OXIDATIVE STABILITY OF STRIPPED AND NON-STRIPPED BORAGE AND EVENING PRIMROSE OILS AND THEIR OIL-IN-WATER EMULSIONS

MUHAMMAD AHMAD KHAN

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OXIDATIVE STABILITY OF STRIPPED AND NON-STRIPPED BORAGE AND EVENING PRIMROSE OILS AND THEIR OIL-IN-WATER EMULSIONS

BY

©Muhammad Ahmad Khan

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ABSTRACT

Borage and evening primrose oils have been used for the treatment of a wide range of nutritional and clinical disorders. However, very little information is available about the oxidative stability of these nutritional oils and virtually nothing is known regarding their minor components-stripped counterparts. Therefore, this research was initiated to evaluate the oxidative stability of borage and evening primrose oils and their oil-in-water emulsions as well as their minor component stripped counterparts in the dark at 60 C and under fluorescent light at 27 C. Several analytical methods were used to assess the oxidative stability of oil and emulsion systems. The correlations between peroxide values (PV) and conjugated dienes (CD) as well as thiobarbituric acid reactive substances (TBARS) and hexanal content for all samples have been examined. Moreover, the effectiveness of natural and synthetic antioxidants as well as phospholipids to inhibit the oxidation of stripped borage and evening primrose oils was evaluated in the dark at 60 C.

The results indicate that minor components play a major role in the oxidative stability of borage and evening primrose oils and their emulsions in water in the dark as well as in the light. Moreover, the endogenous antioxidants in the emulsion system may or may not behave according to the "polar paradox" theory. Correlations have been observed ($r>0.514$, $P<0.05$, $n=15$) between PV and CD as well as TBARS and hexanal content for most oils and emulsion systems stored in the dark or under fluorescent light.

Delta-tocopherol was more effective as an antioxidant than alpha-tocopherol in stripped borage and evening primrose oils. Meanwhile, the most effective natural

antioxidant was Tenox GT-2 (which is a mixture of α-, γ-, and δ-tocopherols) at 500 ppm while TBHQ at 200 ppm was the most effective synthetic antioxidant in both oils. Moreover, the oxidative stability of stripped borage and evening primrose oils was increased by a mixture of tocopherols and phospholipids. These results may be used to: (1) provide simple and reliable analytical methods to follow the oxidation of stripped and non-stripped borage and evening primrose oils, (2) enhance our understanding of the parameters involved in the oxidation of nutritional and medicinal oils and their emulsions in water, (3) design proper refining processes to retain optimum amounts of minor components and (4) improve the oxidative stability of stripped borage and evening primrose oils through natural and synthetic antioxidants.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AOCS	American Oil Chemists' Society
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CD	Conjugated dienes
DAG	Diacylglycerol
EPUFA	Essential polyunsaturated fatty acids
FAME	Fatty acid methyl esters
FID	Flame ionization detection
GL	Glycolipids
GLA	gamma-Linolenic acid
HPLC	High performance liquid chromatography
ISC	Intersystem crossing
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
MA	Malonaldehyde
MAG	Monoacylglycerol
NBE	Non-stripped borage oil emulsion
NBO	Non-stripped borage oil
NEPE	Non-stripped evening primrose oil emulsion
NEPO	Non-stripped eveninig primrose oil

NL	Neutral lipids
OS	Oxidative stability
PA	Phosphatidic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Propyl gallate
PI	Phosphatidylinositol
PL	Phospholipids
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
SBE	Stripped borage oil emulsion
SBO	Stripped borage oil
SEPE	Stripped evening primrose oil emulsion
SEPO	Stripped evening primrose oil
TAG	Triacylglycerol
TBARS	Thiobarbituric acid reactive substances
TBHQ	tert-Butylhydroquinone
TLC	Thin layer chromatography
UV	Ultraviolet

CHAPTER 1
INTRODUCTION

Many oilseed crops are cultivated for extraction and production of edible oils. The major oils and fats on a world production basis are soybean, palm, rapeseed, sunflower, and tallow, respectivly (Hammilton, 1994). Meanwhile, special varieties of herbaceous oilseed crops are grown for the production of nutritional and medicinal oils (Hammond, 1995). The oils from seeds of borage (*Borago officinalis*) and evening primrose (*Oenothera biennis*) are rich in gamma-linolenic acid (GLA) and, thus have become major commercial sources of this essential polyunsaturated fatty acid (PUFA) (Syed Rahmatullah et al, 1994).

Borage and evening primrose oils as well as GLA have been used for the treatment of a wide range of nutritional and clinical disorders. The possible gastric cancer protective action of borage oil was reported by Gonzalez (1993). Furthermore, borage oil has been used for possible treatment of endotoxin-induced lung injury as reported by Muncuso (1997) and treatment of infantile seborrhoeic dermatitis (Tollesson, 1993) as well as cystic fibrosis (Christophe, 1994). Evening primrose oil was used in the treatment of atopic dermatitis (Andreassi et al, 1997). Furthermore, anti-diabetic and anti-ulcerogenic properties of this oil were observed by Garland et al (1997) and Andreassi et al (1997), respectively. GLA concentrates have been used in the treatment of rheumatoid arthritis (Zurier et al, 1996) and management of impaired nerve function in diabetes (Horrobin, 1997). In addition, the anti-infective activity of the same concentrate was reported by Giamarellosbourboulis et al (1995). Meanwhile, n-3 and n-6 PUFA emulsions have been

used in the treatment of different clinical disorders (Zadak et al, 1997; Tashiro et al, 1998).

The nutritional and medicinal applications of borage and evening primrose oils have attracted much research in these areas. The chemical composition has been reported by Sensidoni et al (1996) and Hudson (1989). Enzymatic enrichment of GLA from these oils was accomplished by Syed Rahmatullah et al (1994). Recently, reversed phase high performance liquid chromatography and ^{13}C nuclear magnetic resonance spectroscopy were used to determine the composition and positional distribution of fatty acids in borage and evening primrose oils (Redden et al, 1995; Marti and Thersa, 1996). Furthermore, kinetic and mass-transfer effects of supercritical-fluid extraction of evening primrose oil have been studied by King et al (1997).

Borage and evening primrose oils are readily susceptible to oxidation due to their high levels of PUFA (Sensidoni et al, 1995; Hudson, 1989). Moreover, oxidation of nutritional and pharmaceutical emulsions affects safety and efficiency of these products (Nijveldt et al, 1998). Nevertheless, little is known about the oxidative stability (OS) of borage and evening primrose oils, and virtually nothing is known about the OS of these oils when stripped of their minor-components or the OS of their oil-in-water emulsions. Recently, Shahidi and Shukla (1996) have reported that minor components play an important role in the oxidative stability of vegetable oils. Meanwhile, the behaviour of antioxidants in edible oil-in-water emulsions can be explained by the polar paradox theory proposed by Porter et al (1989). This theory suggests that the non-polar lipophilic antioxidants, such as tocopherols, are more active in oil-in-water emulsions than the polar

hydrophilic antioxidants, such as ascorbic acid. The opposite trend has been observed in bulk oil systems. In this dry system, the lipophilic antioxidants will remain in the oil, while the hydrophilic antioxidants will be oriented in the air-oil interface and thus being more effective in reducing or preventing oxygen accessibility for oil oxidation.

Hypotheses considered were: (1) although it is generally accepted that the OS of edible oils is dictated by their degree of unsaturation, minor components might play an important role in this respect and (2) the endogenous antioxidants, if present, in borage and evening primrose oils behave in their bulk and oil-in-water emulsions according to the well established "polar paradox theory'' proposed by Porter et al (1989) and supported by Frankel et al (1994).

To examine these hypotheses and to fill an important gap in the scientific literature regarding the OS of nutritional and medicinal oils, this research was designed to: (1) evaluate the OS of stripped and non-stripped borage and evening primrose oils, (2) determine the minor components in stripped and non-stripped borage and evening primrose oils and (3) study the impact of minor components, mainly tocopherols and phospholipids, on the OS of stripped borage and evening primrose oils.

CHAPTER 2
LITERATURE REVIEW

2.1 Nutritional and medicinal oils

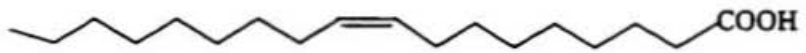
Vegetable and marine oils are frequently used to correct nutritional deficiencies and treat different clinical disorders (Ackman, 1995; Tenwolde et al 1997; Wang et al, 1997). The effects of dietary oils rich in lauric, mystric, palmitic, oleic or linoleic acid on plasma, hepatic and biliary lipids in cholesterol-fed hamsters have been reported by Trautwein et al (1997). Furthermore, substantial depletion of PUFA, both n-6 and n-3, was observed in patients with schizophrenia, a serious mental disorder (Laugharne, 1997). Dietary supplementation of patients for six weeks with 10 g per day of fish oil led to significant improvement in schizophrenic symptoms.

Over the last two decades, understanding of the role and nutritional importance of PUFA and vegetable oils rich in them has improved. Therefore, this section will focus on PUFA, particularly gamma-linolenic acid (GLA) as well as on borage and evening primrose oils, the major commercial sources of GLA.

2.1.1 Essential polyunsaturated fatty acids (EPUFA)

EPUFA are nutrients that must be provided through the diet because they cannot be synthesized in the human body. Polyunsaturated fatty acids contain two or more double bonds (Figure 2.1). These fatty acids are biosynthesized via an extension of the saturated fatty acid pathway, in which stearate is converted to oleate (18:1 $\Delta 9$) and then

Figure 2.1 Chemical structure of oleic, linoleic, α-linolenic, and γ-linolenic acids.



Oleic acid

Linoleic acid

alpha-linolenic acid

gamma-linolenic acid

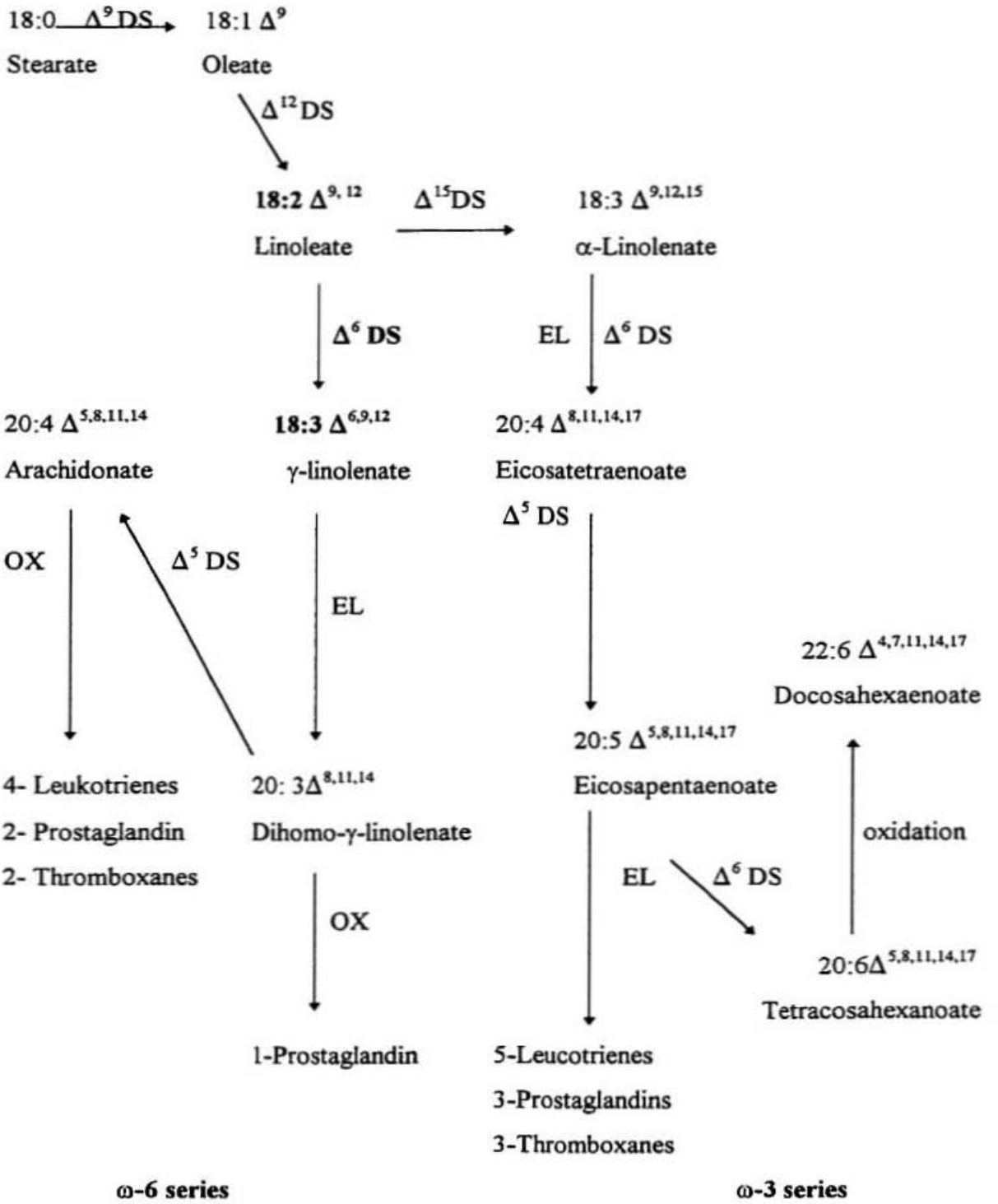
linoleate, which is the central precursor for the n-6 and n-3 series (Figure 2.2; Gill and Rao, 1997; Horrobin, 1992).

