (*trans*-9-OOH) 10-hydroperoxy-*trans*-8-octadecenoate (*trans*-10-OOH)
Linoleate	9-hydroperoxy-*trans*-10-*cis*-12-octadecadienoate (*trans,cis*-9-OOH) 10-hydroperoxy-*trans*-8-*cis*-12-octadecadienoate (*trans,cis*-10-OOH) 12-hydroperoxy-cis-9-trans-13-octadecadienoate (*cis,trans*-12-OOH) 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoate (*cis,trans*-13-OOH)
Linolenate	9-hydroperoxy-*trans*-10, -*cis*-12, *cis*-15-octadecatrienoate (*trans,cis,cis*-9-OOH) 10-hydroperoxy-*trans*-8, -*cis*-12, *cis*-15-octadecatrienoate (*trans,cis,cis*-10-OOH) 12-hydroperoxy-*cis*-9, -*trans*-13, *cis*-15-octadecatrienoate (*cis,trans,cis*-12-OOH) 13-hydroperoxy-*cis*-9, -*trans*-11, *cis*-15-octadecatrienoate (*cis,trans,cis*-13-OOH) 15-hydroperoxy-*cis*-9, -*cis*-12, *trans*-16-octadecatrienoate (*cis,cis,trans*-15-OOH) 16-hydroperoxy-*cis*-9, -*cis*-12, *trans*-14-octadecatrienoate (*cis,cis,trans*-16-OOH)

Primary products are measured by peroxy value (PV) and conjugated dienes method. There are different methods for measuring PV including the titration method, the colorimetric ferro method, micromethod and FOX2 method but the most reliable one is the colorimetric ferric thiocyanate method, also called the IDF method. In this method, absorbtion of a red colored complex of ferric thiocyanate which is formed by reaction of ammonium thiocyanate and oxidized ferrous ions, is measured at 500nm (Rustad, 2009).

The reaction can be seen below;

$$ROOH + Fe^{2+} \rightarrow RO\bullet + Fe^{3+} + OH^{-1} \quad \textbf{(2.15)}$$

$$RO\bullet + Fe^{2+} + H^{+} \rightarrow ROH + Fe^{3+} \quad \textbf{(2.16)}$$

$$Fe^{3+} + 5SCN^{-} \rightarrow Fe(SCN)^{2-}_{5} \quad \textbf{(2.17)}$$

In the study of Che Man et al. (1999), the peroxy value was found to be decreased after it reached the highest value which proves that peroxides are unstable compounds and decompose easily to form the secondary products in oxidation reactions. It means that oil with a low peroxide value does not always guarantee a good quality of oil. Therefore, PV methods are not sufficient to determine the quality of the oil but conjugated dienes and trienes can be used for more reliable and accurate results (Che Man et al., 1999).

Conjugated dienes (CD) are other primary products of lipid oxidation. Free radicals react with hydrogens of the methylene group, causing the double bonds to shift and form conjugated dienes. Both conjugated and non-conjugated diene hydroperoxides are formed from linoleic and linolenic acid in singlet oxygen reactions (Figure 2.2). Conjugated dienes are used together with PV to determine the stability of lipids (Kiokias et al., 2010).

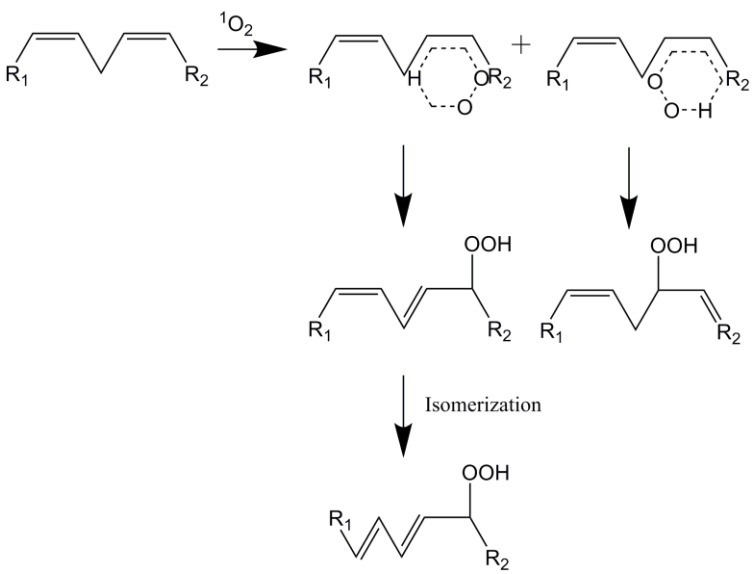

Figure 2.2: Conjugated and non-conjugated diene hydroperoxides formation in singlet oxygen reactions (Kim and Min, 2008).

2.1.3.2 Secondary products

Secondary products including aldehydes, ketones, acid esters, alcohols and short-chain hydrocarbons are formed from alkoxy radicals by decomposition of hydroperoxides (Kim and Min, 2008). The decomposition pathway of hydroperoxides and some secondary products including alkoxyl (RO•), peroxyl (ROO•), hydroxyl (•OH) and new lipid radicals (R•) can be seen in Figure 2.3.

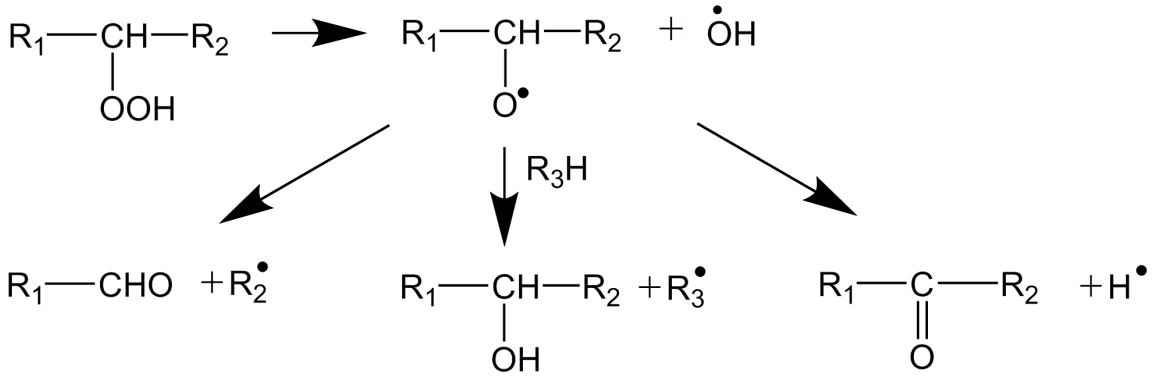

Figure 2.3: Decomposition of hydroperoxides (Shahidi and Zhong, 2010).

The quantity of aldehydes such as 2-alkenals and 2,4-alkadienals can be determined by using the *para*-anisidine value method. This value provides information about the amount of secondary oxidation products formed in oxidation reactions. The principle of this method is the measurement of a colored product which is the result of the reaction between aldehydes and *p*-anisidine under acidic conditions. The problem about the method is the interefence of non-volitile products to the absorbance with volatile products (Kiokinas et al., 2010).

PV and *p*-anisidine value can be combined to determine the oxidative status with so-called TOTOX value which is defined as follows:

$$\text{TOTOX Value} = 2\,(\text{PV}) + p\text{-anisidine} \qquad \textbf{(2.18)}$$

Homolytic β-scission of fatty acid hydroperoxides is the one which is responsible for the formation of volatile compounds including unsaturated *cis* and *trans* aldehydes, alcohols and hydrocarbons. Depending on the polyunsaturated fatty acids present, different kind of compounds are formed, for example proponal, hepta-2,4-dienal from n-3 PUFAs and hexanal, pentane formation from n-6 PUFAs (Kolakowska, 2003).

2.1.3.3 Epoxy fatty acid formation

Epoxides are significant compounds in the oxidation mechanisms as they are highly reactive and hard to analyse by conventional methods. Moreover, they are thought to have a main role in oxidation as the whole lipid oxidation mechanism leads to the formation of epoxy fatty acids (Schaich et al., 2012). Therefore, it is essential to determine the epoxy fatty acids in food systems. The formation of epoxide rings occurs either at the site of the double bond (1st route) or near it (2nd route). In the first route, a peroxy radical is added to an isolated or nonconjugated double bond and the formation of the epoxide occurs by the elimination of an alkoxy radical (LO•) (Figure 2.4). In the second route, the formation of an epoxyallylic radical occurs with 1,2-addition to a double bond which then results in the cyclization of an epoxy radical (Schaich, 2005). One of the epoxides which is formed from oxidized methyl linoleate by double bond cleavage of the epoxy-hydroperoxide is *trans*-2,3-epoxyoctanoic acid (Frankel, 2005). Cyclization of alkoxy radicals is also responsible for the epoxy allylic hydroxy formation from hydropeoxides of linoleate. Formation of epoxy allylic hydroxy, epoxy allylic hydoperoxy, and epoxy allylic keto oleate from linoleate hydroperoxides is shown in Figure 2.5. 11-hydroxy-12,13-epoxy-9-oleate and 9-hydroxy-12,13-epoxy-10-oleate isomers from oxidized linoleic acid can be given as an example. The other compounds that are responsible for the formation of epoxy are oleate and linolenate in which 9,10-epoxy-stearate, 9,10-epoxy ester, 10,11-epoxy ester are formed from corresponding compound (Frankel, 2005).

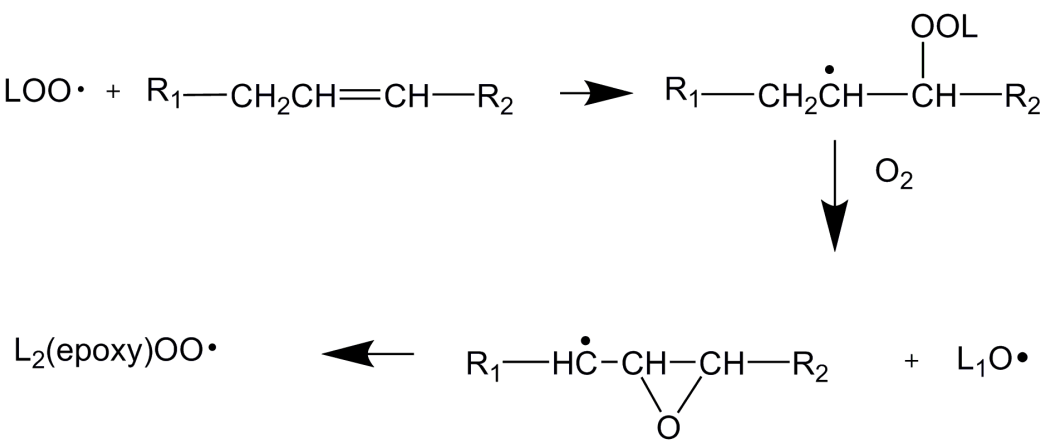

Figure 2.4: Epoxide ring formation during lipid oxidation (Schaich, 2005).