The elongase and desaturase enzymes required for the *de novo* production of PUFA are possessed by algae, fungi, bacteria, insects and some other invertebrates. However, some plants and animals lack the ability to produce fatty acids exceeding 18 carbons, except in rare cases, because of the absence of these enzymes. It also appears that humans lack the $\Delta 4$ desaturase, and thus cannot synthesize linoleic and alpha-linolenic acids (LA & ALA) or longer PUFA *de novo*. Nonetheless, they can further elaborate LA (or GLA) and ALA and, therefore, these PUFA are essential nutrients for the human body (Gill and Rao, 1997).

Normal synthesis of GLA from LA via $\Delta 6$ desaturase can be decreased or blocked in humans by several factors, such as stress, diabetes, obesity and aging (Haro and Rio, 1998). Supplementation of the diet with GLA or vegetable oils rich in it has reversed and corrected the health disorders related to the deficiency of the essential fatty acids and prostaglandins (Table 2.1).

Gamma-linolenic acid is found in the seed oils of many plants (Haro and Rio, 1998). Seed lipids of certain Mongolian and other Ranuculaceae have shown the presence of unusual fatty acids including GLA (up to 20 %). However, only seed oils of borage, black current and evening primrose are commercially available for human consumption (Syed Rahmatullah et al, 1994).

Figure 2.2 Biosynthesis and interconversion of polyunsaturated fatty acids.



Abrreviations are: DS, desaturase; EL, elongase; OX, oxidase enzymes.

Table 2.1 Nutritional and clinical applications of gamma-linolenic acid, borage and evening primrose oils.

Material	Application	References
GLA	Management of impaired nerve function in diabetes	Horrobin, 1997
	Treatment of rheumatoid arthritis	Zurier et al, 1996
	Anti-infective activity	Giamarellosbourboulis et al, 1995
GLA, EPO	Treatment of atopic dermatitis	Andreassi et al, 1997
EPO	Anti-diabetic properties in rats	Garland et al, 1997
	Anti-diabetic and anti-ulcerogenic properties	Alshabannah, 1997
BO	Possible treatment of endotoxin -induced acute lung injury	Muncuso, 1997
	Treatment of cystic-fibrosis	Christophe,1994
	Treatment of infantile seborrhoeic dermatitis (ISD)	Tollesson, 1993
	Possible gastric cancer protective factor	Gonzalez, 1993
BO, EPO	Increased production of prostaglandin E(2) in growing rats	Quoc and Pascaud, 1996

GLA, gamma linolenic acid; BO, Borage oil; and EPO, Evening primrose.

2.1.2 Borage (*Borago officinalis*)

Borage belongs to the Boraginaceae family and is distributed throughout temperate and subtropical areas of the world, particularly in the Mediterranean region. It is rarely found in cool temperate and tropical regions (Heywood, 1979). In Canada, borage is cultivated on an average of 2000 acres in Saskatchewan. An average of 400 lb of seeds are produced per acre, but only 80 lb are harvested and the rest end up on the ground. This occurs due to indeterminate growth habit, non-uniform seed ripening, and its shattering habit; a large percentage of ripened seeds shatters and falls to the soil before and during the harvest (Haro and Rio, 1998). Innovative techniques have been developed to overcome this serious problem and to enhance recovery of as much as 150 lb of seeds per acre.

The total oil content of borage seed ranges between 27 and 37 % with a GLA content in the oil of 20 to 26 % (Tyler, 1994). Chemical analysis of the unsaponifiable fraction of the oil revealed that it contains beta(β)-, delta(δ)- and gamma(γ)- tocopherols as well as various amounts of chlorophyll, depending on the extraction technique employed (Sensidoni et al, 1996). Meanwhile, chemical analysis of borage leaves, flowers, seeds and seed oil showed that the leaves and flowers contain some toxic pyrrolizidine alkaloids. However, no alkaloids were detected in borage seed oil (Larson et al, 1984; Dodson and Stermitz, 1986).

Attempts have been made to concentrate GLA from the oil. Huang et al (1997) have concentrated GLA from borage oil by a three step enzymatic method. The first step was selective hydrolysis in isooctane by *Candida rugosa* lipase. The next step involved

selective esterification of the free fatty acids obtained from saponified borage oil and n-butanol by lipozyme IM-20, and the last step was acidolysis of the products of the two reactions to obtain the purified GLA. Similarly, Shimada et al (1997) have purified GLA from borage oil using *Pseudomonas spp.* lipase for hydrolysis and *Rhizopus delemar* lipase for selective esterification with lauryl alcohol. These conditions esterified 74.4 % of the borage free fatty acid fraction and the GLA content in this fraction was enriched from 22.5 % to 70 .2 %.

2.1.3 Evening primrose (*Oenothera biennis*) oil

Evening primrose belongs to Onagraceae family which is found all over the world, but it is more diverse in the western United States of America and Mexico, where all known genera of this family occur (Heywood, 1979). In Canada, it is more frequently found in the eastern and western provinces (Brandle et al, 1993). The nutritional and pharmaceutical applications of evening primrose oil have resulted in cultivation and production of higher amounts of this herbaceous oilseed crop (Hamilton,1994). The seeds are being cultivated in at least 15 countries, including Canada. Furthermore, the oil is currently available in more than 30 countries as a nutritional supplement (Carter,1988).

Evening primrose seeds contain between 22 and 28 % of oil which on average contain 73 % of LA (which is unusually high for vegetable oils) and an average of 10.4 % of GLA, which might be variable within the species (Hudson, 1984). GLA from evening primrose is readily converted in the body to prostaglandin E1, and thus it can be employed in the treatment of any and every condition for which this prostaglandin might be

beneficial, including premenstrual syndrome, obesity, mental disorder and childhood hypersensitivity. Horrobin and Manku (1990) have reported a study where clinical trials with GLA-rich borage, black currant, fungal as well as evening primrose oils were carried out. These oils have different fatty acid compositions with the first three having more GLA than evening primrose. Nevertheless, evening primrose oil was more effective than the other oils on the flow of eicosanoids, including prostaglandin, from arterial tissue and therefore more beneficial in the treatment of clinical disorders associated with abnormalities in eicosanoid metabolism.

2.2 Minor component-stripped vegetable oils

Edible vegetable oils consist mainly (95 %) of triacylglycerols. Non-triacyglycerols (also known as minor components) make up the remaining 5 %. The minor components of vegetable oils, in general are primarily composed of phospholipids, tocopherols, tocotrienols, flavonoids, other phenolic pigments (carotenoids, chlorophyll), sterols as well as free fatty acids and di and mono acylglycerols (Hamilton, 1994; Shahidi and Shukla, 1996). Several classes of these components might be present in each oil and contribute to its oxidative stability (Shahidi and Shukla, 1996).

The profound effect of minor components on the colour stability and red colour development during the oxidation of tall oil fatty acids was reported by Min (1973). Furthermore, monoacylglycerol and diacyglycerol, which are present in minor amounts in soybean oil, were reported to act as prooxidants (Mistry and Min, 1988).

2.2.1 Preparation of stripped oils

Several chromatographic techniques have been developed and used to remove (strip) the vegetable oils from their minor components. Mistry and Min (1988) introduced and applied a dry-column (packed with silicic acid, charcoal, sugar and celite in different proportions) technique to prepare large amounts of stripped soybean oil. The stripped oil was colourless, odourless, tasteless and free of tocopherols, phospholipids, carotenoids, free fatty acids, mono- and diacylglycerols or oxidized compounds. Similarly, Baldiol et al (1996) used this technique to prepare stripped olive oil.

Lampi et al (1992) applied the dry-column technique to prepare stripped rapeseed oil, however, only 70 % of α-tocopherol and 40 % of γ-tocopherol were removed. Therefore, a new technique was developed and optimized during the course of this work to prepare stripped vegetable oils. The column, used in this study is attached to a water pump and packed with activated silicic acid (top and bottom layers) and mixtures of charcoal and celite as well as sugar and celite (middle layers). The column was conditioned with hexane and the oil was dissolved in an equal volume of this solvent and passed through the column. The water pump reduced the time required for packing the column as well as for passing the oil through it. The solvent is removed from the eluted oil using a rotary evaporator; the product so obtained is defined as stripped oil. This technique was successfully applied to remove the minor components from soybean, virgin olive, sunflower and rapeseed as well as butter oils (Lampi et al, 1992) and refined oilve oil (Blekas, 1995).

Hall and Cuppett (1993) have stripped soybean oil using a chromatographic method. Meanwhile, Yoshida et al (1993) used a column packed with aluminum oxide to prepare stripped rapeseed, soybean and palm oils. Recently, Roedig-Penman and Gordon (1998) used the same type of column to obtain stripped sunflower oil.

High performance liquid chromatography may also be used to prepare stripped oils (Park et al, 1983). However, the major drawback of this technique is the small amounts of the stripped oil that can obtained. Meanwhile, stripped corn oil may be acquired from commercial sources (Frankel et al, 1994)

2.2.2 Determination of the minor components

The minor components in the edible oils can be determined by various instrumental methods. Similar techniques may also be used to determine these components (in stripped oils) in order to confirm their removal by the stripping procedures employed.

The major lipid classes (neutral, glyco- and phospholipids) can be fractionated by silicic acid column chromatography and the subclasses in each fraction may be determined by a combination of TLC and spectrophotometric methods (Christie,1982). Determination of the main lipid classes and subclasses in edible oils can also be accomplished by thin layer chromatography-flame ionization detection (TLC-FID) (Du Plessis and Pretorius, 1983; Imbs and Pham, 1997).

Tocopherols can be assayed by thin layer chromatography (TLC), gas liquid chromatography (GLC) and reverse/normal phase high performance liquid chromatography (HPLC) (Grzegorz et al, 1996). The latter techniques have been used to

determine tocopherols in stripped corn oil (Huang et al, 1996), stripped rapeseed, soybean and sunflower oils (Yoshida et al, 1993) as well as stripped and non-stripped olive, soybean and butter oils (Blekas, 1995; Lampi et al, 1992).

Pigments, such as chlorophyll and carotenoids, can be determined qualitatively by measuring the absorbence at 430-460 nm for carotenoids and 550-710 for chlorophyll (Belkas et al , 1995) or quantitatively according to Hall and Cuppet (1994).

The efficiency of the stripping procedure employed may also be checked by HPLC using an ultraviolet (UV)-visible detector (Neff et al, 199). The minor components and their maximum absorption wavelengths used for the purity checks in soybean oil were: chlorophyll (670 nm), carotene (436 nm), xanthophyll (436 nm), tocopherols (298 nm), squalene (254 nm), TAG hydroperoxides (232 nm), phospholipids, sterols, mono- and diacylglycerol and free fatty acid (215 nm).

2.2.3 Applications of stripped oils

The stripped edible oils are mainly used to evaluate the anti- and prooxidant activities of their minor components as well as to study the effectiveness of synthetic and natural antioxidants to control lipid oxidation. Results from these studies may also be utilized to eliminate or minimize the prooxidants in edible oils via proper processing steps. Alternatively, it possible to design techniques to stabilize the oils by selecting the optimum concentration and combination of antioxidants in order to improve their stability, safety, nutritional quality, and thus their shelf life.

Stripped vegetable oils have been used to study the effect of mono- and diacylglycerols on autoxidation (Mistry and Min, 1988), the influence of tocopherols on photooxidation (Jung et al, 1991), and the effect of fatty acids on the stability of tocopherols during microwave heating (Yoshida et al, 1992). Stripped corn oil and oil-in-water emulsions have been used to compare the antioxidant and prooxidant properties of green tea (Frankel et al, 1997). Meanwhile, stripped sunflower oil and oil-in-water emulsions have been used to study the antioxidant properties of myricetin and quercetin (Roedig and Gordon, 1998).

Stripped oils have also been used in nutritional studies. Stripped corn oil has been used as a placebo to study the effects of dietary oils and methyl ethyl ketone peroxide on *in vivo* lipid peroxidation and antioxidants in rat heart and liver (Skuladottir et al, 1994).

2.3 Edible oil emulsions

Polyunsaturated fatty acid (PUFA) emulsions belonging to n-3 and n-6 series have been used in the treatment of different clinical disorders (Zadak et al, 1997; Yeh et al, 1997; Tashiro et al, 1998). These PUFA are susceptible to oxidation and production of undesirable toxic hydroperoxides as well as secondary oxidation products, which in turn affects safety and efficacy of these products (Nijveldt et al, 1998). Furthermore, Roozen et al (1994) have reported the enzymatic and non-enzymatic oxidation of low-fat food emulsions. Therefore, demands for better and more stable emulsions will continue. Meanwhile, a precise understanding of chemical and physical characterization of emulsions

is required in order to obtain stable preparations with reliable quality (Coupland and McClements, 1996a).

2.3.1 Basic theory

An emulsion is a two phase oil-water system where one phase is dispersed as droplets in the other using a third component known as an emulsifier (Figure 2.3). The droplet size of stable emulsions ranges between 0.1 and 50 μm. Emulsions may be classified into three types: 1) oil-in-water (O/W), where oil droplets are dispersed in an aqueous system; 2) water-in-oil (W/O), which consists of water droplets dispersed in an oil system (Figure 2.4), and 3) multiple emulsions such as (W/O/W) consisting of small water droplets entrapped within large oil droplets, which in turn are suspended in a continuum of water (Pal, 1994). This section will concentrate on O/W emulsions.

Oil-in-water emulsions consist of three regions. The interior of the droplet, the continuous phase (dispersion medium), and the interfacial (air-oil-interface) region (Coupland and McClements, 1996a; Frankel, 1996a). The interface surrounds each emulsion droplet and consist of a mixture of oil, water and emulsifier molecules. The various molecules partition themselves between these three regions. Non-polar molecules in the oil phase, polar molecules in the aqueous phase and amphiphilic molecules at the interface (Coupland and McClements, 1996a).

Figure 2.3 Schematic diagram of oil-in-water emulsion droplet.

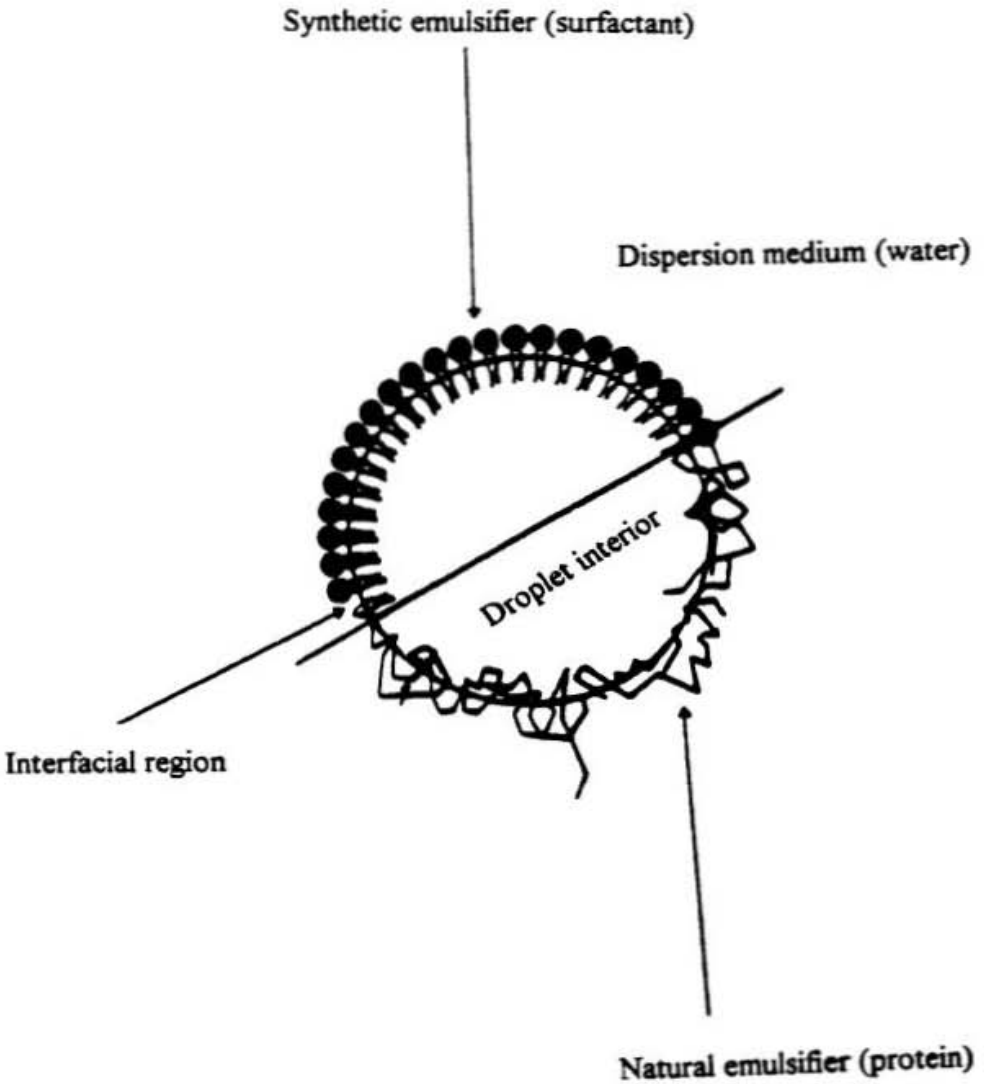
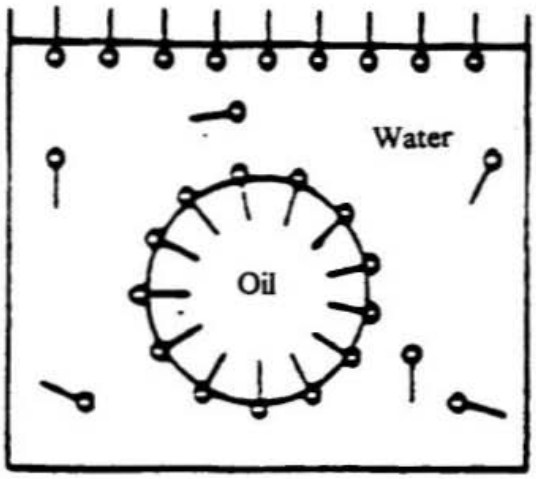
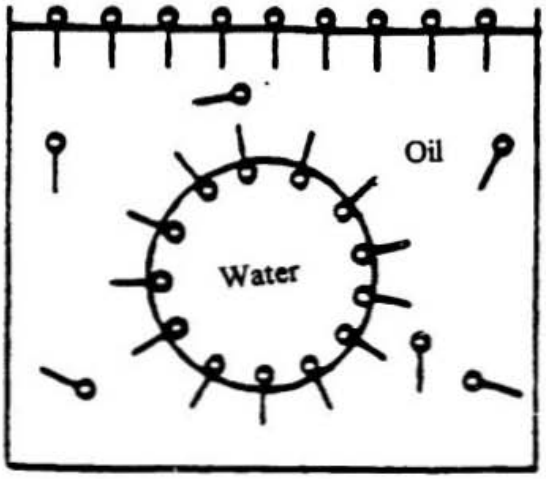

Synthetic emulsifier (surfactant)
Dispersion medium (water)
Droplet interior
Interfacial region
Natural emulsifier (protein)

Figure 2.4 Schematic diagrams of oil-in-water (O/W) and water-in-oil (W/O) emulsions.



Oil-in-water (O/W)



Water-in-oil (W/O)

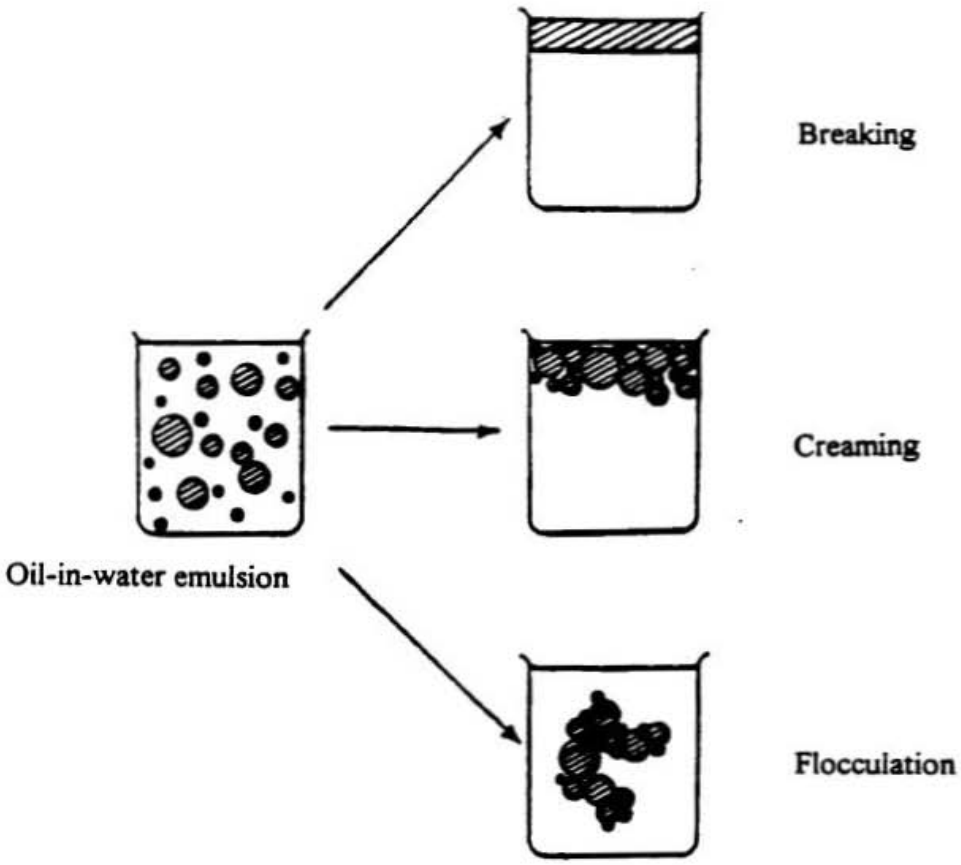
2.3.2 Emulsion stability

Emulsions are inherently unstable. The primary processes leading to instability are creaming, flocculation and breaking (Figure 2.5; Kitchner, 1968; Dickinson and Stainsby, 1988). Creaming is the rising of dispersed droplets under the action of gravity (Kitchner, 1968). Flocculation is the assembly of small droplets to form a cluster separated by regions of dispersing medium, while breaking is merging of small spherical globules (coalescence), leading ultimately to the appearance of free oil at the surface (Dickinson and Stainsby, 1988).

Several factors affect the flow characteristics or rheology, and thus the stability of O/W emulsions. These factors are: the nature of the oil (Parris et al, 1994), the volume fraction of the dispersed phase (Tadros, 1994), the droplet size and distribution (Ivanov and Kralchevsky, 1997), chemical composition of the medium such as pH and electrolytes (Demetriadeset et al, 1997) as well as the interfacial rheology of the emulsifier film and the concentration and nature of the emulsifier (Tadros,1994; Chow and Ho, 1996; Ogden and Rosentval, 1997).

Emulsifiers are surface-active molecules that are adsorbed to the surface of the formed droplets during homogenization, forming protective membranes that prevent the droplets from aggregating. The most common emulsifiers in the food, cosmetic and pharmaceutical industries are amphiphilic proteins such as those of casein, whey, soy or egg, and phospholipids from egg or soy lecithin and small-molecule surfactants such as Spans, Tweens or fatty acids. The nature and concentration of the protective membrane

Figure 2.5 Schematic diagram of major types of instability of emulsions.


Breaking
Creaming
Oil-in-water emulsion
Flocculation

(emulsifier) surrounding the emulsion droplets may have important implications for the lipid oxidation process (Coupland and McClements, 1996a). The oxidation of methyl linoleate as well as methyl docosahexaenoate was strongly inhibited in emulsions prepared with sodium caseinate and 60 % dextrin, and Tween 20, respectively (Fugii et al, 1995; Miyashita et al, 1997). Furthermore, the stability of palm oil-in-water emulsions was strongly dependent on the various types and concentrations of ethoxylated nonionic emulsifiers used for their preparation (Ahmad et al, 1997).

2.3.3 Applications in food and pharmaceutical industries

Emulsions are used in foods (Baldwin et al, 1997), nutrition (Oliveira, et al, 1997), and in the pharmaceutical industries (Washington, 1996; Stone, 1997; Trissel et al, 1997). Emulsions present in our daily diet include milk, ice cream, sauces, dressings and ready-to-use products. These emulsions are complex mixtures which contain a variety of components such as proteins, polysaccharides, sugars, acidifiers and minerals in a continuous phase that can stabilize them. However, the presence of egg phospholipids is enough to stabilize some of these emulsions (Chappat, 1994).

Triacylglycerol emulsions are administrated intravenously to patients that cannot meet their nutritional requirements by conventional parental therapies, and thus provide the necessary energy requirement for different tissues of the body as well as prevent or correct essential fatty acid deficiencies (Henwood et al, 1997; Carpentier et al, 1997). Furthermore, emulsions are useful as carriers of active material (that can reach the target site in a proper form and without any side effects) in pharmaceutical applications

(Constantinides, 1995; Liu and Liu, 1995; Chappat, 1994). Therefore, lipid emulsions are being used increasingly for the delivery of lipophilic drugs (Decker et al, 1995). Meanwhile, solubilization of hydophilic drugs in oil carriers can improve the delivery of these molecules by affording protection against intestinal proteases, improving satiability in the presence of gut surfactants and altering cell permeability and lymphatic secretion (New and Kirby, 1997). Furthermore, animal, vegetable and synthetic oils were tested as potential replacements for mineral oil in Newcastle disease oil emulsion vaccines (Stone, 1997), and the structure-function relationship of non-ionic surfactants in emulsion-mediated gene delivery was reported by Liu et al (1996).