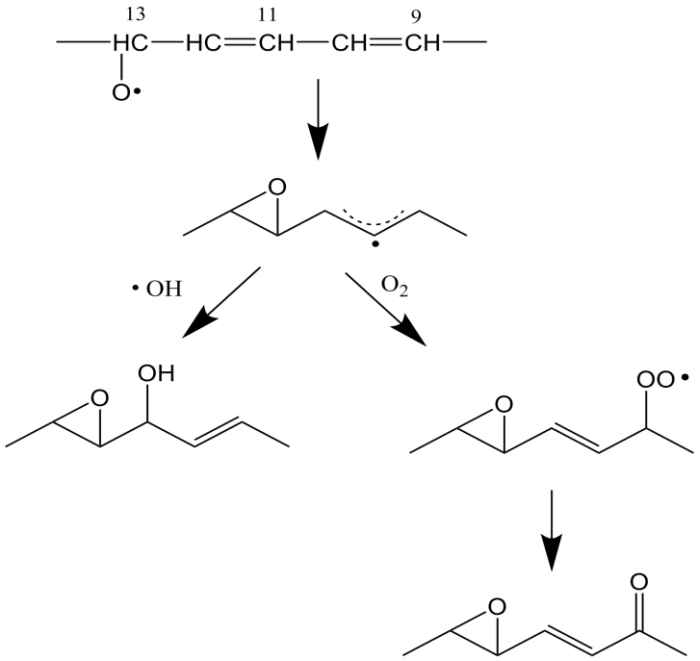

Figure 2.5: Formation of epoxy allylic hydroxy, epoxy allylic hydoperoxy, and epoxy allylic keto oleate from linoleate hydroperoxides (Frankel, 2005).

2.2 Effects of Lipid Oxidation on Health

Many studies showed that lipid oxidation products have negative effects on health as well as on foods. Hydroperoxide decomposition products including hydroxy acids, keto acids, and hydroxyaldehydes are considered to be detrimental non-volatile compounds for the health (Morales and Przybylski, 2013). Oxidized phospholipids which are one of the products of lipid oxidation, is indicated to have a role in aging, neurological disorders including Alzheimer's, Parkinson's and multiple sclerosis (MS) (Leitinger, 2008). It affects living cells by inactivation of enzymes, disturbtion of DNA and the cell membrane (Schaich et al., 1012). Moreover, epoxy fatty acids of C18 are known as leukotoxic and some isomers of them are indicated to cause acute respiratory distress syndrome (Greene et al., 2000). In addition to this, consumption of oxidized oils have some negative health effects on people including diarrhea, poor growth rate, myopathy, hepatomegaly, yellow fat disease hemolytic anemia and secondary deficiencies of vitamins A and E (Khan nad Shahidi, 1999). Especially, the secondary oxidation products of oxidation, long chain epoxy acids are reported to be protoxins which can turn into a toxin after metabolized by humans (Mubiru et al., 2013).

3. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Silica gel 60 (0.063 – 0.100 mm) and boron trifluoride-methanol solution were obtained from Merck Chemicals (Overijse, Belgium). Hexane (>95%), iso-octane, sodium chloride (99.8% purity), sodium sulfate, sea sand, diethylether, barium chloride, hydrochloric acid (37%) and ammonium thiocyanate were purchased from Chem-Lab NV (Zedelgem, Belgium). Aluminum oxide, sodium methoxide (25% w/v) and iron sulfate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Petroleum ether, hexane (>95%), methanol, sulfuric acid (>95%) and chloroform were bought from Fisher Scientific (Tournai, Belgium), methyl-tert-butylether was obtained from Acros Organics (Geel, Belgium). Finally, GLC 68D was obtained from Nu-Chek Prep (Inc., USA).

3.2 Samples

Oil samples were bought from Bio-Planet (an organic bio-supermarket) in Ghent, Belgium. Three different kind of oils, namely linseed oil, sunflower oil and virgin olive oil are stripped, formulated in a 2:1 ratio w/w with non-stripped and stripped virgin olive oil as a non-control and control model, respectively (Table 3.1).

Virgin olive oil was added for photooxidation as virgin olive oil contains chlorophyll pigment which is needed for reaction of photooxidation. All the incubation experiments were done in triplicates.

Table 3.1: Oil models.

Model	Component	Abbreviation
A	Stripped Linseed Oil + Virgin Olive Oil	SLO+VOO
B	Stripped Sunflower Oil + Virgin Olive Oil	SSO+VOO
C	Stripped Virgin Olive Oil + Virgin Olive Oil	SVOO+VOO
D	Stripped Linseed Oil + Stripped Virgin Olive Oil	SLO+SVOO
E	Stripped Sunflower Oil + Stripped Virgin Olive Oil	SSO+SVOO
F	Stripped Virgin Olive Oil + Stripped Virgin Olive Oil	SVOO+SVOO

3.2.1 Sample preparation

3.2.1.1 Oil stripping

Oil stripping was done in two steps

In the first step, 25g of silica was weighed in a beaker and mixed with hexane in the glass column with a stop valve. The column (40 cm x 2 cm) was refilled with hexane until well packed. Then, it was wraped with aluminum foil to prevent light-induced oxidations during the purification process. An amount of 50g of oil was weighed to a beaker, dissolved with 50ml hexane and loaded onto the column. Then, it was rinsed three times with 50ml of hexane and the eluate was collected in a round bottom flask. Finally, hexane was evaporated in a rotary vacuum evaporator (Heidolph Instruments GmbH & Co, Schwabach, Germany).

In the second step, aluminum oxide was activated at 200°C overnight in a furnish. Then, 100g of aluminum oxide was taken to a beaker and mixed with petroleum ether. Glass column (40 cm x 2 cm) which has been covered with aluminum foil was packed with this mixture. 50g of oil recovered from the first step was weighed to a beaker, mixed with 60ml of petroleum ether and finally loaded onto the column. It was rinsed with 100ml of hexane and the eluate was collected in a round buttom flask wrapped with aluminium foil. Finally, it was evaporated in a rotary vacuum evaporator, flushed with nitrogen and stored in a brown bottle in the freezer until it is used.

3.2.1.2 İncubation of the samples

Stripped LO, stripped SO and stripped OO were blended with VOO in ratio 2:1 (w/w) and homogenized by using Ultraturax (IKA-Werke GmbH & Co, Staufen, Germany). A similar batch of oil blends were prepared in the same way but non-stripped virgin olive oil used instead of stripped ones to compare the levels of photooxidation (Table 3.1). Transparent SPME (Solid Phase Microextraction) vials were filled with 4 ml of samples, closed with cotton and stored in the cold room (T= 6 ±1°C). Samples in transparent SPME vials were put into the shaker (Edmund Bühler GmbH, Hechingen, Germany) and exposed to fluorescent light (intensity of light were measured 1848 Lux on the corners, 2239 Lux on the surface, and 1877 Lux at the buttom of the shaker where samples were placed).

3.3 Methods

3.3.1 Chlorophyll content

Chlorophyll content was determined according to the standard methods of IUPAC by using spectrophotometry (Agilent Technologies, Diegem, Belgium). The method is based on the measurement of absorbance at 650, 670, and 710nm in which chlorophylls absorb the visible light. The total chlorophyll content was expressed in mg of pheophytin a/ kg of oil. Calculation was made according to the formula below:

$$\text{Chlorophyll content} = 345.3 \times (A_{670} - (0.5 \times A_{630}) - (0.5 \times A_{710})) / L \qquad \textbf{(3.1)}$$

where,

A_{670}= absorbance at 670nm

A_{630}= absorbance at 630nm

A_{710}= absorbance at 710nm

L= width of the cuvette (cm)

3.3.2 Fatty acid composition

The American Oil Chemists' Society Official Method Ce 1b-89 for marine oils was used for determination of fatty acid composition of the oil samples (AOCS, 1990).

Triacylglycerols were saponified with a methanolic NaOH solution. Subsequently, the fatty acids were esterified with BF_3/ MeOH- reagent in the presence of sodium hydroxide (NaOH) as catalyst.

$$RCOOR' + CH_3OH \longrightarrow RCOOCH_3 + R'OH \quad \textbf{(3.2)}$$

$$RCOOH + CH_3OH \longrightarrow RCOOCH_3 + H_2O \quad \textbf{(3.3)}$$

Fatty acid methyl esters (FAMEs) were identified by comparing their retention times with the retention times of standard fatty acids (GLC 68D).

Briefly, the internal standard solution was prepared by dissolving 500 mg of nonadecanoic acid in 100 mL of iso-octane. An exactly 1 mL of internal standard was pipetted into a test tube and subsequently dried under gentle flow of nitrogen gas.

Approximately 50mg of oil blend was dissolved in 2ml of 0.5N methanolic NaOH-solution. Samples were placed in a boiling water bath for 7 minutes and cooled in cold water. A volume of 2ml BF_3/ MeOH- reagent was added, vortexed for 30 seconds and placed in the boiling bath for 5 minutes. Samples were transfered to the cold water for cooling. A volume of 3ml iso-octane was added to the test tube, vortexed for 30 seconds, and immediately 5 ml of saturated NaCl- solution was added and vortexed again for 30 seconds. After phase seperation, the iso-octane phase were collected with a Pasteur pipette. It was repeated with 3ml of iso-octane one more time. A little amount of dry sodium sulphate was added to the test tube containing the iso-octane phase and vortexed for some seconds. After sodium sulphate was settled down 30µl of the organic layer and 970µl of iso-octane were transferred to a vial for GC-analysis. Results were expressed in g/100 g of oil.

3.3.2.1 GC analysis

Epoxy fatty acids and the FAMEs in the sample were analyzed by using an Agilent 6890N series gas chromotography (Agilent, USA). The samples were dissolved in iso-octane, and 0.1 µL was injected directly into the column using a cold on-column injector (coc), separation was performed in a CP-Sil 88 column for FAME (60 m × 0.25 mm i.d.) capillary column coated with a 0.2 µm film. A deactivated fused silica precolumn, 3 m × 0.25 mm i.d. (Agilent, Belgium), was fitted to protect the column.

The oven temperature program was set to 50 °C hold for 4 min, then ramp to 225 °C at 20 °C min^{-1}, and hold for 25 min. The flame ionization detector temperature was set at 300°C. The detector flow rates for hydrogen, air, and helium (makeup) were 40, 400, and 20 mL min^{-1}, respectively. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. Identification of individual epoxy FAMEs was carried out by comparison of retention times with the standards (GLC 68D, Nu-Chek Prep, Inc., USA).

3.3.2.2 Calculation

Calculations were done according to formula below;

$$\text{FAME amount (g/ 100 g of oil)} = [(A_c / A_{IS}) \times M_{IS}) \ / (C \times M_s)] \times 100 \quad \textbf{(3.4)}$$

where,

A_c= area of component

M_{IS}= weight of internal standard (nonadecanoic acid) (µg)

A_{IS}= area of internal standard

C= correction factor

M_s= weight of sample (g)

3.3.3 Peroxide value determination

The peroxide value (PV) has determined using the photometric determination of the amount of red iron (III) complex which is formed after the addition of iron (II) chloride and ammonium thiocyanate to a solution of the oil sample in a dicloromethane/ methanol mixture. PV was determined according to International Dairy Federation (IDF) method proposed by FAO with slight modifications. Briefly, dichloromethane and anhydrous methanol solution (7:3, v/v) was prepared as a solvent. Iron (II) solution was prepared by using barium chloride ($BaCl_2.2H_2O$) and iron sulphate ($FeSO_4.7H_2O$). Approximately 0.4g of barium chloride ($BaCl_2.2H_2O$) was dissolved in 50ml of distilled water. Meanwhile approximately 0.5g of iron sulphate ($FeSO_4.7H_2O$) was dissolved in 50ml of distilled water. The barium chloride solution was poured slowly with constant stirring into the iron (II) sulphate solution.

Then, approximately 2ml of 10N hydrochloric acid was added. Barium sulphate was allowed to settle down and precipitate. After precipitation, the mixture was filtered, and the clear solution was collected in a brown bottle and flushed with nitrogen. This solution was prepared every week freshly. Precautions were taken to protect them from photodegradation.

To prepare the ammonium thiocyanate solution, approximately 30g of ammonium thiocyanate (NH_4SCN) was dissolved in 100ml of distilled water and a colorless solution was obtained. All test tubes were covered with aluminium foil to protect from light.

Standard solutions of iron(III) chloride were prepared by dissolution of 0.5g iron powder in 50ml of 10N hydrochloric acid and addition of 1-2ml of about 30% (m/m) hydrogen peroxide solution. Then, excess of hydrogen peroxide was removed by boiling for 5 minutes, cooling down to room temperature and diluting with water to 500ml. This solution was transfered by using 1ml pipette to a measuring flask and diluted with the mixture of dichloromethane and methanol (7:3, v/v) to 100ml (concentration of Fe (III) is 10 microgram per ml). Standard Fe (III) samples containing 1 to 40 microgram Fe (III) solutions were prepared from above stock solution for analysis by IDF method for calibration. Composition of the solutions necessary of the calibration curve was explained in Table 3.1.

Table 3.2: Amount of solutions for calibration curve.

Amount of Fe (III) (µg)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
Stock solution (ml)	0	0.5	1	1.5	2	2.5	3	3.5	4
Solvent (ml)	10	9.5	9.0	8.5	8.0	7.5	7.0	6.5	6.0

A volume of 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml and 4ml of the standard iron solution were transfered to the test tubes to obtain a series with 5, 10, 15, 20, 25, 30, 35, 40µg of iron (III) ion. Then, 9.5ml, 9.0ml, 8.5ml, 8.0ml, 7.5ml, 7.0ml, 6.5ml, and 6.0ml of dichloromethane/methanol mixture were added and vortexed for some seconds to have homogeneous mixture.

For the measurement, 50µl of ammonium thiocyanate solution was added, vortexed for some seconds and after exactly 5 minutes measured at 500nm with the spectrophotometer by using a solvent (dichloromethane/methanol (7:3) solution) as a blank. The calibration curve was obtained by plotting the extinction of the series against the added amount of iron (III) ion, expressed as µg Fe(III).

For sample analysis, 0.01g of oil sample was weighed to the test tubes and 10ml of dichloromethane and methanol has added by using volumetric pipette. 50µl of ammonium thiocyanate solution was added, vortexed for some seconds, then immediately 50µl of Fe (II) solution was added, vortexed for some seconds and absorbance was measured at 500nm exactly after 5 minutes.

Results were expressed as milliequivalents per kg of oil according to the formula below;

$$PV = (\text{Corrected absorbance} \times m) / (55.84 \times w \times 2) \qquad \textbf{(3.5)}$$

where,

Corrected absorbance= $E_{sample} - E_{reagent\ blank}$

m= slope of the calibration curve

w= mass in grams of the sample taken

55.84= atomic weight of Iron

3.3.4 Conjugated dienes and trienes

Conjugared dienes and trienes were measured directly by utraviolet absorption after the oil is dissolved in iso-octane.

For determination of conjugated dienes, 10mg of oil samples were dissolved in 10ml of iso-octane and finally vortexed. Samples were put in a quartz cuvette one by one and the absorbance was measured at 233nm by using a UV-visible spectrophotometer (Agilent Technologies, Diegem, Belgium). Iso-octane was used as a blank. The experiment was performed in triplicate.

For the conjugated trienes, depending on the oxidation status, 30-50mg of the oil samples were dissolved in 10ml of iso-octane and vortexed. Absorbance was measured at 268nm by using a UV-visible spectrophotometer (Agilent Technologies, Diegem, Belgium). Iso-octane was used as a blank. The experiment was performed in triplicate.

Calculation of both conjugated dienes and trienes were done according to the formula below by using absorbance and weights recorded before;

$$A = \varepsilon \times b \times C \quad \textbf{(3.6)}$$

where,

A= corrected absorbance

ε= molecular extinction coefficient of 29000 $M^{-1}cm^{-1}$

b= width of the cuvette (cm)

C= concentration (CD and CT were expressed as micromoles CD/CT per gram of oil, μmol CD/CT kg^{-1}oil)

3.3.5 *p*-Anisidine value

The principle of *p*-anisidine (*p*-AV) method is the measurement of colored product which is a result of the reaction between aldehydes and *p*-anisidine reagent under acidic conditions. The *p*-AV of the oil samples was determined quantitatively by using the AOCS Official Method Cd 18-90 (AOCS, 1990). For preparation of the reagent, 0.125g of *p*-anisidine was weighed into a 50ml volumetric flask and dissolved in a glacial acetic acid to the mark. Depending on the oxidation status, 0.1-2.5g of oil samples were weighed into 25ml volumetric flasks and adjusted with iso-octane to the mark. Exactly 5ml of the solutions were transfered to the test tubes and 5ml of iso-octane to the other test tube. Absorbance of the solution was measured at 350nm, using iso-octane as a blank. A volume of 1ml of *p*-anisidine reagent was added to each test tube and vortexed. Exactly after 10 minutes, absorbance was measured at 350nm, using iso-octane tube as a blank.

Calculations were done according to the formula below;

$$p\text{-AV} = 25*(1{,}2*A_s - A_b) / w \qquad \textbf{(3.7)}$$

where,

A_s=(absorbance of the solution after addition of *p*-anisidine reagent – absorbance of the iso-octane after addition of *p*-anisidine reagent)

A_b= (absorbance of the solution before addition of *p*-anisidine reagent – absorbance of the iso-octane before addition of *p*-anisidine reagent)

w= weight of the oil sample (g)

3.3.6 Epoxy fatty acid determination

3.3.6.1 Base-catalyzed transmethylation with sodium methoxide at room temperature

Transmethylation is the method for transesterification of fatty acids to form methyl esters in the presence of a catalyst, in this case, sodium methoxide. Base-catalyzed transmethylation with sodium methoxide at room temperature is done according to the method developed by Muribu et al. (2014). An amount of 30µg methyl cis-10,11-epoxyheptadecanoate, internal standard, was dried under nitrogen. Then, an amount of 200 mg oil samples were weighed to that test tubes, and 5ml of tert butyl methyl ether was added. A volume of 2.5ml of 0.2M sodium methoxide solution in methanol was added, vortexed 1 minute and allowed to stand for 2 minutes in room temperature. Immediately after 2 min, 0.17ml of 0.5M sulphuric acid was added and vortexed for some seconds. 5ml of distilled water was added and vortexed for 30 seconds. The organic layer was collected from the top and extraction was repeated with 5ml of tert butyl methyl ether. The organic layer was dried under nitrogen and the resultant FAMEs were dissolved in 2ml of n-hexane/diethyl ether (98:2, v/v) before loading onto a silica column.

3.3.6.2 Solid phase extraction (SPE)

Solid phase extraction is a seperation method of methyl esters from non-polar fractions and impurities for following GC anaylsis. It was performed according to the method developed by Mubiru et al. (2014). Briefly, silica gel was dried at 450°C in a muffle furnace (Heraeus, Belgium) overnight, then put in desicator to cool down. Then, the moisture content was adjusted to 10% and the mixture was put to the shaker, later stored in desicator until it is used.

To pack the column, around 1g of activated silica gel was weighed into an empty SPE catridge column (6ml, 6.5cm x 1.3cm). A volume of 5ml of hexane:diethylether solution in a ratio of 98:2 v/v was added for conditioning. Air bubbles are avoided for uniform packing of the column. Then, 1 cm of sea sand was added to finalize the column packing.

Samples were dissolved in 2ml of n-hexane/diethylether solution (98:2, v/v) and loaded onto the prepared silica columns. A volume of 15ml of hexane:diethylether solution (98:2, v/v) was added to the column to remove non-polar fractions. Then, a volume of 15ml of n-hexane/diethylether solution (90:10, v/v) was loaded onto the column to elute the epoxy FAMEs. The collected solution was dried under nitrogen with a gentle flow of nitrogen and dissolved in 200µl of iso-octane which is then injected to the vials for GC-FID analysis. GC-FID analysis was performed by using the same procedure for the analysis of FAMEs which was explained before, in Section 3.3.2.1.