2.4 Lipid Oxidation

Oxidative deterioration is a major problem associated with extraction, processing, storage and usage of fats and oils. Lipid oxidation may compromise the safety, nutritional quality and shelf life of edible oils (Chow and Gupta, 1994). Furthermore, lipid oxidation in the human body, occurring via free radical reactions, may lead to oxidative damage of tissue cells which, in turn, can cause various diseases and clinical disorders (Table 2.2). In fact, almost any disease is likely to be accompanied by increased formation of reactive oxygen species (ROS) (Halliwell, 1991). Lipid oxidation products are implicated in the disruption of biological membranes, formation of age pigment in damaged membranes, inactivation of enzymes and damage to proteins, oxidative damage of the lungs by atmospheric pollutants, and cancer.

Therefore, lipid oxidation has become a major concern for food scientists as well as nutritionists and biochemists (Arouma, 1998). Proper understanding of lipid oxidation mechanisms and measurement techniques as well as synthetic and natural antioxidants will improve the ability to control lipid oxidation and therefore prevent or minimize oxidative deterioration of foods as well as oxidative damage in the human body.

Many edible fats and oils contain unsaturated fatty acids, primarily oleic, linoleic and linolenic. The susceptibility to oxidation depends largely on the degree of unsaturation; thus linolenic acid (3 double bonds) oxidizes 100 time faster than oleic acid (one double bond) (Hamilton, 1994). Fats and oils can be oxidized through autoxidation, photooxidation, thermal oxidation and enzymatic oxidation, however, only the first two mechanisms will be addressed in this thesis.

2.4.1 Autoxidation

Autoxidation of fats and oils proceeds via a free radical chain reaction which involves initiation, propagation and termination steps (Chow and Gupta, 1994). In the initiation step (eq.2.1 and 2.2), a hydrogen atom is abstracted from the α-methylenic carbon of an unsaturated fatty acid, RH, and a free radical $R^{\bullet}$ is formed. The formation of free radicals in this step is catalyzed and accelerated by external energy sources such as heat, light, high energy radiation, metal ions, metalloporphyrins (haem) and other radical compounds (Hamilton, 1994).

$$RH + O_2 \xrightarrow{\text{catalyst}} R^{\bullet} + {}^{\bullet}OOH \qquad \text{(eq.2.1)}$$

$$RH \xrightarrow{\text{catalyst}} R^{\bullet} + H \qquad \text{(eq.2.2)}$$

Table 2.2 Some clinical disorders attributed to free radicals and reactive oxygen species.

Target organ	Clinical disorders
Brain	Parkinson's disease
	Vitamin E deficiency
Eye	Cataractogenesis
	Degenerative retinal damage
Heart	Atherosclerosis
Kidney	Metal- ion mediated nephrotoxicity
Gastrointestinal tract	Oral ion poisoning
	Endotoxin liver injury
Red blood cells	Malaria
	Sickle cell anemia
Lung	Cigarette smoke effect
	Mineral dust pneumoconiosis

The free radicals formed in the initiation step react with the atmospheric oxygen to form peroxy radicals, which in turn abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide, ROOH, and another free radical $R^{\bullet}$ (eq.2.3 and 2.4).

$$R^{\bullet} + O_2 \xrightarrow{\text{catalyst}} RO^{\bullet}_2 \qquad \text{(eq.2.3)}$$

$$RO^{\bullet}_2 + RH \longrightarrow RO_2H + R^{\bullet} \qquad \text{(eq.2.4)}$$

The free radicals formed can initiate and promote oxidation of large amounts of lipids (Porter et al,1995). The free radical chain reaction can be terminated either by self-quenching or polymerization of free radicals to form non-radical dimers, trimers and polymers (eq. 2.5 and 2.6) or by antioxidants which react competitively with alkyl free radicals and/ or peroxy radicals and remove them from the system (King et al, 1995).

$$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R \qquad \text{(eq.2.5)}$$

$$R^{\bullet} + RO^{\bullet}_2 \longrightarrow RO_2R \qquad \text{(eq.2.6)}$$

Hydroperoxides (or primary oxidation products) formed during the autoxidation of oleate, linoleate and linolenate (Table 2.3) have been reported by Frankel et al (1984) and Gunstone (1984). The autoxidation of oleate starts by abstraction of a hydrogen from the allylic methylenes on carbon-8 and carbon-11 to produce 2 allylic radicals which in turn react with O_2 at the end positions to produce a mixture of 8-, 9-, 10- and 11-hydroperoxides in different proportions. The autoxidation of linoleate proceeds similarly. However, the hydrogen abstraction will occur on carbon-11 only to produce a delocalized pentadienyl moiety which in turn is attacked by the oxygen to produce an equal mixture of conjugated 9- and 13-hydroperoxide isomers. The abstraction of hydrogen from carbons -

11 and -14 in the autoxidation of linolenate produces a mixture of 9-,12-, 13- and 16-conjugated diene-triene hydroperoxide isomers.

Unsaturated fatty acid hydroperoxides (or primary oxidation products) are colourless, odourless and tasteless (King et al, 1995). Nonetheless, undesirable flavours associated with lipid oxidation arise from further decomposition of these hydroperoxides. The decomposition of hydroperoxides occurs via homolytic cleavage of oxygen-oxygen bonds to yield a hydroxyl and an alkoxy radical, which in turn decompose by carbon-carbon cleavage to yield aldehydes or hydrocarbons which are formed from alkyl radical abstracted hydrogen from appropriate donors. The alkyl radicals can undergo further reaction to produce ketones or alcohols (Frankel,1984).

Major volatiles produced from the decomposition of methyl oleate, linoleate and linolenate hydroperoxides have been reported by Frankel (1984) (see Table 2.3). Meanwhile, hexanal is the major volatile observed during the oxidation of oils rich in n-6 PUFA while propanal is an abundant volatile detected during the oxidation of oils rich in n-3 PUFA (Frnakel et al, 1993; Shahidi and Wanasundara, 1998).

Unsaturated fatty acid hydroperoxides can also react with oxygen to form epoxy hydroperoxides, ketohydrperoxides, dihydroperoxides, cyclic peroxides and bicyclic endoperoxides. Further breakdown of these products produces aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Frankel, 1984; Hamilton, 1994).

Table 2.3 Fatty acid hydroperoxides produced during autoxidation of oleate, linoleate and linolenate and the major volatiles formed from these hydroperoxides.

Fatty acid	Hydroperoxides	Relative %[1]	Major volatiles [2]	Relative %[1]
Oleic	8-OOH	27	Me 8-oxooctanoate	1.5
	9-OOH	23	Nonanal	15
	10-OOH	23	Nonanal	15
	11-OOH	27	Me 10-oxodecanoate	11
Linoleic	9-OOH	50	Me 9-oxooctanoate	19
	13-OOH	50	Hexanal	15
Linolenic	9-OOH	30	Me octanoate	22
	12-OOH	12	2,4- Heptadienal	9.3
	13-OOH	12	2-/ 3-Hexenal	1.4
	16-OOH	46	Ethane/ ethene	10

Adapted from Frankel et al (1981) and Frankel (1984); Me: methyl
[1] based on GC-MS analysis.
[2] each hydroperoxide may produce several volatiles.

2.4.2 Photooxidation

Photooxidation occurs in the presence of photosenitizers and visible light. Natural pigments in edible oils include chlorophyll, pheophytin, flavins, dyes and haem compounds which may absorb visible light energy efficiently due to their conjugated double bonds and form an excited singlet sensitizer ($^1Sen^*$; see eq.2.7), (Chow and Gupta, 1994; King et al, 1995). This excited product can drop back to the ground state or form an excited triplet sensitizer ($^3Sen^*$) via intersystem crossing processes (ISC). The energy generated in this step is transferred to the more stable triplet oxygen ($^3O_2$) to form the excited singlet oxygen ($^1O^*_2$) (eq.2.8). Singlet oxygen, due to its electrophilic nature, will attack moieties with high electron densities (C=C) to produce peroxy

$$^1Sens \xrightarrow{h\nu} {}^1Sen^* \xrightarrow{ISC} {}^3Sen^* \qquad \text{(eq.2.7)}$$

$$^3Sen^* + {}^3O_2 \longrightarrow {}^3O^*_2 \longrightarrow {}^1O^*_2 + {}^1Sens \qquad \text{(eq.2.8)}$$

radicals and eventually hydroperoxides. This reaction is known as the "ene" reaction. (Hall and Cuppet,1993; Hamilton,1994). It has been observed that ($^1O_2$) can react with linoleic acid 1450 times faster than ($^3O_2$) and the relative reactivity of oleic, linoleic and linolenic acids with ($^1O_2$) are in the ratio of 1:2:3 (King et al, 1995).

Hydroperoxides formed during photooxidation of oleic, linoleic and linolenic acids have been reported by Frankel (1984). Oleate has been shown to produce 9- and 10-hydroperoxides with allylic trans double bonds, while linoleate produces 4 isomers, 2 conjugated 9- and 13- diene hydroperoxides (as in autoxidation) and 2 conjugated 10- and 12-diene hydroperoxides (different from autoxidation). The photooxidation of

linolenic acid produced 6 isomers; 9-,12-,13-, and 16- hydroperoxides (as in autoxidation) and 10- and 15- hydroperoxides (different from autoxidation) (Table 2.4).

Photooxidized hydroperoxides can decompose, as in autoxidation, to form flavour-active secondary oxidation products (Frankel, 1983). Thermal breakdown products of pure hydroperoxides from photosensitized oxidized methyl oleate, linoleate and linolenate are summarized in Table 2.4. Recently, it has been observed that the most abundant volatile produced from thermal decomposition of photooxidized soybean triacylglycerols was 2-heptenal while propanal was the predominant volatile detected in photooxidized high linolenic acid soybean oil (Neff et al, 1993).

2.4.3 Effects of consuming oxidized oils

Lipid oxidation reduces the nutritional value and shelf life of edible oils. Furthermore, primary and secondary oxidation products are shown to have adverse effects on human health (Chow and Gupta,1994). The main symptoms associated with the consumption of oxidized fats and oils are diarrhea, poor rate of growth, myopathy (replacement of healthy muscle with scarred tissue), hepatomegaly (liver enlargement), steatitis or yellow fat disease (deposition of lipofunction pigments in adipose tissue), haemolytic anemia and secondary deficiencies of vitamins A and E (Sander, 1994).

2.5 Measurement of lipid oxidation

Lipid oxidation is a set of complicated catalytic processes. The four hydroperoxides or primary oxidation products of linoleic acid are degraded to over 100 secondary

Table 2.4 Fatty acid hydroperoxides produced during photooxidation of oleate, linoleate and linolenate and the major volatiles formed from these hydroperoxides.

Fatty acid	Hydroperoxides	Relative %[1]	Major volatiles [2]	Relative %[1]
Oleic	9-OOH	50	2-Decenal	12
	10-OOH	50	Me 9-oxononanoate	11
Linoleic	9-OOH	31	Me 9-oxooctanoate	22
	10-OOH	18	Me 10-Oxo-8-decanoate	14
	12-OOH	18	Hexanal	17
	13-OOH	33	Pentane	4.3
Linolenic	9-OOH	21	Me octanoate	15
	10-OOH	13	Me 10-Oxo-8-decanoate	13
	12-OOH	13	2,4 Heptadienal	8.8
	13-OOH	14	2-/ 3-Hexenal	3.4
	15-OOH	13	2-Butenal	11
	16-OOH	25	Propanal / acrolein	9

Adapted from Frankel et al (1981) and Frankel (1984); Me, methyl
[1] based on GC-MS analysis.
[2] each hydroperoxide may produce several volatiles.

products, and thus there is no universal technique which can be applied to monitor the progress of lipid oxidation (Prior and Loliser, 1994). Several chemical, instrumental and sensory techniques are used to monitor the oxidation of foods and thus predict their shelf life. These, techniques can also be used to evaluate the effectiveness of antioxidants in different lipid systems (King et al, 1995). Sensory methods are the most accurate to predict the stability of lipids, but these are not used for routine analysis (Wanasundara et al, 1995).

While some techniques measure the loss of reactants such as oxygen or the substrates such as fatty acids, others measure primary oxidation products (free radicals, hydroperoxides or conjugated dienes) or secondary oxidation products (alcohols, aldehydes, hydrocarbons or ketones (Lampi et al, 1997b). Table 2.5 summarizes some chemical, spectrophotometric and chromatographic methods which are routinely used to monitor the oxidation of edible oils.

Determinations of peroxide value (PV) and conjugated dienes (CD) are frequently used to measure primary oxidation products, while thiobarbituric acid reactive substances (TBARS) and headspace volatiles are determined as indices for monitoring secondary oxidation products.

2.5.1 Measurement of primary oxidation products

During the initial stage of lipid oxidation, formation of hydroperoxides exceeds their decomposition. However, this trend is reversed during latter stages. Therefore, measuring the amount of hydroperoxides as a function of time can indicate the stage of oxidation.

Table 2.5 Recent applications of different chemical and instrumental techniques to monitor oxidation of edible oil.