3.3.6.3 Calculation

The amount of epoxy fatty acids per gram of oil (µg g^{-1}) was calculated on the basis of the amount of added internal standard and sample weight together with the peak area. The correction factor based on C17:0 as an internal standard and was 1.22 depending on the paper of Mubiru et al. (2014).

Calculations were done according to formula below;

$$\text{Epoxy fatty acid amount } (\mu g\ g^{-1} \text{ oil}) = [(A_c \times M_{IS}) / A_{IS} \times C)] / M_s \qquad \textbf{(3.8)}$$

where,

A_c= area of component

M_{IS}= weight of internal standard (µg)

A_{IS}= area of internal standard

C= correction factor

M_s= weight of sample

Statistical analyses were performed by using one-way ANOVA in SPSS program to compare the means of epoxy fatty acids.

4. RESULTS AND DISCUSSION

4.1 Fatty Acid Composition

Fatty acid composition of oil samples were identified by GC-FID based on the use of methyl nonadecanoate (19:0) as internal standard. Fatty acid compositions of the olive, sunflower and linseed oil blends are shown in Table 4.1. Juita et al. (2012) indicated that palmitic acid, stearic acid as saturated fatty acid, oleic, linoleic and linolenic acid as unsaturated fatty acid are present in linseed oil. According to the current results, linseed oil blends which were represented as model A and D had the highest amount of C18:3 (linolenic acid) among the other oil samples. Both two linseed oil blends (model A and D) showed around 38% of linolenic acid with an amount of around 33 g/ 100g of oil, 32% of oleic acid (C18:1) with around 28 g/ 100g of oil, 9% of C16:0 (palmitic acid) with around 8 g/ 100g of oil and trace amount of other compounds. On the other hand, linoleic acid (C18:2) was the primary component found in sunflower oil blends (model B and E) which had around 45% with an amount of 38 g/ 100g oil. Other prominent compound was oleic acid with percentage of 41% (35 g/ 100g of oil). Olive oil blends, namely model C and F, had oleic acid, palmitic acid and linoleic acid with percentage of around 62%, 17%, and 15%, respectively.

Although the same oil models showed the same type of fatty acid composition, all stripped olive oil added models (namely D, E and F) had lower amount of compounds than the non-stripped olive oil added ones (namely A, B and C) probably due to the loss of some compounds during the stripping process.

Table 4.1: Fatty acid composition of oil blends.

	Fatty Acid Composition (g/ 100g of oil)[a]							
Model	C16:0	C16:1	C18:0	C18:1c11	C18:2	C20:0	C18:3n3	C22:0
A	8.29±0.19	0.70±0.03	3.09±0.08	28.05±0.71	13.68±0.32	0.22±0.02	33.56±0.65	0.20±0.02
B	8.44±0.07	0.66±0.09	2.47±0.05	34.77±0.53	38.02±0.47	ND	0.25±0.02	0.40±0.04
C	16.95±1.77	1.86±0.19	2.55±0.34	60.30±6.64	14.27±1.54	0.25±0.03	0.64±0.06	ND
D	7.90±0.67	0.54±0.06	2.99±0.24	26.97±2.20	12.96±1.01	0.18±0.01	32.64±2.43	ND
E	8.11±0.21	0.56±0.02	2.40±0.07	33.89±0.86	36.87±1.05	0.06±0.1	0.2*	0.25±0.22
F	14.98±1.10	1.66±0.10	2.32±0.15	54.34±3.76	12.49±0.85	0.25±0.01	0.55±0.07	ND

*standard deviation (std) < 0.01, ND not determined

[a] Experimental data are presented as mean of three independent replicates ± standard deviations

A: stripped linseed oil-non-stripped olive oil blend **B**: stripped sunflower oil-non-stripped olive oil blend **C**: stripped-non-stripped olive oil blend

D: stripped linseed oil-stripped olive oil blend **E**: stripped sunflower oil-stripped olive oil blend **F**: stripped olive oil blend

4.2 Chlorophyll Content

Chlorophyll was used as a sensitizer of the reaction for photooxidation. Chlorophyll content was measured to determine the level of photosensitizer in the oil. The chlorophyll content of the oil blends during 56 days incubation can be seen in the Table 4.2. Chlorophyll pigments were completely removed in oil blends with stripped olive oil (model D, E and F). In the study of Khan and Shahidi (1999), chlorophyll and caretonoid pigments were also not found in stripped virgin olive oil which was due to the stripping process. Chlorophyll content of linseed, sunflower and olive oil blends with non-stripped olive oil (A, B and C models) were reduced or removed after the end of 56 days incubation. This is due to the degredation of chlorophyll and transformation to the other compounds such as pyropheophytin, 13^2 hydroxy-pheophytin a, 15^1 hydroxy-lactone pheophytin a and others (Giuliani et al., 2011). In addition, the color of the oil blends became lighter and the oil blends became thicker over the time of incubation. In the study of Psomiadou and Tsimidou (2002) conducted with olive oil, total chlorophyll content was demonstrated as gradually reduced during exposure to light and also pheophytin *a* was found to be destructed. However, chlorophyll content in sunflower and olive oil blends showed fluctiotions probably due to some analytical errors. The initial concentration of chlorophyll was around 6.80 mg of pheophytin/ kg oil in all non-stripped virgin olive oil added samples. Giuliani et al. (2011) indicated that chlorophyll content differs from region to region depending on the olive cultivar and in different geographical areas, total chlorophyll amount varies between 2.41–64.1 mg/kg oil.

Table 4.2: Chlorophyll content of the oil samples in photooxidation.

	Chlorophyll Content (mg of pheophytin / kg oil)•					
	A (SLO&VOO)	B (SSO&VOO)	C (SVOO&VOO)	D (SLO&SVOO)	E (SSO&SVOO)	F (SVOO&SVOO)
Day 0	6.80 ± 0.01	6.85*	6.66*	-	-	-
Day 4	6.84 ± 0.01	6.83 ± 0.01	6.30 *	-	-	-
Day 7	6.62 ± 0.01	5.93 ± 0.1	2.61*	-	-	-
Day 14	6.05 ± 0.01	3.11 ± 0.01	3.35 ± 0.03	-	-	-
Day 21	5.57 ± 0.05	6.23 ± 0.03	1.98 ± 0.18	-	-	-
Day 28	2.43 ± 0.06	6.19 ± 0.04	1.73 ± 0.05	-	-	-
Day 42	0.02*	5.81 ± 0.03	1.31 ± 0.03	-	-	-
Day 56	ND	4.47 ± 0.1	0.85 ± 0.03	-	-	-

*standard deviation (std) < 0.01

• Experimental data are presented as mean of three independent replicates ± standard deviations

4.3 Peroxy Value, Conjugated Dienes and Trienes

The primary oxidation products of photooxidation were determined by peroxide value (PV), conjugated dienes (CD) and trienes (CT). Analyses were performed in triplicates and results were expressed by means. PV, CD and CT results of all oil models were summarized in Table 4.3, 4.4, and 4.5, respectively.

PV of linseed oil blend with chlorophyll (A model) increased over time (Table 4.3). However, linseed oil blend without chlorophyll (D model) showed fluctuations during incubation. CD and CT of these blends generally increased over time except some fluctuations.

PV, CD and CT values of sunflower oil blends can be seen in Table 4.4. Peroxide value of both sunflower oil blends showed a reduction after 42nd day of incubation because of the formation of secondary products. The formation of CD was higher in sunflower oil blend with chlorophyll (B model) during the first month of oxidation but CD value of the blend without chlorophyll suddenly increased after one month. Formation of CT was mostly higher in the oil blend with chlorophyll till the end of incubation period. However, CT value of the oil blend without chlorophyll showed a sudden increase in 56 days of incubation.

Peroxide, CD and CT values of olive oil blends are shown in Table 4.5. Peroxide values of the olive oil blend with non-stripped olive oil (C model) which contains more chlorophyll were higher than the stripped olive oil blend (F model). Similarity was indicated in the paper of Psomiadou and Tsimidou (2002). Initial amount of CD and CT in of the olive oil blend with non-stripped olive oil was also higher than the stripped olive oil blend.

Table 4.3, 4.4, and 4.5 illustrate that PV increased in all oil models during the entire experimental period despite some fluctuations. Peroxide value of linseed oil blends were higher both in the beginning and during the oxidation compared to the other oils as linseed oil is highly unsaturated and very sensitive to oxidation.

It can be observed that levels of conjugated dienes increased with time except in linseed oil blend without chlorophyll. CD values of this model decreased after 28 days of incubation. Since linseed oil blend without chlorophyll contained not only high level of linolenic acid but also stripped olive oil without antioxidants. Therefore, it is highly susceptible to lipid oxidation and capable of fast forming secondary products.

Conjugated dienes formed in olive oil blends were less than in oil models rich in polyunsaturated fatty acids (A, B, D and E models). As olive oil blends contain high level of oleic acid, which is less susceptible to lipid oxidation, and low levels of linoleic acid and linolenic acid, which are capable of forming more CD.

CT content in oil blends during photo-oxidation given in Table 4.3, 4.4, and 4.5 did not change considerably with time, with the exception of linseed oil blends (A and D model). The highest amount of CT were formed in linseed oil blends, followed by sunflower oil blends. Olive oil blends had the lowest levels of formed CT as they contained low amount of linolenic acid which is capable of forming conjugated trienes.

Initial amount of CD values were found to be higher than CT values in all type of oils analyzed. Moreover, the amount of CD formed during photo-oxidation was also higher than CT in all blends.

Table 4.3: Peroxide value (PV), conjugated diene (CD) and conjugated triene (CD) of linseed oil blends.

Model	Time (day)	PV (mEq O2 kg-1)[a]	CD (µmol kg^{-1})[a]	CT (µmol kg^{-1})[a]
A	0	0.04*	5.37±0.06	0.30±0.03
	4	0.35±0.02	19.17±0.42	0.44±0.01
	7	0.64±0.03	29.18±1.97	0.48*
	14	1.07±0.05	58.93±0.85	0.89±0.04
	21	1.79±0.2	81.61±3.48	1.62±0.05
	28	0.01*	86.94±3.18	1.89±0.08
	42	7.46±0.1	110.90±2.7	42.32±1.88
	56	8.74±0.22	96.39±7.56	46.47±0.2
D	0	0.04*	5.30±0.35	0.56±0.06
	4	1.08±0.02	70.36±0.14	3.53±0.07
	7	0.57±0.03	98.98±4.27	15.99±0.43
	14	4.25±0.39	112.68±2.52	37.75±1.18
	21	5.16*	112.29±2.14	5.34±0.02
	28	0.01*	103.76±1.98	4.59±0.16
	42	7.81±0.71	98.70±1.32	40.27±0.19
	56	5.60±0.46	97.84±1.1	32.36±0.41

* standard deviation (std) < 0.01

[a] Experimental data are presented as mean of three independent replicates ± standard deviations

A: Linseed oil blend with chlorophyll; **D**: Linseed oil blend without chlorophyll

Table 4.4: Peroxide value (PV), conjugated diene (CD) and conjugated triene (CD) of sunflower oil blends.

Model	Time (day)	PV (mEq O2 kg-1)[a]	CD (µmol kg^{-1})[a]	CT (µmol kg^{-1})[a]
B	0	0.02*	6.33±0.02	2.48±0.05
	4	0.2±0.01	10.63±0.22	2.21*
	7	0.34*	15.87±0.27	2.15±0.04
	14	0.79±0.06	20.22±0.98	1.62±0.1
	21	1.39±0.22	40.95±0.01	2.26*
	28	1.97±0.05	50.94±0.29	2.17±0.02
	42	2.92±0.36	76.84±2.16	2.36±0.1
	56	2.76±0.15	84.74±1.43	2.59±0.07
E	0	0.01*	5.08±0.13	2.04±0.21
	4	0.01*	5.35±0.13	2.10±0.01
	7	0.04*	6.21±0.07	1.87±0.04
	14	0.06*	4.74±0.01	1.68±0.02
	21	0.13±0.01	14.63±0.24	1.98±0.08
	28	0.52±0.01	36.76±2.03	1.58±0.05
	42	2.43±0.01	87.63±1.64	1.88±0.1
	56	2.36*	89.04±1.37	3.44±0.43

*standard deviation (std) < 0.01

[a] Experimental data are presented as mean of three independent replicates ± standard deviations

B: Sunflower oil blend with chlorophyll

E: Sunflower oil blend without chlorophyll

Table 4.5: Peroxide value (PV), conjugated diene (CD) and conjugated triene (CD) of olive oil blends.

Model	Time (day)	PV (mEq O2 kg-1)[a]	CD (µmol kg^{-1})[a]	CT (µmol kg^{-1})[a]
C	0	0.02*	4.52±0.09	0.20*
	4	0.1±0.01	5.32*	0.18*
	7	0.18±0.02	3.17±.0.05	ND
	14	0.38±0.04	7.89±0.03	0.19*
	21	0.41±0.04	10.48±0.52	0.32±0.01
	28	0.57±0.03	11.48±0.34	0.32*
	42	0.75±0.05	13.35±1.03	0.41*
	56	1.07±0.05	19.16±0.44	0.59±0.03
F	0	0.02*	3.14±0.03	0.12*
	4	0.01*	4.06±0.04	0.09*
	7	0.53±0.05	2.41±0.02	ND
	14	0.12*	9.18±0.05	0.07*
	21	0.18*	14.77±0.55	0.23*
	28	0.23±0.01	18.59±1.2	0.33±0.01
	42	0.44±0.04	31.06±0.5	0.77±0.05
	56	0.64±0.05	43.21±0.22	1.31±0.06

*standard deviation (std) < 0.01

[a] Experimental data are presented as mean of three independent replicates ± standard deviations

ND, not detected

C: Stripped olive oil blend with non-stripped olive oil

F: Stripped olive oils blend

4.4 *p*-Anisidine Value

p-Anisidine value (*p*-AV) indicates the amount of secondary products of oxidation. It gives the idea about the formation of saturated and unsaturated carbonyl compounds (Juita et al., 2012). *p*-AV of linseed, sunflower and olive oil samples during photooxidation can be seen in Table 4.6. Linseed oil samples showed higher *p*-anisidine value than the other oil blends during incubation time due to the high unsaturation of linolenic acid in linseed oil. However, sunflower oil blend without chlorophyll (model E) had the highest initial *p*-AV with 8.65±0.28 g^{-1}, and sunflower oil blend with chlorophyll followed with 7.76±0.01 g^{-1}.

Table 4.6: *p*-Anisidine of the oil blends.

	***p*-Anisidine (1/g oil)**[•]					
Day	Model A	Model B	Model C	Model D	Model E	Model F
0	3.5±0.25	7.76±0.01	6.26±0.83	2.14±0.17	8.65±0.28	4.27±0.18
4	14.28±0.36	2.96±0.39	2.16±0.27	14.17±0.10	3.27±0.09	0.78±0.02
7	16.11±1.34	6.27±0.44	1.97±0.11	14.0±0.36	4.55±0.03	0.8±0.05
14	12.33±0.62	3.62±0.11	2.49±0.22	82.37±3.18	4.54±0.07	1.03±0.06
21	19.51±0.67	6.54±0.15	1.55±0.05	24.93±0.06	6.45±0.14	1.36±0.06
28	10.80±0.79	3.36±0.30	1.36±0.03	128.29±2.15	3.48±0.48	1.61±0.02
42	74.61±6.49	4.76±0.22	0.54±0.04	202.91±3.24	6.90±0.44	3.28±0.08
56	156.64±5.19	3.82±0.07	1.33±0.19	199.68±0.61	9.18±0.85	6.28±0.14

[•] Experimental data are presented as mean of three independent replicates ± standard deviations

4.5 Epoxy Fatty Acids

Epoxy fatty acid (EFA) content in the oil samples was determined by GC-FID according to the method developed by Mubiru et al. (2014). Identification of individual epoxy FAMEs was carried out by comparison of retention times with the standards. Results of the linseed, sunflower and olive oil samples are given in Table 4.7, 4.8 and 4.9, respectively. Analyses were performed in triplicates. Limit of detection (LOD) was defined as 2.8 μg g^{-1} and limit of quantification (LOQ) was 5.5 μg g^{-1} according to the paper of Mubiru et al. (2014). There was an obvious increase in the amount of total epoxy among all type of oils analyzed during photo-oxidation.

In the end of 56 days, total amount of epoxy of linseed oil blend with stripped olive oil (D model) was almost two times than the non-stripped olive oil blend of linseed oil (A model). However, initial total amount of epoxy was founded to be higher in linseed oil blend with chlorophyll (A model). Non-stripped olive oil blend (A model) was expected to have higher amount of epoxy formation due to the photosensitizer effect of chlorophyll in olive oil but the results showed the opposite trend. Linseed oil blend without chlorophyll (D model) had higher content of EFA which was attributed to high reactivity due to the lack of antioxidants in stripped model. Furthermore, the richness of linolenic acid in this blend (D model) makes it more sensitive to oxidation. In singlet oxygen oxidation, reaction rate of linolenic acid was found to be faster than linoleic acid because of two pentadienyl group of linolenic acid (Kim and Min, 2008). Comparison of the amount of epoxy formed during photo-oxidation in all three different type of oil blends demonstrates that linseed oil which consists of primarly linolenic acid has the highest reaction rate among the others. In the end of incubation period, methyl *cis*-12, 13 and *trans*-9, 10-epoxylinoleate (12, 13 *cis* & 9, 10 *trans* EOL) which is the epoxy formed in both linseed oil blends, had the highest amount among the other types due to the high unsaturation of linseed oil. The other epoxy types which were abundant in both models were *trans*-9, 10-epoxystearate (9, 10 ES *trans*), *cis*-9, 10-epoxystearate (9, 10 ES *cis*), *trans*-12, 13-epoxylinoleate (12, 13 EOL *trans*) and *trans*-15, 16-epoxylinoleate (15, 16 EOL *trans*). Methyl *cis*-9, 10-epoxystearate (9, 10 ES *cis*), and methyl 12, 13 *cis* and 9, 10 *trans* EOL were present in fresh samples of both models despite under LOQ in the linseed oil blend without chlorophyll (D model).

Mubiru et al. (2013) suggested some reasons for the high amount of EFA present in the fresh samples, which are unspecificity of PV method due to the lack of information about the concentration of the different hydroperoxides and possibility of another route for the formation of EFA other than decomposition of hydroperoxides.

Epoxy fatty acid content in sunflower oil models is present in Table 4.8. Initial high total amount of epoxy present in the sunflower oil blend with chlorophyll is probably due to the same reasons with linseed oil models explained in the paper of Mubiru et al. (2013). Comparison between two models of sunflower oil blends showed that 12, 13 *cis* & 9, 10 *trans* EOL was the epoxy fatty acid which only present in the sunflower oil blend with non-stripped olive oil (B model) during photo-oxidation. However, it was not found in the blend with stripped olive oil, which means this epoxy originated from olive oil. Moreover, at the end of incubation period, methyl *cis*-9, 10-epoxystearate and *cis*-9, 10-epoxyoleate were present in higher amount in the sunflower oil blend with chlorophyll (B model) than the other type of epoxy fatty acids. On the contrary, *trans*-9, 10-epoxystearate and *trans*-9, 10-epoxyoleate were present in higher amount in the blend without chlorophyll (E model) than the others. It indicates that the sunflower oil blend which contains non-stripped olive oil (B model) is prone to form *cis* configuration while the model which contains the stripped olive oil tends to form *trans* configuration. In the paper of Mubiru et al. (2013), the initial amount of methyl *cis*-9, 10-epoxystearate and *cis*-9, 10-epoxyoleate were also found to be higher among the other epoxy fatty acid in sunflower oil. Contrary to linseed oil blends, the sunflower oil blend with chlorophyll (B model) had higher total amount of epoxy fatty acid than the blend without chlorophyll (E model) during photo-oxidation. This is probably due to the act of chlorophyll as a photosensitizer.

Epoxy fatty acid content in olive oil samples is shown in Table 4.9. It can be clearly seen that methyl *cis*-9, 10-epoxystearate is the major epoxy fatty acid formed during photooxidation in the olive oil blend with chlorophyll (C model), whereas methyl *trans*-9, 10-epoxystearate is in the stripped olive oil blend (F model). Methyl 12, 13 *cis* & 9, 10 *trans* EOL was detected in every analysis in the olive oil blend with chlorophyll (C model) even if it was under the LOQ.