Technique	Application	References
TBARS	Evaluating the antioxidant effectiveness of myriectin and quercetin	Roedig-Penman and Gordon (1998)
	Evaluating the antioxidant properties of commercial phospholipids on the oxidation of salmon oil.	King et al (1995)
	Studying antioxidant activity of dry bean extracts in soybean oil	Ganthavorn and Hughes (1997)
Hexanal	Examining the stability of sesame oil as affected by processing and storage	Shahidi et al (1997)
PV+CD	Evaluating tocopherol activity in olive oil	Blekas et al (1995)
PV+ TBARS	Assessing the antioxidant activity of mung bean hulls in refined, bleached and deodorized soybean oil	Duh et al (1997)
PV+hexanal	Evaluating the effect of virgin olive oil natural antioxidants on oxidative stability of olive oil	Satu et al (1995)
CD+hexanal	Comparing the antioxidant activity of green teas in different lipid systems	Frankel et al (1997)
	Examining the antioxidant activity of green tea in different lipid systems	Hopia et al (1996)
PV+CD+ TBARS	Assessing the oxidative stability of sesame oil extracted from intact and dehulled seeds.	Abou-Gharbia et al (1997)
PV+CD+ headsspace	Evaluating the antioxidants properties of catechins and green tea extracts in sunflower oil-in-water emulsion	Roedig-Penman et al (1997)

TBARS, thiobarbituric acid reactive substances; PV, peroxide value; and CD, conjugated dienes;

Moreover, monitoring the induction period before the appearance of these primary oxidation products can be used to examine the effectiveness of antioxidants in edible oils (Hudson and Gordon, 1994; Shahidi and Wanasundara, 1998). Primary lipid oxidation products are frequently measured by the classical iodometric or conjugated dienes methods.

2.5.1.1 Peroxide value (PV)

Determination of peroxide value is based on the reduction of hydroperoxides (ROOH) with iodide (I^-). The liberated iodine is titrated with a standardized sodium thiosulphate ($Na_2S_2O_3$) solution (eqs.2.9 and 2.10). Therefore, the amount of released iodine is proportional to that of peroxides present (Shahidi and Wanasundara, 1998). The main disadvantages of this method are the absorption of iodine at the unsaturated bonds of fatty acids which affects the peroxide values and the liberation of iodine from potassium iodide by oxygen present in the solution. However, many of these disadvantages were taken into consideration in the AOCS method Cd-8-53 (Rosell, 1994).

$$2ROOH + 2H^+ + 2KI \longrightarrow I_2 + 2ROH + H_2O + K_2O \qquad \text{(eq.2.9)}$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2\,NaI \qquad \text{(eq.2.10)}$$

The peroxide value is a common method of measurement of lipid oxidation and good correlations have been observed between PV and sensory scores of several vegetable oils (Frankel,1993b). Nonetheless, oils with low PV have not always received high flavour scores. Therefore, it has been recommended that additional analytical methods be used to assess the stability of edible oils (King et al, 1995).

2.5.1.2 Conjugated dienes (CD)

It has been observed that the hydroperoxides formed during oxidation of linoleic and linolenic acids at the initial stages contain conjugated diene groups which absorb UV light strongly at 234 nm. This observation forms the basis of their spectrophotometric determination (Prior and Loliger, 1994).

Edible oil oxidation has been followed by measuring the absorbance at 234 nm by the IUPAC method 2.505 (Wanasundara et al, 1995; Abou-Gharbia et al, 1997) or by calculating the conjugated dienes as mmol of methyl linoleate hydroperoxide per kg of oil using a molar absorptivity of 26,000 (Hopia et al, 1997). The presence of molecules containing double bonds such as carotenoids, that can absorb UV light at 234 nm, might interfere with CD determination (Shahidi and Wanasundara, 1998). Nonetheless, this method is very useful in evaluating the oxidative stability of refined or stripped edible oils because dienes, trienes and tetraenes, which have absorbance at 234, 274 and 392 nm, respectively, are not normally present in the system (Prior and Loliger, 1994; Blekas, 1995).

2.5.2 Measurement of secondary oxidation products

Undesirable flavours associated with lipid oxidation arise from further decomposition of lipid hydroperoxides to yield aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Hamilton,1994). Some analytical techniques used to measure these products relate to the amount of total aldehydes in the oxidized

lipid such as TBARS, while others can precisely determine individual secondary oxidation products such as determination of headspace volatiles.

2.5.2.1 2-thiobarbituric acid reactive substances (TBARS)

The 2-thiobarbituric acid reactive substances (TBARS) or the TBA test is frequently used for the quantitation of lipid oxidation in foods. The proposed reaction is shown in Figure 2.6. One molecule of malonaldehyde (MA), which is a secondary product of lipid oxidation, reacts with two molecules of 2-thiobarbituric acid (TBA) to afford the

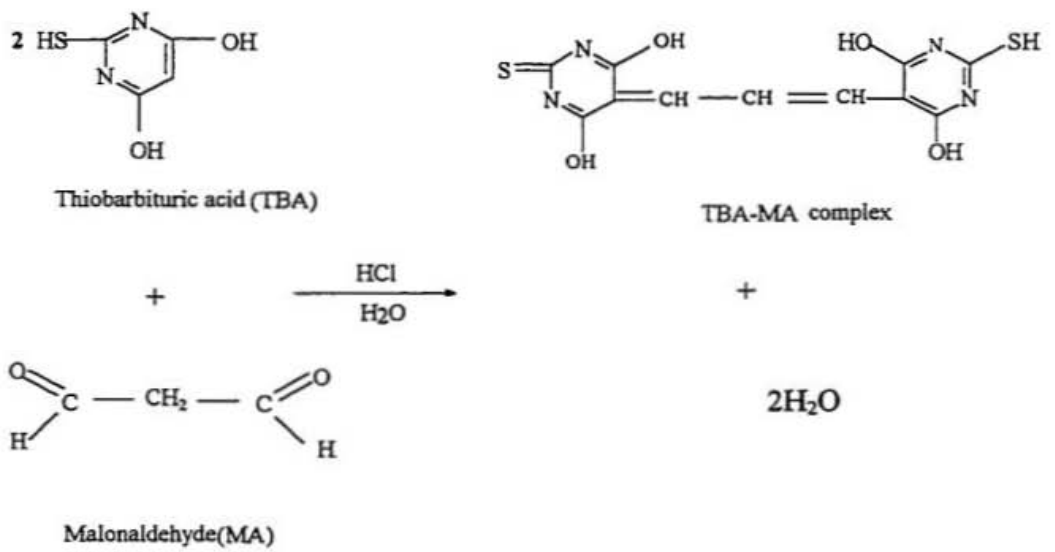


Figure 2.6 The formation of malonaldehyde-thiobarbituric acid (TBA-MA) chromogen.

TBA-MA complex which has a maximum absorbance at 532 nm (King et al, 1995). The accuracy of the TBA test has been questioned due to the reaction of TBA with other molecules such as sugars and oxidized proteins (Rossell, 1994). However, these products are normally absent in edible oils, thus TBA is frequently used to assess their oxidation (Kaitaranta, 1992; Ganthavorn and Hughes, 1997). Meanwhile, TBA might also react with other oxidation products such as 2-alkenals and 2,4-alkadienals present in edible oil, which might reflect the total amount of aldehydes rather than MA alone (Rossell, 1994; Shahidi and Wanasundara, 1998).

2.5.2.2 Headspace analysis of volatiles

Various types of headspace analyses, using gas chromatography, have been used to assess the oxidation of edible oils. In most of these techniques, the oil is heated at 180 C in closed vials. The volatiles, from the decomposition of the hydroperoxides as well as those present before heating, collected in the headspace above the oil, are analyzed by gas chromatography. The total peak area of the volatiles in this technique increases with the length of storage period of an oil. Therefore, gas chromatography can provide useful data about the origin of flavour volatiles and their precursors (Frankel, 1993; Rossell, 1994).

Gas chromatographic analyses of edible oils have revealed that hexanal and pentane are the major volatiles of oxidation of n-6 PUFA, while propanal is the predominant volatile derived from n-3 PUFA (Frankel, 1993b; Shahidi and Wanasundara, 1998). Moreover, King et al (1995) have reported that an excellent correlation has been

observed between GC data and sensory scores in photooxidized soybean oil, while pentanal and hexanal correlated well with flavour scores of autoxidized soybean oil.

2.5.3 Measurement of oxidation in emulsions

Many of the analytical techniques used to measure oxidation of edible oils are also useful to monitor lipid oxidation in emulsions. However, it is often necessary to extract the oil phase before performing the analysis (Coupland and Mc Clements, 1996a). This can be achieved by mixing the emulsions with a nonpolar solvent such as hexane to extract the lipid (Frankel,1994). Isolation of oil from emulsions can also be accomplished by freezing, thawing and centrifugation (Yen and Chen, 1995).

Primary and secondary oxidation products can also be measured without the extraction step. Huang et al (1996a) have determined the conjugated diene hydroperoxides by dispersing stripped corn oil in methanol and measuring the absorbance at 234 nm. Furthermore, volatiles such as hexanal can be measured directly from the emulsion samples using static headspace gas chromatography (Frankel et al, 1994).

2.5.4 Selection of several methods to evaluate oxidative rancidity

Most of the analytical methods used to follow lipid oxidation such as PV, CD and TBARS have limitations (Wanasundara et al, 1995). Furthermore, the methodologies used to evaluate oxidative stability of edible oils must be carefully interpreted based on the analytical procedure used to determine the extent and the end point of the oxidation. Therefore, it is recommended that progress of oxidation be followed by more than one

method and by measuring different types of products, including initial and decomposition products of lipid oxidation (Frankel, 1993; Lampi et al, 1997). It is also possible to determine the extent to which the various methods agree with one another by using carefully controlled model systems. This can be achieved by calculating the correlation coefficients (Hudson and Gordon, 1994) or by employing linear regression analysis (Lampi et al,1997).

2.5.5 Accelerated oxidation tests

In order to estimate the stability and susceptibility of fats and oils towards oxidation, and thus their quality and shelf life, a number of accelerated oxidation tests are used, including the Schaal oven test, the Sylvester test and its automated form using Oxidograph equipment, and the Swift test and its automated forms using the Rancimat and Oxidative Stability Instrument (Rossel, 1994). Table 2.6 summarizes standard accelerated stability tests at different temperatures. The information in this table indicates that heating is the most commonly used and effective means of accelerating oxidation, although fluorescent light can also be used to study the role of the sensitizers on the stability of edible oils (Frankel, 1994). Furthermore, all tests have limitations and some of the automated techniques such as FIAR-Astell are known to suffer from several drawbacks and, therefore, such equipment is no longer manufactured (Rossel, 1994). During the stability tests, induction period (IP) is measured as the time required to reach the end point of oxidation that is corresponding to detectable activity or a sudden change in the rate of oxidation (Hamilton, 1994; Frankel, 1993a).

Table 2.6 Some standard accelerated Oxidative stability tests.

Test	Conditions	Characteristics
Metal catalyst	Room temperature, atmospheric pressure	More decomposition
Weight gain method	30-80 C	The end point is questionable
Schaal oven test	60-70 C atmospheric pressure	Fewest problem
Oxygen uptake	80-100 C atmospheric pressure	Different mechanisms
Active oxygen	98 C air bubbling	Different mechanisms
Rancimat	100-140 C	End point questionable
Light	Room temperature atmospheric pressure	Different mechanisms

The Schaal oven test (60-70 C) suffers from very few limitations. The induction period (IP) represents a lower degree of oxidation and results correlate well with evaluation of actual shelf lives. Using higher temperatures, however, might interfere with the proper prediction of shelf life. Therefore, the conditions of accelerated oxidation tests should be maintained as close as possible to those under which the food is stored (Frankel, 1993b).

2.6 Controlling lipid oxidation

The oxidation of edible oils and fats can be controlled by proper application of synthetic antioxidants, natural antioxidants and/or use of inert gas or vacuum packaging. Ideal synthetic and natural antioxidants for food applications, should meet certain criteria such as safety, ease of incorporation, effectiveness at low concentration, absence of undesirable odour, flavour and colour, resistance to high temperature experienced during baking and frying as well as availability at low cost (Coppen, 1994). The effects of several commercial antioxidants and packaging conditions on the oxidative stability of carp fillets have been evaluated. Results have indicated that antioxidants together with vacuum packaging are more effective in retarding oxidation of the fillets than antioxidants alone without vacuum packaging (Khalil and Mansour, 1998).

Synergism between antioxidants, where combination of two or more antioxidants has shown better success in controlling lipid oxidation than equivalent quantities of any one antioxidant, has also been observed. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been shown to act synergistically (Omura, 1995).

Furthermore, some acidic compounds such as ascorbic acid, citric acid, phosphoric acid as well as ethylenediaminetetraacetic acid (EDTA) and phospholipids have the ability to quench traces of metal ions or act synergistically in the presence of other antioxidants, thus preventing lipid oxidation (Lambelet et al, 1994; Coppen et al, 1994). In addition to the antioxidants and proper packaging, undesirable lipid oxidation of edible oil emulsions can be retarded by controlling factors that affect the stability of emulsions as described previously.

2.6.1 Synthetic antioxidants

Phenolic antioxidants, AH, inhibit lipid oxidation by acting as hydrogen or electron donors, and interfere with the free radical, $R^{\bullet}$, chain reaction by forming non-radical compounds that will not propagate further radical reactions (eqs. 2.11-2.16). Synthetic antioxidants such as BHA, BHT and tert-butylhydroquinone (TBHQ) are examples of commonly used phenolic antioxidants (Figure 2.7). The effectiveness of these antioxidants is attributed to the stability of the phenoxy radical which is due to resonance delocalization. This stability is increased by interactions between phenolic hydroxy groups and neighboring methoxy or tertiary-butyl groups (Berger and Hamilton, 1995).