However, it was detected only in the last analysis of the stripped olive oil blend (F model) as it was above the LOQ. Although *cis*-15, 16-EOL; *cis*-9, 10-epoxylinoleate (9, 10 EOL *cis*); *trans*-12, 13-EOL and *trans*-15, 16-EOL were not formed in the olive oil blend with chlorophyll (C model); *trans*-12, 13-EOL were detected at the end of incubation period in the olive oil blend without chlorophyll (F model) as it was above the LOQ.

In fresh samples of all oil blends, *cis* configuration dominated as indicated in the study of Mubiru et al. (2013). Since *cis* isomers are naturally occuring isomers and double bonds found in most fatty acids have *cis* configuration. Epoxystearate in all oil blends showed *trans* domination in absence of singlet oxygen and *cis* domination in the presence of singlet oxygen during incubation time. Moreover, *trans* configuration in all oil blends without chlorophyll dominated and *cis* configuration of epoxyoleate in oil blends with chlorophyll dominated except linseed oil blend. For linseed oil blend with chlorophyll, *trans* configuration became dominant after one month of incubation.

Linseed oil blends contained high levels of polyunsaturated epoxy FAMEs. Those are 9, 10- EOL *cis* and *trans*; 12, 13- EOL *cis* and *trans*; 15, 16- EOL *cis* and *trans* isomers. Sunflower oil blends had more monounsaturated epoxy FAMEs, namely 12, 13- EO *cis* and *trans*; 9, 10- EO *cis* and *trans* isomers, while higher levels of saturated monoepoxy FAMEs (9, 10- ES *cis* and *trans* isomers) formed in olive oil blends (C and F models).

In the end of 56 days, PV of the oil blends were still low but epoxy fatty acids were much more higher than PV. It demonstrates that PV is not sufficient alone to determine the quality of the oil.

Table 4.7: Epoxy fatty acids of linseed oil blends.

Model	Time (day)	9, 10 ES *trans*	9, 10 ES *cis*	12, 13 EO *trans*	9, 10 EO *trans*	12, 13 EO *cis*	9, 10 EO *cis*	12, 13 EOL *trans*	15, 16 EOL *trans*	12, 13 *cis* & 9, 10 *trans* EOL	15, 16 EOL *cis*	9, 10 EOL *cis*	Total Epoxy
		Epoxy Fatty Acid Content (μg g^{-1}) •											
A	0	<LOQ	32.53±0.26^{a}	ND	ND	<LOQ	8.51±0.10^{a}	ND	ND	14.79±0.21^{a}	12.67±0.06^{a}	13.92±0.55^{a}	82.42
	4	<LOQ	42.48±1.37^{a}	ND	ND	8.04±0.58^{a}	13.69±0.54^{b}	ND	ND	25.77±0.64^{b}	20.87±0.99^{b}	29.76±1.39^{ab}	140.61
	7	<LOQ	42.25±2.06^{a}	ND	ND	7.92±0.54^{a}	13.66±0.77^{b}	ND	ND	24.10±1.06^{ab}	19.64±0.67^{b}	26.08±1.04^{ab}	133.66
	14	<LOQ	37.25±1.80^{a}	ND	ND	<LOQ	10.27±0.89^{a}	ND	ND	26.41±2.83^{ab}	13.20±1.25^{a}	17.10±0.87^{a}	104.23
	21	<LOQ	47.56±0.95^{a}	ND	ND	12.45±0.17^{b}	19.59±0.54^{c}	ND	<LOQ	40.23±0.41^{c}	29.60±0.68^{c}	47.58±0.41^{b}	197.01
	28	56.24±1.38^{a}	225.99±0.92^{b}	22.56±0.18^{a}	46.24±0.50^{a}	53.42±1.32^{c}	87.75±0.29^{d}	38.35±0.04^{a}	37.72±0.32^{a}	244.28±7.02^{d}	112.21±1.73^{d}	203.51±2.03^{c}	1128.29
	42	480.65±10.59^{b}	411.26±3.75^{c}	203.25±1.20^{b}	196.78±2.39^{b}	103.39±1.09^{d}	132.34±0.09^{e}	498.50±5.31^{b}	526.27±6.95^{b}	648.79±2.0^{e}	139.26±0.89^{e}	294.27±11.85^{d}	3634.76
	56	853.80±8.93^{c}	676.09±25.53^{d}	392.15±9.68^{c}	384.50±3.86^{c}	201.75±1.93^{e}	215.04±0.40^{f}	882.74±15.1^{c}	832.13±11.6^{c}	1109.05±9.7^{f}	254.16±2.27^{f}	398.76±25.67^{e}	6200.16
D	0	ND	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	0
	4	20.00*^{a}	19.33±0.66^{a}	9.78±0.21^{a}	10.54±0.05^{a}	6.91±0.57^{a}	9.14±0.42^{a}	27.35±0.05^{a}	29.06±0.36^{a}	49.55±0.04^{a}	22.84±0.05^{a}	27.65±0.72^{a}	232.15
	7	109.27±4.42^{b}	40.0±0.65^{b}	49.43±2.48^{b}	52.24±2.15^{b}	14.05±0.28^{a}	13.98±0.41^{a}	127.94±7.13^{b}	129.56±6.78^{b}	160.52±4.98^{b}	36.0±1.38^{a}	39.02±3.10^{a}	772.02
	14	350.43±0.15^{c}	158.38±3.63^{c}	152.27±2.50^{c}	154.77±1.36^{c}	68.47±3.01^{b}	68.21±3.74^{b}	377.45±3.00^{c}	334.03±7.01^{c}	449.85±11.27^{c}	119.15±4.34^{b}	117.90±2.84^{b}	2350.90
	21	559.82±0.52^{d}	322.68±4.83^{d}	170.59±9.82^{c}	175.80±14.2^{d}	118.84±3.54^{c}	111.75±4.57^{c}	497.28±2.30^{d}	494.16±18.78^{d}	686.89±19.05^{d}	144.86±3.30^{b}	140.82±5.27^{b}	3423.49
	28	1261.41±2.51^{e}	762.56±9.16^{e}	519.82±3.89^{d}	520.79±2.72^{e}	251.12±5.07^{d}	238.40±1.19^{d}	1155.64±3.68e	1012.06±6.53^{e}	1400.12±0.30^{e}	376.10±4.68^{c}	371.14±0.91^{c}	7869.15
	42	1455.05±3.82^{f}	1130.42±2.70^{f}	616.76±3.45^{e}	585.51±2.72^{f}	339.53±3.89^{e}	349.30±3.29^{e}	1246.33±23.19^{f}	1046.90±10.05^{f}	1517.81±16.08^{f}	511.58±3.26^{d}	534.53±6.66^{d}	9333.73
	56	1608.47±26.41^{g}	1638.06±6.18^{g}	641.01±14.17^{f}	623.68±4.92^{g}	460.62±9.3^{f}	489.57±0.77^{f}	1303.12±18.37^{g}	1171.40±4.02^{g}	1730.25±29.11^{g}	547.28±41.15^{d}	652.81±37.71^{e}	10866.28

ND, not detected; <LOQ, under the limit of quantification (LOQ=5.5 μg g^{-1}); ES, epoxystearate; EO, epoxyoleate
• Experimental data are presented as mean of three independent replicates ± standard deviations
A: Linseed oil blend with chlorophyll; D: Linseed oil blend without chlorophyll

Table 4.8: Epoxy fatty acids of sunflower oil blends.

		Epoxy Fatty Acid Content (μg g^{-1}) •											
Model	Time (day)	9, 10 ES *trans*	9, 10 ES *cis*	12, 13 EO *trans*	9, 10 EO *trans*	12, 13 EO *cis*	9, 10 EO *cis*	12, 13 EOL *trans*	15, 16 EOL *trans*	12, 13 *cis* & 9, 10 *trans* EOL	15, 16 EOL *cis*	9, 10 EOL *cis*	Total Epoxy
B	0	ND	33.70±3.11[a]	ND	ND	11.96±1.75[a]	17.31±1.91[a]	ND	ND	<LOQ	ND	ND	62.97
	4	<LOQ	49.02±1.51[ab]	ND	ND	22.55±0.81[ab]	31.64±1.56[b]	ND	ND	<LOQ	ND	ND	103.21
	7	<LOQ	57.92±0.02[b]	ND	ND	28.93±0.1[b]	41.55±0.34[c]	ND	ND	<LOQ	ND	ND	128.40
	14	<LOQ	48.94±0.21[ab]	ND	ND	24.26±3.73[b]	37.36±1.87[bc]	ND	ND	<LOQ	ND	ND	110.56
	21	11.30±1.15[a]	165.28±2.39[c]	14.02±1.30[a]	7.17±1.57[a]	89.60±0.10[c]	134.51±2.59[d]	ND	ND	21.74±0.75[ab]	ND	ND	443.63
	28	15.03±0.73[b]	180.67±1.23[d]	11.13±0.58[a]	8.32±0.01[a]	81.56±1.27[c]	126.29±3.76[d]	ND	ND	20.51±1.96[a]	ND	ND	443.51
	42	16.72±1.22[ab]	105.40±10.42[e]	21.83±1.55[b]	9.17±2.18[a]	127.77±5.49[d]	146.45±2.95[e]	ND	ND	21.35±0.39[ab]	ND	ND	448.69
	56	97.73±3.56[c]	199.12±7.90[f]	23.93±1.63[b]	37.34±3.54[b]	163.82±1.54[e]	260.35±7.21[f]	ND	ND	23.34±1.20[b]	ND	ND	805.63
E	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
	4	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	0
	7	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	0
	14	ND	9.49±0.29[a]	ND	ND	6.71±0.30[a]	11.67±0.45[a]	ND	ND	ND	ND	ND	27.87
	21	<LOQ	9.57±0.33[a]	<LOQ	<LOQ	7.12±0.07[a]	11.67±0.32[a]	ND	ND	ND	ND	ND	28.37
	28	12.29±1.01[a]	7.29±0.42[a]	10.75±1.71[a]	13.14±1.01[a]	<LOQ	7.34±0.25[a]	ND	ND	ND	ND	ND	50.81
	42	108.69±3.80[b]	56.50±6.79[b]	105.21±1.77[b]	105.98±0.59[b]	80.43±7.78[b]	55.18±2.16[b]	ND	ND	ND	ND	ND	511.98
	56	241.43±19.75[c]	149.30±7.67[c]	222.87±16.31[c]	238.54±18.02[c]	141.41±1.32[c]	130.47±7.14[c]	ND	ND	ND	ND	ND	1124.02

ND, not detected; <LOQ, under the limit of quantification (LOQ=5.5 μg g^{-1}); ES, epoxystearate; EO, epoxyoleate
• Experimental data are presented as mean of three independent replicates ± standard deviations
B: Sunflower oil blend with chlorophyll; E: Sunflower oil blend without chlorophyll

Table 4.9: Epoxy fatty acids of olive oil blends.