BHA and BHT are highly soluble in animal fats and vegetable oils. However, they are more effective antioxidants for animal fats. Meanwhile, TBHQ, which has been approved in the USA and some other countries since 1972, but not in Canada, is a powerful antioxidant for vegetable oils. Propyl gallate, another synthetic phenolic antioxidant, is effective in both animal fats and vegetable oils, however, the major

drawback of this antioxidant is the formation of blue-black reaction products with iron in the presence of water (Coppen, 1994).

$AH + R^{\bullet} \longrightarrow RH + A^{\bullet}$ (eq.2.11)

$AH + RO^{\bullet} \longrightarrow ROH + A^{\bullet}$ (eq.2.12)

$AH + ROO^{\bullet} \longrightarrow ROOH + A^{\bullet}$ (eq.2.13)

$A^{\bullet} + R^{\bullet} \longrightarrow AR$ (eq.214)

$A^{\bullet} + RO^{\bullet} \longrightarrow AOR$ (eq.2.15)

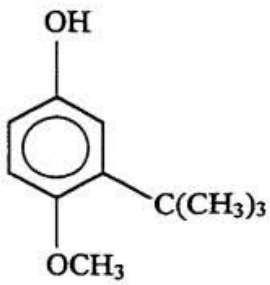
$A^{\bullet} + ROO^{\bullet} \longrightarrow AOOR$ (eq.2.16)

2.6.2 Natural antioxidants

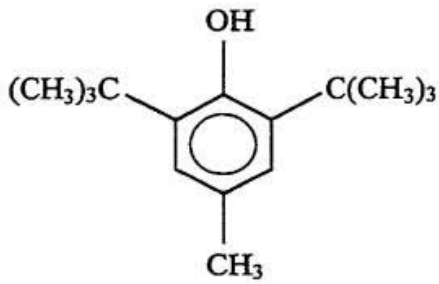
Application of natural antioxidants to prevent oxidation of food lipids is growing rapidly due to increasing consumer demands and controversy over the use of synthetic antioxidants (Bruun-Jensen et al, 1996). Moreover, consuming foods rich in natural antioxidants may improve health status of humans since many diseases are developed due to oxidation reactions in the body (Ramarathnam et al, 1995; Wanasundara et al, 1997).

Most of the natural antioxidants are derived from plant sources (Table 2.7). The ability of herbs and spices to control lipid oxidation has been known for several decades (Madsen and Bertelsen, 1995). Thirty five species of edible pulses were extracted with methanol. Extracts of 11 species showed strong antioxidant activity (Tsuda et al, 1993). Furthermore, the radical scavenging abilities of methanol extracts of 51 spices have been reported, where mustard varieties, thyme, oregano, cloves and allspice exhibited

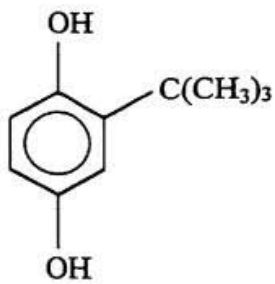
Figure 2.7 Chemical structure of commonly used synthetic antioxidants in food industry.



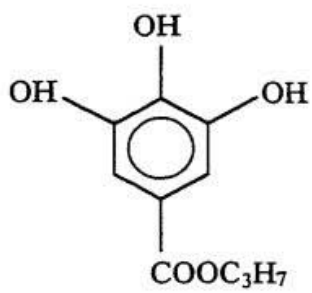
Butylated hydroxyanisole (BHA)



Butylated hydroxytoluene (BHT)



tertiary-Butylhydroquinone (TBHQ)



Propyl gallate (PG)

strong hydroxyl radical ($^{\bullet}OH$) scavenging activities (Chung et al, 1997). Rosemary extracts, containing the active ingredients carnosol and carnosic acid, effectively inhibited lipid oxidation in corn, soybean, peanut and fish oils. However, these compounds were inactive or promoted oxidation in the vegetable oil-in-water emulsions (Frankel, 1996b). Antioxidant activity of tea and its catechins has been observed in a fish meat model system (He and Shahidi, 1997) as well as in a sunflower oil-in-water emulsion (Roedig-Penman and Gordon,1997). However, when green tea extracts from India, China and Japan were compared in different lipid systems, they were active antioxidants in bulk corn oil, oxidized at 50 C, but were prooxidants in the corresponding oil-in-water emulsions (Frankel et al, 1997).

It has been observed that carotenoids, tocopherols and phospholipids in edible oils, although occurring in minor amounts, have profound effects on their oxidative stability (Shahidi et al, 1996). This observation has encouraged the industry to use these compounds, particularly mixed tocopherols, as natural antioxidants to prevent or control lipid oxidation.

Carotenoids have been shown to serve as excellent antioxidants in singlet oxygen oxidation (Mortensen and Skibsted, 1997). Moreover, chain breaking activities of carotenoids during non-illuminated storage of foods have been observed (Nielsen et al, 1996). Flavour deterioration of soybean oil treated with citric acid stored under light was inhibited by the addition of 5-10 ppm of β-carotene (Warner and Frankel, 1987). This inhibition has been attributed to the ability of β-carotene to quench singlet oxygen and

Table 2.7 Different lipid oxidation inhibitors found in plants and seeds

Type of inhibitor	Major chemical compound (s)
Antioxidants	Flavonoids, tocopherols, carotenoids
Synergists	Flavonoids, tocopherols, phospholipids
Retarders	Catalase, Maillard reaction products
Metal scavengers	Flavonoides, amino acids, phytic acid
Singlet-oxygen quenchers	Superoxide dismutase, ascorbic acid, carotenoids

thus minimize chlorophyll photosensitized oxidation of soybean oil (Lee and Men, 1988). Meanwhile, potential prooxidant activities of carotenoids have been discovered in vegetable oils and oil-in-water emulsions and tocopherols were suggested to protect β-carotene from light (Haila et al, 1996 ; Heinonen, 1997).

2.6.3 Tocopherols

The main biochemical function of tocopherols is belived to be protection of PUFA against peroxidation. The correlations between fatty acid composition and tocopherol levels in 14 vegetable oils have been reported. Principal component and linear regression analyses showed positive correlation between linoleic acid and α-tocopherol as well as linolenic acid and γ-tocopherol (Kamal-Eldin and Appelqvist, 1997). Tocopherols are synthesized only by plants; they are present in oilseeds, leaves, and other green parts of higher plants, and, therefore, are important nutrients for animals and humans (Kamal-Eldin and Appelqvist, 1996, Wanasundara et al, 1997).

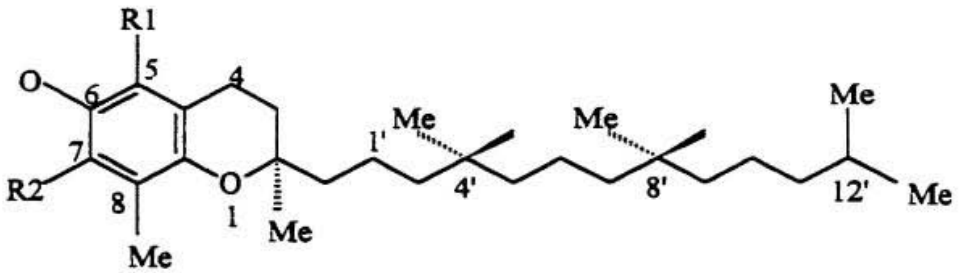
Tocopherols consist of a chromane head with two rings (one is phenolic and the other is heterocyclic) and phytyl tail (Kamal-Eldin and Appelquist, 1996). They are designated α, β, γ, and δ based on the number and position of methyl groups on the chromane ring (Shahidi and Shukla, 1996). Therefore, the four tocopherols have saturated tails and vary only in the number of methyl substituents and the pattern of substitution in the phenolic ring (Figure 2.8).

The antioxidant activity of tocopherols is mainly due to their ability to donate their phenolic hydrogens, similar to the previously described phenolic antioxidants, to lipid free radicals generated during free radical chain reactions (Yamauchi et al, 1997). This would generate a neutral lipid and a tocopheryl semiquinone radical. Subsequently, two of these radicals form a tocopheryl quinone molecule and a tocopherol, thus terminating the chain reaction (Wanasundara et al, 1997). Meanwhile, tocopheryl radicals in the human body can be converted back to tocopherol by ubiquinone , ascorbate, or enzymes such as ferrocytochrome C (Thomas, 1995).

The chemical structure of the various tocopherols supports a hydrogen donating power in the order $\alpha > \beta > \gamma > \delta$. Therefore, it has been observed that the relative antioxidant activity of tocopherols *in vivo* is in the order $\alpha > \beta > \gamma > \delta$. Nonetheless, the reverse order of activities has been reported for *in vitro* systems. The controversy over this phenomenon has not yet been fully resolved (Kamal-Eldin and Appelqvist, 1996; Bruun-Jensen et al, 1996). Meanwhile, tocopherols are known to be strong singlet oxygen scavengers and thus are able to prevent or control lipid photooxidation. The effects of α-, γ- and δ-tocopherols, at different concentrations, on chlorophyll photosensitized oxidation of soybean oil have been studied. The results indicate that α-tocopherol exhibits the highest singlet oxygen quenching activity followed by γ- and δ- tocopherols (Jung and Min, 1991).

Tocopherols might act as antioxidants or prooxidants based on their concentration. The effects of different concentrations (0, 100, 250 and 500 ppm) of various tocopherols

Figure 2.8 Chemical structures of tocopherols.



Tocopherol	R1	R2
α	Me	Me
β	Me	H
γ	H	Me
δ	H	H

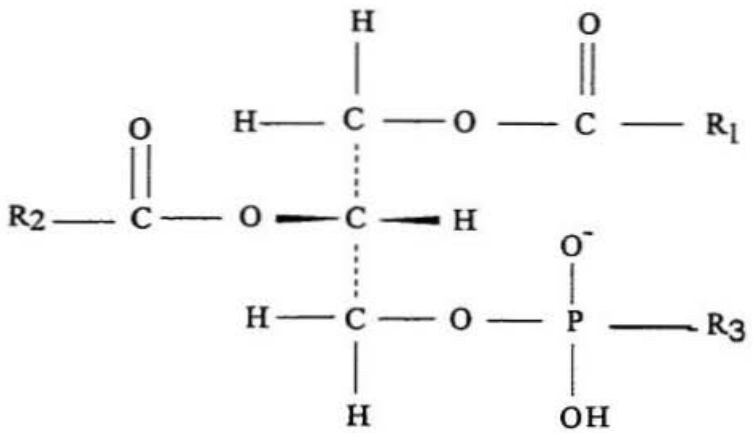
(α, γ and δ) on the oxidative stability of stripped soybean oil have been reported (Jung and Min, 1990). Tocopherols above 500 ppm exhibited prooxidant activity. Similarly, Yoshida et al (1993) have evaluated the effects of α-, β-, γ- and δ-tocopherols at different concentrations in a stripped vegetable oil. Antioxidant activity at concentrations above 500 ppm was not significant. Moreover, Blekas et al (1995) have studied the effects of 100, 500 and 1000 ppm of α-tocopherol on the oxidation of stripped olive oil at 40 C. Although α-tocopherol acted as an antioxidant at all levels, at 100 ppm it exhibited the greatest effect.

2.6.4 Phospholipids

Phospholipids (PL) occur in edible oils at levels ranging from 0.1 to 10%. The main PL subclasses occurring in edible oils are phosphatidylcholine (PC), lysophos-phosphtidylcholine (LPC), phosphotidylethanolamine (PE), lysophosphatidylethanolamine (LPE), pohsphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (Figure 2.9) (Du Plessis and Pretorius, 1984; Wanasundara et al, 1997; Imbas and Pham, 1997). Phospholipids are considered as important factors influencing the flavour stability of edible oils due to their higher degree of unsaturation as compared to triacylglycerols (King,1995).

Phospholipids can act as antioxidants or prooxidants as well as synergists and chelating agents. The effects of total lipids, neutral lipids (NL) and phospholipid fractions (PL) extracted from bluefish have been examined using a salmon oil model system

Figure 2.9 Chemical structures of main phospholipid subclasses in edible oils.



R_3 =	Phospholipid subclasses
-OH	Phosphatidic acid (PA)
$-O-CH_2-CH(NH_2)-COOH$	Phosphatidylserine (PS)
$-O-CH_2-CH_2-NH_2$	Phosphatidylethanolamine (PE)
$-O-CH_2-CH_2-N^+(CH_3)_3$	Phosphatidylcholine (PC)

(King et al, 1992). PL fractions at 2.5 and 5% (w/w) improved the oxidative stability of this model system incubated at 55 or 180 C. However, Nwosu et al (1997) have reported that SP, PC and PE have little or no antioxidant activity in food grade menhaden oil while the same three PL subclasses had significant antioxidant activity in salmon oil. The relative antioxidant activities of commercial PL on the oxidation of salmon oil are in the order of sphingomyelin (SP)= LPC=PC=PE>PS>PI>PG> control salmon oil (Shahidi and Shukla, 1996). Meanwhile, Hildebrand (1984) observed that combinations of tocopherols and PC, PE and PI increased the stability of soy bean oil; PI and PE were more effective than PC. Similarly, tocopherols together with PE were more effective than tocopherol with PC or PS in controlling lipid oxidation in sardine and mackerel lipids (Ohshima et al, 1993).

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

Cold-pressed borage oil was obtained from Bioriginal Food & Science Co. (Saskatoon, SK). The evening primrose oil was obtained from Scotia Pharmaceuticals (Kentville, NS). Natural antioxidants, namely α-tocopherol, and δ-tocopherol, as well as synthetic antioxidants, mainly butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and phospholipids, phosphatidylcholine (PC), lysophosphatidylethanolamine (LPE), phosphatidylinositol (PI), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidic acid (PA) as well as oleic acid, monoolein, diolein, triolein, linoleic acid ethyl ester, 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), sucrose, and Tween 40 were obtained from Sigma Chemical Co. (St. Louis., MO). The food grade natural antioxidant Tenox GT-2 was acquired from Eastman Chemical Co. (Kingsport, TN). Compressed air, hydrogen and UHP helium were obtained from Canadian Liquid Air Ltd. (St. John's, NF). Activated charcoal was acquired from BDH Inc. (Toronto, ON). Celite 545 was obtained from Fisher Scientific (Fair Lawn, NJ). Silicic acid powder (100 mesh) was purchased from Mallinckrodt Canada, Inc. (Point-Claire, PQ). All other chemicals were ACS grade or better.

3.2 Preparation of minor-component stripped oils

Borage and evening primrose oils were stripped of their minor components by following the method of Lampi et al (1992) with modifications. A chromatographic column (3.4 cm internal diameter x 40 cm height) was connected to a water-pump vacuum and packed sequentially with four adsorbents. The bottom layer consisted of 40 g of activated silicic acid, the next layers were 20 g of a 1:2 (w/w) mixture of Celite 545-activated charcoal and 80 g of a 1:2 (w/w) mixture of Celite 545-powdered sugar (sucrose), and the top layer was 40 g of activated silicic acid. All adsorbents were suspended in n-hexane. The silicic acid was activated as described by Min (1973). Silicic acid (100 g) was washed three times with a total of 3 L of distilled water. After each treatment the silicic acid was left to settle for 30 min, then the suspended silicic acid was decanted. Finally, the silicic acid was washed with methanol and the supernatant decanted. Traces of methanol were removed by filtering through a Buchner funnel under vacuum and the semi-dried material was activated at 200 C for 22 h.

Oil (100 g) was diluted with an equal volume of n-hexane and passed through the chromatographic column. The solvent in the eluent (stripped oil) was evaporated under vacuum at 30 C and traces of the solvent were removed by flushing with nitrogen. This procedure was repeated until 800 g of stripped borage (SBO) or stripped evening primrose (SPO) oil were obtained. Oils (SBO or SPO) obtained from several runs were pooled together, transferred into a 250 mL amber bottle, flushed with nitrogen and kept at -70 C for subsequent studies.

3.3 Preparation of oil-in-water emulsions

Ten percent (w/v) borage and evening primrose oil-in-water emulsions were prepared as described by Frankel et al (1994). Oil (5 g) was weighed into a precleaned 125 mL Pyrex flask, the volume was made up to 50 mL with distilled water and the mixture was emulsified with 0.5 g of Tween 40. The emulsions were mixed gently for 5 min, using a magnetic stirrer, and sonicated at maximum power in an ultrasonic sonicator (model 6 QT NEYO, Yucaipa, CA) filled with cooled water (1 C) for 25 min.

3.4 Preparation of samples for accelerated oxidation tests

Stripped and non-stripped oil samples (5 g in 25 mL flasks), and oil-in-water emulsions (50 g in 125 mL flasks), were used to study their oxidative stability in the dark with heating (autoxidation) and in the light (photooxidation). For autoxidation studies, the sample containers were covered with aluminum foil and kept at 60 C in a forced air Precision oven Model 2 (Precision Scientific Co., Chicago, IL). For photooxidation studies the samples were placed in a box (70 cm lengh X 35 cm width X 25 cm height) equipped with two 40 watt cool white fluorescent lights which were suspended approximately 10 cm above the surface of the oil containers. The remaining open space was covered with aluminum foil. The fluorescent radiation was at a level of 2650 Lux and the temperature inside the container was 27± 1 C.

Oil and emulsion samples were removed from the oven after 24, 72, 120 and 168 h, and from the box after 12, 24, 48 and 72 h, flushed with nitrogen, covered with parafilm and kept at -70 C for oxidative stability tests. Prior to oxidation stability tests, the oil was

extracted from the emulsion (50 g) by adding 50 mL methanol and 50 mL hexane in a 250 mL Pyrex separatory funnel (Frankel et al, 1994). After removing the hexane layer, the methanol layer was washed twice with hexane. The hexane extracts were combined and evaporated under vacuum at 30 C. Traces of the solvents were removed by flushing with nitrogen. The extracted oil was transferred to 10 mL screw capped vials, flushed with nitrogen and stored for no more than one week before subsequent analysis.

3.5 Oxidative stability tests

The oxidative stability of stripped and non-stripped oil and oil-in-water emulsions described in Section 3.4 was evaluated by determining peroxide value (PV), conjugated dienes (CD), 2-thiobarbituric acid reactive substances (TBARS) and headspace volatiles. However, progress of oxidation for stripped oil samples in section 3.7 was followed by determining only their CD and TBARS.

3.5.1 Determination of peroxide value (PV)

Peroxide values of the stripped and non-stripped oils were determined according to the American Oils Chemists' Society', (AOCS) method Cd-8-35 (1990). Oil samples (1.5-2.0 g) were weighed into 125 mL glass-stoppered Pyrex flasks. 30 mL of an acetic acid-chloroform (3:2, v/v) mixture were added to each flask and after thorough mixing, 0.5 mL of saturated potassium iodide (KI) was added and the mixture was kept in the dark, with occasional shaking, for exactly 1 min. 30 mL of distilled water were then added and the liberated iodine was titrated with 0.01 or 0.1 N sodium thiosulphate solution, until the

yellow colour had almost disappeared. Then, approximately 0.5 mL of starch indicator solution (1% w/v) was added and the titration was continued with vigorous mixing until the blue colour of the solution had disappeared. A blank titration was conducted each time. Peroxide value was expressed as the uptake of milliequivalents of active oxygen per kilogram of oil and calculated as follows:

$$PV = \frac{(V \text{ sample} - V \text{ blank}) \times N\ Na_2S_2O_3 \times 1000}{\text{Mass of sample (g)}}$$

where V= volume of sodium thiosulphate solution (mL) and N = normality of sodium thiosulphate.

3.5.2 Determination of conjugated dienes (CD)

Conjugated dienes of the oil samples were determined by the IUPAC method 2.505 (1987). Oil samples (0.02-0.04 g) were weighed into 25 mL volumetric flasks, dissolved in iso-octane (2,2,4-trimethylpentane) and made up to volume with the same solvent. The contents were mixed thoroughly and the absorbance was read at 234 nm in a 10 mm Hellma quartz cell using a Hewlett-Packard 8452A diode array spectrophotometer. Pure isooctane was used as the blank. Conjugated dienes value was calculated as

$$CD = A/(c \times d)$$

where, A = absorbance of the solution at 234 nm, c = concentration of the solution in g/100 ml solution, and d = length of the cell.

3.5.3 Determination of 2-thiobarbituric acid reactive substances (TBARS)

Oil samples (0.05 - 0.20 g) were analyzed for their content of TBARS according to the AOCS (1990) method Cd 19-90. The samples were accurately weighed into 25 mL volumetric flasks and dissolved in a small volume of 1-butanol and made up to volume with the same solvent. Five mL of this mixture were transferred into a dry test tube, and 5 mL of fresh TBA reagent (200 mg TBA in 100 mL 1-butanol) were added. The contents were mixed and heated in a water bath at 95 C for 120 min. The intensity of the resultant coloured complex was measured at 532 nm using a Hewlett-Packard 8452A diode array spectrophotometer.

The TBARS values were calculated by multiplying the absorbance readings by a factor of 0.145 determined from a standard regression line prepared using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde (He and Shahidi, 1997). The results are recorded in Figure A.1.

3.5.4 Headspace analysis of volatiles

A Perkin-Elmer 8500 gas chromatograph equipped with an HS-6 headspace sampler (Perkin-Elmer Co., Montreal, PQ) was used for volatile analysis of oil and emulsion samples (Shahidi et al, 1997). The column used to separate the volatiles was a SUPLECOWAX-10 fused-silica capillary (30 m x 0.32 mm i.d., 0.10 μm film: Supleco Canada Ltd., Mississauga, ON). Helium (UHP) was the carrier gas employed at an inlet column pressure of 20 psig with a split ratio of 7:1. The injector and flame ionization detector (FID) temperatures were 280 C. The oven temperature was maintained at 40 C

for 5 min, and then increased to 115 C at 10 C/min and subsequently ramped to 200 C at 30 C /min and held there for 5 min.

Oil and emulsion samples (0.2 g) were transferred into special glass vials which were capped with special butyl septa and preheated in the HS-6 magazine assembly at 90 C for a 45 min equilibrium period. Pressurization time was 6 s and the volume of the vapour phase drawn was approximately 1.5 mL. Individual volatile compounds were tentatively identified by comparing the relative retention times of GC peaks with those of commercially available standards. Quantitative determination of dominant aldehyde and hexanal, was accomplished using 2-heptanone as an internal standard. Formation of several volatiles (pentanal, hexanal, 2-heptenal and 2,4-heptadienal) was monitored as a measure of the oxidation of the oil and emulsion samples.

3.6 Chemical and instrumental analysis

3.6.1 Analysis of fatty acid composition

Fatty acid composition of the oils was analyzed according to the method described by Wanasundara and Shahidi (1997). Oil samples (10 mg) were weighed into 6 mL chromerge-cleaned (Fisher Scientific, Fair Lawn, NJ), screw capped, Teflon-lined, conical vials. Subsequently, 2 mL of transmethylation reagent were added to each vial and mixed thoroughly. This reagent was prepared freshly each day by adding 6 mL of concentrated sulphuric acid to a 100 mL volumetric flask, then making up to volume with spectral-grade methanol and adding 15 mg of BHT as antioxidant. The mixture of fatty acids and the reagent was incubated at 60 C for 17 h. At the end of the incubation period, the vials

were removed, cooled to room temperature and 1 mL of distilled water was added. The fatty acid methyl esters (FAME) were extracted 3 times with 1.5 mL of n-hexane. A few crystals of BHT were added to each vial before the extraction step to prevent oxidation of unsaturated fatty acids. Hexane layers were separated, combined, transferred to a clean dried tube and washed two times with distilled water. The hexane layer was separated and evaporated under a stream of nitrogen at room temperature.

3.6.1.1 Analysis of fatty acid methyl esters by gas chromatography

Fatty acid methyl esters, prepared in the previous section, were analyzed using a Hewlett Packard 5890 II gas chromatograpgh (Hewlett packard, Toronto, ON) equipped with a 30 m x 0.25 mm supelcowax-10 column (SP 2330, Supelco Canada Ltd., Oakville, ON). The injector and flame ionization detector temperatures were both at 270 C. The oven temperature was initially 220 C for 10.25 min and then increased to 240 C at 30 C/min and held there for 9 min. Helium (UHP) was used as the carrier gas. The FAMEs were identified by comparing their retention times with those of an authentic standard mixture (PUFA1; Supelco, Canada Ltd. Oakville, ON) and quantified using area normalization.

3.6.2 Separation of lipid classes

Neutral, glyco- and phospholipids were separated by silicic acid column chromatography (Christie, 1982). Borage and evening primrose oils were applied to an activated silicic acid column (2.5 cm internal diameter and 30 cm height). Neutral lipids

were eluted with chloroform (10 times the column volume), then monogalactosyl diacylglycerol with 8 times the column volume of chloroform-acetone (50:50, v/v). Digalactosyl diacylglycerols were eluted with 10 times the column volume of acetone. Finally, the phospholipid fraction was eluted using 10 times the column volume of methanol. Solvents were removed under vacuum using a rotary evaporator at 40 C and traces of each solvent were removed under a stream of nitrogen at room temperature. The fractions were weighed and the weight percentages of neutral lipids (NL), glycolipids (GL) (both mono- and digalactoacyl diacylglycerols) and phospholipids (PL) were calculated.

3.6.3 Analysis of neutral and phospholipids composition

3.6.3.1 Sample and standard curve preparation

The NL and PL fractions obtained in the previous section (3.6.2) were used to determine the concentration of NL and PL subclasses according to Du Plessis and Pretorius (1984) and Imbas and Phame (1997), with modifications. Free fatty acids as oleic acid equivalents and individual neutral lipids monoacylglycerol (monoolein), diacylglycerol (diolein), triacylgycerol (trioelien), as well as individual phospholipids, namely phosphatidylserine, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylcholine, lysophosphatidylcholine, phosphatidylinositol, and phosphatidic acid were dissolved in chloroform - methanol (2:1, v/v) to obtain standard stock solutions. The purity checks for all standard solutions revealed a single peak with thin layer chromatography-flame ionization detection (TLC-FID). A range of standards at different

concentrations (2-20 μg) were prepared and used to obtain the R_f values and establish the standard curves.