		Epoxy Fatty Acid Content (μg g^{-1}) •											
Model	Time (day)	9, 10 ES *trans*	9, 10 ES *cis*	12, 13 EO *trans*	9, 10 EO *trans*	12, 13 EO *cis*	9, 10 EO *cis*	12, 13 EOL *trans*	15, 16 EOL *trans*	12, 13 *cis* & 9, 10 *trans* EOL	15, 16 EOL *cis*	9, 10 EOL *cis*	Total Epoxy
C	0	ND	46.95±0.29[a]	ND	ND	5.97±0.28[a]	8.86±0.43[a]	ND	ND	<LOQ	ND	ND	61.77
	4	ND	66.90±1.33[bc]	ND	ND	8.28±0.21[ab]	13.28±0.36[bc]	ND	ND	<LOQ	ND	ND	88.46
	7	ND	64.07±1.10[bc]	ND	ND	7.49±0.22[ab]	12.14±0.15[b]	ND	ND	<LOQ	ND	ND	83.70
	14	ND	58.72±0.78[ab]	ND	ND	7.81±0.12[ab]	11.77±0.03[ab]	ND	ND	<LOQ	ND	ND	78.29
	21	ND	74.15±1.42[c]	ND	ND	10.14±0.18[b]	15.03±0.30[c]	ND	ND	<LOQ	ND	ND	99.31
	28	<LOQ	113.65±13.35[d]	ND	ND	18..27±1.73[c]	26.20±2.07[d]	ND	ND	6.80±0.45[a]	ND	ND	164.92
	42	28.26±2.41[a]	214.55±4.30[e]	7.40±1.12[a]	9.06±0.44[a]	26.91±2.22[d]	50.09±1.11[e]	ND	ND	25.02±0.04[b]	ND	ND	361.29
	56	18.56±1.24[b]	235.06±4.13[f]	15.55±0.51[b]	18.35±2.72[b]	31.28±1.89[e]	59.37±0.09[f]	ND	ND	27.63±4.45[b]	ND	ND	405.80
F	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
	4	<LOQ	8.08±0.61[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	8.08
	7	<LOQ	8.22±0.38[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	8.22
	14	11.64±0.44[a]	9.02±0.41[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	20.66
	21	22.40±0.53[a]	15.71±0.16[b]	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	ND	ND	38.10
	28	64.77±0.51[b]	33.73±2.36[c]	14.83±0.04[a]	13.30±0.09[a]	<LOQ	9.25±0.61[a]	ND	ND	ND	ND	ND	135.88
	42	223.98±21.21[c]	80.35±2.64[d]	49.96±4.95[b]	49.96±4.50[b]	11.18±0.38[a]	17.74±1.05[b]	ND	ND	5.81±0.22[a]	ND	ND	438.98
	56	567.86±2.69[d]	136.36±0.28[e]	116.49±0.14[c]	112.80±2.85[c]	18.14*[b]	25.64±0.75[c]	6.16±0.30	<LOQ	19.72±0.40[b]	ND	ND	1008.11

*standard deviation (std) < 0.01; ND, not detected; <LOQ, under the limit of quantification (LOQ=5.5 μg g^{-1})
ES, epoxystearate; EO, epoxyoleate
• Experimental data are presented as mean of three independent replicates ± standard deviations
C: Stripped olive oil blend with chlorophyll; F: Stripped olive oil blend without chlorophyll

4.6 Correlations

SPSS Pearson's Correlation (2-tailed) and one-way ANOVA were used for statistic analysis. The relations between epoxy formation and PV, CD, CT determined by Pearson's Correlation can be seen in Table 4.10.

There was no significant correlation between total epoxy and CT in the sunflower oil blend with chlorophyll and linseed oil blend without chlorophyll (B and D models) ($p > 0.05$, $n=8$). No correlation was found between total epoxy and CD in both linseed oil blends (A and D models) ($p > 0.05$, $n=8$).

For linseed oil blend with chlorophyll, correlation of 0.944 was found between PV and total epoxy fatty acid ($n= 8$). However, in the blend without chlorophyll, there was no significant correletaion between PV and total epoxy fatty acid ($p > 0.05$, $r= 0.602$, $n= 8$).

Sunflower oil blend with chlorophyll (model B) showed a possitive correlation of 0.889 between PV and total epoxy fatty acid ($n= 8$). Similarly, there was a possitive correlation of 0.90 between PV and total epoxy fatty acid ($n=8$) in the oil blend without chlorophll (model E).

Statistically, total epoxy fatty acid of the olive oil blend with chlorophyll (C model) was significantly correlated with PV ($r= 0.917$, $n= 8$). There was a possitive correlation between PV and total epoxy of the stripped olive oil blend (F model) but not as significant as in the oil blend with chlorophyll (C model) ($r= 0.734$, $n= 8$).

Strong correlation was found between PV and CD in sunflower oil blends (B and E models) and olive oil blend with chlorophyll. However, there was no correlation in linseed oil blends (A and D models) and olive oil blend without chlorophyll between PV and CD ($p > 0.05$, $n= 8$).

Table 4.10: Correlation of PV, CD, and CT with epoxy fatty acid.

Model		Epoxy Fatty Acid	PV
A (n=8)	PV	0.944**	1
	CD	0.673	0.705
	CT	0.959**	
B (n=8)	PV	0.889**	1
	CD	0.933**	0.989**
	CT	0.511	
C (n=8)	PV	0.917**	1
	CD	0.886**	0.970**
	CT	0.851**	
D (n=8)	PV	0.602	1
	CD	0.452	0.486
	CT	0.534	
E (n=8)	PV	0.900**	1
	CD	0.889**	0.990**
	CT	0.854**	
F (n=8)	PV	0.734*	1
	CD	0.939**	0.672
	CT	0.985**	

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

5. CONCLUSION

Lipid oxidation is considered to be a health issue for years. Photooxidation is a type of lipid oxidation which causes the deterioration of foods and formation of some toxic products. Hence, it is important to monitor the process of oxidation and formed products. In this study, photooxidation of linseed, sunflower and olive oils were examined. Primary and secondary oxidation products were determined during 56 days under the effect of fluorescence light. Epoxy fatty acid formation was given a special attention because of negative health effects and the difficulties in determination.

For primary oxidation products, PV, CD and CT values were determined and initial amount of CD value was found to be higher than CT value in all type of oils. Furthermore, the amount of CD formed during photooxidation was also higher than CT in all samples. Peroxide value of linseed oil blends was higher both in fresh samples and samples analyzed during the oxidation compared to the other oils because of high unsaturation and sensitive to oxidation.

FAME and epoxy fatty acid content of the oil blends were identified by GC-FID. Linseed oil blends which consist of primarly linolenic acid had the highest reaction rate among the others. It demostrates that reaction rate is related with the number of double bond present in oil. There was an obvious increase in the amount of total epoxy among all type of oils analyzed during photooxidation. Although this study only emphasises the importance of photooxidation, it leads up the future researchs about autoxidation and thermoxidation. In the future, more researches should be done to understand the exact route of the epoxy fatty formation during oxidation reactions. Depending on the results and consideration of the effect of photooxidation, precautions including light protective packages should be taken for the human health.

REFERENCES

Che Man, Y.B., Liu, J.L., Jamilah,B., Abdul Rahman, R., 1999. Quality changes of refined-bleached- deodorized (RBD) palm olein, soybean oil and their blends during deep-fat frying. *Journal of Food Lipids* 6, 181-193, Food & Nutrition Press, Inc.

Eldin, A. K., and Pokorny, J., 2005. Lipid oxidation products and methods used for their analysis. Analysis of Lipid Oxidation edited by Afaf Kamal-Eldin and Jan Pokorny, AOCS Press

FAO, n.d. 5.3 International Standard FIL-IDF 74: 1974 - Anhydrous milk fat1 – Determination of the peroxide value, http://www.fao.org/docrep/003/X6537E/X6537E05

Frankel, E. N., 2005. Photooxidation of unsaturated fats. Lipid Oxydation, Woodhead Publishing

Giuliani, A., Cerretani, L., and Cichelli, A., 2011. Chlorophylls in Olive and in Olive Oil: Chemistry and Occurrences. *Critical Reviews in Food Science and Nutrition*, 51:7, 678-690

Gordon, M.H., 2004. Factors affecting lipid oxidation. Understanding and Measuring the Shelf-life of Food edited by R. Steele. CRC Press

Greene, J. F., Newman, J. W., Williamson, K. C., Hammock, B. D., 2000. Toxicity of epoxy fatty acids and related compounds to cells expressing human soluble epoxide hydrolase. *Chem. Res. Toxicol*, 13 (4), 217−226

Juita, Dlugogorski, B. Z., Kennedy, E. M., Mackie, J. C., 2012. Low temperature oxidation of linseed oil: a review. *Fire Science Reviews*, 1:3

Khan, M. A., Shadihi, F., 1999. Rapid oxidation of commercial extra virgin olive oilstored under fluorescent light. *Journal of Food Lipids* 6, 331-339

Kim, H. J., and Min, D. B., 2008. Chemistry of lipid oxidation. Food Lipids: Chemistry, Nutrition, and Biotechnology edited by Casimir C. Akoh and David B. Min, CRC Press

Kiokias, S., Varzakas, T. H., Arvanitoyannis, I. S., and Labropoulos, A. E., 2010. Lipid Oxidation and Control of Oxidation. Advances in Food Biochemistry edited by Fatih Yıldız, CRC Press

Knothe, G., Kenar, J. A., Gunstone, F. D., 2007. Chemical properties. The Lipid Handbook, third edition, edited by Frank D. Gunstone, John L. Harwood, Albert J. Dijkstra, CRC Press

Kolakowska, A., 2003. Lipid oxidation in food systems. Chemical and Functional Properties of Food Lipids, edited by Zdzislaw E. Sikorski and Anna Kolakowska, CRC Press

Lehninger, A. L., 2008. Principles of Biochemistry edited by W.H. Freeman

Leithinger, N., 2008. The role of phospholipid oxidation products in inflammatory and autoimmune diseases. Lipids in Health and Disease Subcellular Biochemistry Volume 49, pp 325-350

Marquez- Ruiz, G., Holgado, F., and Velasco, J., 2013. Mechanisms of oxidation in food lipids. Food Oxidants and Antioxidants: Chemical, Biological, and Functional Properties edited by Grzegorz Bartosz CRC Press

Morales, M. T., Przybylski, R., 2013. Olive oil oxidation. Handbook of Olive Oil: Analysis and Properties edited by Roman Aparicio and John Harwood, Springer

Mubiru, E., Shrestha, K., Papastergiadis, A., and Meulenaer, B., 2013. Improved gas chromatography-flame ionization detector analytical method for the analysis of epoxy fatty acids. *Journal of Chromatography A*, 1318, 217-225

Mubiru, E., Shrestha, K., Papastergiadis, A., and Meulenaer, B., 2014. Development and Validation of a Gas Chromatography−Flame Ionization Detection Method for the Determination of Epoxy Fatty Acids in Food Matrices. *Journal of Agricultural and Food Chemistry*, 62, 2982-2988

Psomiadou, E., and Tsimidou, M., 2002. Stability of virgin olive oil. 2. Photo-oxidation studies. *Journal of Agricultural and Food Chemistry*, 50, 722-727

Rustad, T., 2009. Lipid oxidation. Handbook of Seafood and Seafood Products Analysis edited by Leo M. L. Nollet and Fidel Toldrá, CRC Press

Schaich, K. M., 2005. Lipid oxidation: Theoretical aspects. In Bailey's Industrial Oil and Fat Products, 6th ed.; Shahidi, F., Ed.; Wiley Interscience: Hoboken, NJ, USA; pp 269−355

Schaich, K. M., Shahidi, F., Zhong, Y., Eskin, N. M. A., 2012. Lipid oxidation. Biochemistry of Foods edited by N. A. Michael Eskin and Fereidoon Shahidi, Elsivier Inc.

Shahidi, F., and Zhong, Y., 2010. Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, 39, 4067-4079

APPENDICES

APPENDIX A: Choromatogram result of linseed oil model with chlorophyll in day 21

APPENDIX B: Choromatogram result of linseed oil model without chlorophyll in day 21

APPENDIX A

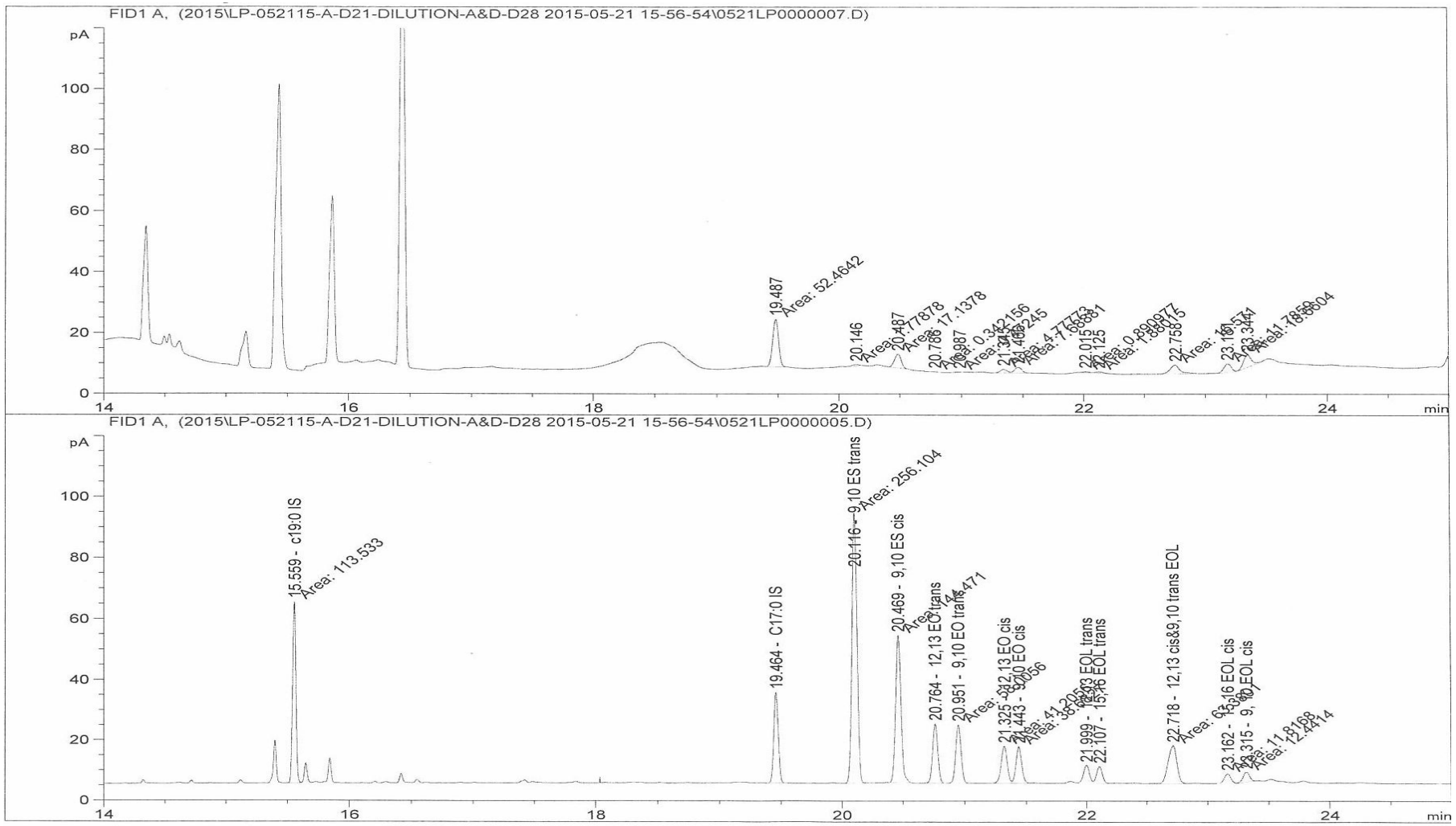

Figure A: Choromatogram result of linseed oil model with chlorophyll in day 21

APPENDIX B

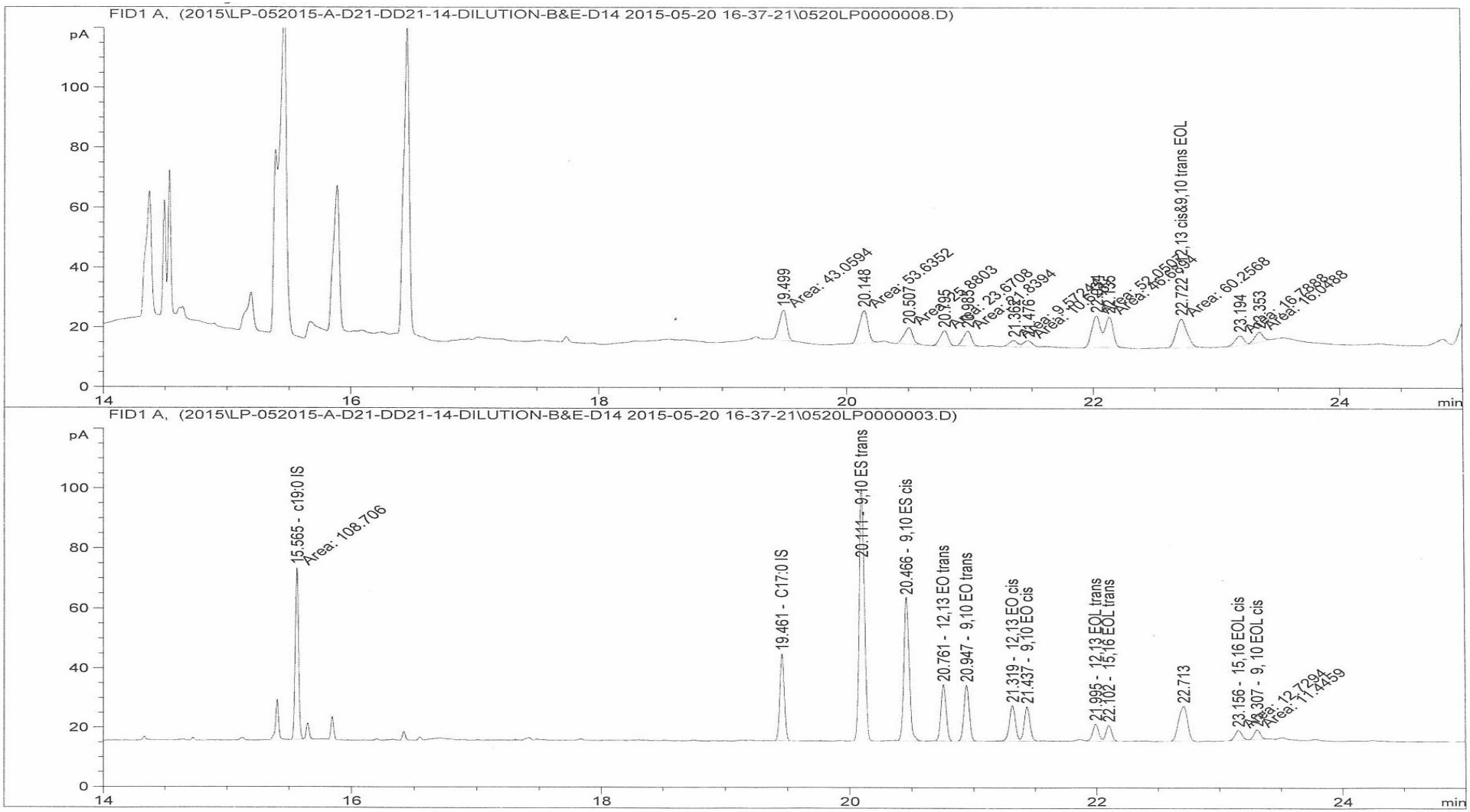

Figure A: Choromatogram result of linseed oil model without chlorophyll in day 21

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