3.6.3.2 Cleaning and activation of the Chromarods

Chromarods-SII (Iatron laboratories Inc., Tokyo, Japan) were used to separate lipid sub-classes. To clean the Chromarods, they were dipped overnight in concentrated sulphuric acid, then washed thoroughly with HPLC grade distilled water and dried with acetone. Residual acetone was removed by placing the rods in an oven at 100 C for 5 min. The cleaned, washed and dried Chromarods were passed through the FID scanner while monitoring the baseline of each rod.

3.6.3.3 Lipid subclasses analysis by thin layer chromatography-flame ionization detection (TLC-FID; Iatroscan)

NL and PL fraction samples (1μL or 10 μg) in chloroform-methanol (2:1, v/v) were spotted on chromarods-SII with a 5μL Hamilton syringe prior to TLC separation of NL and PL classes. The rods were dried for 10 min and then transferred into a developing tank containing benzene-chloroform-acetic acid (70:30:4, v/v/v) in order to separate neutral lipid classes. A chloroform-methanol-water (70:30:3, v/v/v) mixture was used to separate PL classes. After 45 min, the rods were removed from the glass tanks, dried for 10 min and the residual solvents were removed by incubating at 100 C for 3 min. After this period, the rods were removed from the oven and cooled to room temperature.

Analysis of the developed rods was performed on Iatroscan MK-5 TLC/FID (Iatron laboratories Inc., Tokyo, Japan). FID scanning speed was 40 s/rod, hydrogen flow rate was 160 mL/min and the air flow rate was 2000 mL/min. Individual lipids were identified by comparison of their retention times with those of known standards. Quantification, on a percentage basis, was achieved by utilizing external standard curves (prepared as described previously in 3.6.3.1) and TSCAN computer software (S.P.E. Limited, Concord, ON).

3.6.4 Measurement of pigments

Pigments present in the stripped and non-stripped oil samples were determined qualitatively by (1) measuring the absorbance at 430 - 460 nm for carotenoids and 550-710 for chlorophyll and their derivatives (Blekas et al, 1995). Oil samples (3 mL) were transferred into Hellma glass cells and the absorbance was read using a Hewlett-Packard 8452A diode array spectrophotometer and (2) by recording the absorption spectra between 430 and 710 nm. Olive oil, which contains carotenoids and chlorophyll, was used as a reference.

3.6.5 Determination of tocopherols by high performance liquid chromatography

A Shimadzu high performance liquid chromatograph (HPLC) equipped with two LC-6A pumps, SPD-6AV, Lichrosorb Si 60 analytical column (Merck, 3.2x200 mm, 5 μ) and a UV-VIS detector was used to analyze tocopherol content in the stripped and non-stripped oils (Shahidi et al, 1997).

One gram of oil was dissolved in 10 mL diethyl ether-hexane (5:59, v/v); 20 μL of this preparation was used for analysis. The flow rate was 1 mL/min and tocopherols in the samples were detected with the UV detector set at 295 nm and identified by comparing their retention times with those of the authentic standards (Holfman-La Roche Ltd., Basle, Switzerland).

3.7 Evaluating the effect of minor components on oxidative stability of stripped oils

Oil samples (5g) were weighed into screw capped Pyrex tubes (8 mmx150 mm). Selected tocopherols, phospholipids or synthetic antioxidants were dissolved in a minimum amount of ethanol (the same amount was added to the control samples) and added to the sample tubes at appropriate concentrations as given in Table 3.1. The tubes were then oxidized and analyzed as described in Section 3.5. Conjugated dienes (CD) were used to monitor primary oxidation products, while 2-thiobarbituric acid reactive substances (TBARS) were measured to quantitate the secondary oxidation products.

3.8 Statistical analysis

All experiments and/or measurements were replicated three times. Mean ± standard deviation was reported for each case. Normality was examined using Sigmastat. Analysis of variance and Tukeys studentized test were performed at a level of $p<0.05$ to evaluate the significance of differences between mean values. Linear regression analysis was used to establish the relationship between PV and CD as well as between hexanal and TBARS

Table 3.1 Types and concentration (ppm) of different synthetic and natural antioxidants used for oxidation experiments with stripped borage and evening primrose oils.

Purpose of addition	Additives	Concentration (ppm)
Effect of tocopherols	α- tocopherol	200, 500
	δ- tocopherol	200, 500
	Tenox GT-2	200, 500
Effect of phopholipids	PC	500
	PE	500
Effects of phospholipids together with tocopherols	PC+α-tocopherol	500+500
	PE+α- tocopherol	500+500
	PC+δ- tocopherol	500+500
	PE+δ- tocopherol	500+500
Effect of synthetic antioxidants	BHA	200
	BHT	200
	TBHQ	200

Abbreviations are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene and tert-butylhydroquinone, (TBHQ). Tenox GT-2 is a commercial natural antioxidant consist of 7.88 ppm of α-, 39.67 ppm of γ- and 21.77 of δ-tocopherol.

(Wanasundara et al, 1995, Freund and Williams, 1988). Data presented as (%) were transformed, prior to statistical analysis, using the following formula: transformed $x = \arcsin\sqrt{x/100}$.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Chemical and instrumental analysis

4.1.1 Chemical characteristics of stripped and non-stripped borage and evening primrose oils

The Chemical characteristics of cold-pressed non-stripped borage and evening primrose oils are summarized in Table 4.1. Data in this table demonstrate that non-stripped oils have a good oxidative stability, although non-stripped evening primrose oil (NEPO) contained more ($p<0.05$) primary and secondary oxidation products than non-stripped borage oil (NBO). Freshly refined edible oils usually have a peroxide value of less than 1. Nonetheless, peroxide values of up to 10 are often encountered for oils stored after refining (Rossel, 1994). Furthermore, high initial PV values up to 20, have been reported for freshly refined, solvent as well as supercritical carbon dioxide extracted borage oils (Sendodini,1995).

Neutral lipid contents of NBO and NEPO were 95.1±0.1 and 93.2±0.5 respectively. However, NEPO had higher amounts ($p<0.05$) of glyco- and phospholipids than NBO. The main tocopherol classes, as determined by high performance liquid chromatography (HPLC) in NBO were 52 ppm of δ- and 659 ppm of γ- tocopherols, while NEPO contained only 16 ppm of α- and 335 ppm of γ-tocopherls. Thus, NBO had higher amounts ($p<0.05$) of total tocopherols than NEPO. According to previously published data, borage oil may contain 150 ppm of γ-tocopherol and 1350 ppm of δ-tocopherol (Shahidi and Shukla, 1996). Furthermore, borage oil may contain β-tocopherol in

addition to γ- and δ-tocopherols (Sensidoni, 1996). Meanwhile, evening primrose oil has been reported to contain only 187 ppm of γ-tocopherol and 76 ppm of α-tocopherol (Hudson, 1984). Nonetheless, the same oil may contain higher amounts of α- (160 ppm), γ- (420 ppm), and δ-(65 ppm) tocopherols (Shahidi and Shukla, 1996) as compared to those obtained in this study.

Variation of tocopherols within the same type of vegetable oil may be attributed to different analytical procedures employed as well as existing genetic differences within the same plant biotype. Brandle et al (1993) characterized genetic variability in Ontario-grown evening primrose plants. Genetic variations have been observed among the 36 biotypes of evening primrose tested for oil content and fatty acid composition except for C 16:1 (n-9). Similarly, Roy et al (1993) found that annual variation in seed yield and oil content as well as the levels of gamma-linolenic acid in seeds may occur. A good correlation was found between fatty acid composition and tocopherol contents of vegetable oils (Kamal-Eldin and Appelqvist, 1996).

Pigments such as carotenoids, with absorbances between 430 and 460 nm, and chlorophylls which absorb light between 550 and 710 nm (Blekas et al, 1995) were significantly ($p<0.05$) higher in NEPO than NBO. According to Sensidoni et al (1996), borage oil contains 0.8 to 3.3 ppm of chlorophyll.

Preliminary experiments using columns packed with several layers of silicic acid and charcoal were not successful for the preparation of stripped borage and evening primrose oils at cold-room temperature (5 C) as well as ambient temperature (27 C).

Table 4.1 Chemical characteristics of non stripped and stripped borage and evening primrose oils.

Characteristics		NBO	SBO	NEPO	SEPO
Oxidative status	Peroxide value (meq/kg)	1.65 ± 0.27^{c}	0^{a}	3.16 ± 0.11^{d}	1.0 ± 0.23^{b}
	Conjugated dienes	1.60 ± 0.1^{b}	0^{a}	3.12 ± 0.4^{d}	2.05 ± 0.05^{c}
	TBARS (μmol/g)	0.56 ± 0.1^{c}	0.2 ± 0.01^{a}	1.65 ± 0.2^{d}	0.68 ± 0.1^{b}
	Hexanal (mg/ kg)	1.92 ± 0.06^{b}	0^{a}	4.14 ± 0.11^{c}	0^{a}
Lipid classes (weight %)	Neutral lipids	95.1 ± 0.1^{a}	99.89 ± 1.1^{b}	93.2 ± 0.5^{a}	99.56 ± 0.89^{b}
	Glycolipids	3.8 ± 0.1^{b}	0^{a}	5.3 ± 0.2^{c}	0^{a}
	Phopsholipids	0.9 ± 0.1^{b}	0^{a}	1.8 ± 0.1^{c}	0^{a}
Tocopherols [1] (mg/kg)	α	0^{a}	0^{a}	0^{a}	0^{a}
	δ	52^{c}	0^{a}	16^{b}	0^{a}
	γ	659^{d}	0^{a}	335^{c}	84^{b}
	Total	711^{a}	0^{a}	341^{c}	84^{b}
Pigments (absorbance)	430 nm	0.07 ± 0^{c}	0^{a}	0.62 ± 0.01^{d}	0.01 ± 0^{b}
	460 nm	0.02 ± 0^{b}	0^{a}	0.27 ± 0^{c}	0^{a}
	550 nm	0^{a}	0^{a}	0.09 ± 0^{b}	0^{a}
	620 nm	0^{a}	0^{a}	0.05 ± 0^{b}	0^{a}
	670 nm	0^{a}	0^{a}	0^{a}	0^{a}

Values are means of three determinations ± standard deviations. Values with different superscripts in each row are different ($p<0.05$) from one another. [1]Average of duplicate analysis. Abbreviations are: NBO, non-stripped borage oil; NEPO, non-stripped evening primrose oil; SBO, stripped borage oil; SEPO, stripped evening primrose oil.

The flow rate was very slow at 5 C (24 hours to strip 100 g of oil). Although the flow rate was increased by stripping the oils at the laboratory temperature, the stripped oils were partially oxidized. This may be due to the long duration of the stripping procedure (12 hours to strip 100 g of oil).

NBO was stripped from its minor components effectively by using a modified multi-layer column chromatographic technique developed by Lampi et al (1992). This procedure required only 2 hours to strip 100 g of borage and evening primrose oils. No peroxides, conjugated dienes, hexanal, pigments, tocopherols, phospholipids or glycolipids were detected in stripped borage oil (SBO). However, some primary and secondary oxidation products as well as γ-tocopherol (84 ppm) were retained in the stripped primrose oil (SEPO). The original γ-tocopherol content of NEPO was 335 ppm. Similar difficulties were experienced by Lampi et al (1992) in removing all γ-tocopherol from vegetable oils when present at more than 300 ppm, using similar techniques. Moreover, Finnish rapeseed oil stripped by this procedure showed a reduction in its γ-tocopherol content from 700 ppm to ≤ 24 ppm (Lampi et al, 1997b).

The precise mechanisms of interaction as well as adsorbtion of minor components of vegetable oils on multi-layer adsorbents employed in this study are not fully understood. Nonetheless, silicic acid has been used to fractionate the minor components in soybean, corn and canola oils as well as those contributing to colour development in tall oil during oxidation (Endo et al, 1991; Min, 1972). Removal of phospholipids from crude soybean oil miscelles by silicic acid has been reported by Brown and Snyder (1989). Moreover,

during stripping of vegetable oils, the mixture of Celite 545 and activated charcoal was especially effective in adsorbing tocopherols as only 15 % of tocopherols were adsorbed without this mixture (Lampi et al, 1992). Meanwhile, filtering of the oil through various activated carbons or charcoals has been reported to decrease peroxide values (PV), 2-thiobarbituric acid reactive substances (TBARS), carbonyl value (CV) as well as tocopherols of autooxidized soybean oil (Boki et al, 1991). These authors suggested, based on chemical and physical properties of activated carbons such as acidity, basicity, specific surface area and pore volume, that hydroperoxides, aldehydes and ketones in soybean oil were adsorbed on the acid sites distributed over the surface or within the pores of charcoal, in a packed column. Some types of charcoal reduced 88 % of PV, 87 % of TBARS, 34 % of CV, as well as 50 % of total tocopherols. Furthermore, chlorophyll molecules are believed to be adsorbed as protonated species on acid activated clay (Mokaya et al, 1994).

4.1.2 Fatty acid composition

The fatty acid composition of stripped and non-stripped oils are given in Table 4.2. The results in this table indicate that stripped and non-stripped evening primrose oil contained higher amounts ($p<0.05$) of PUFA than stripped and non-stripped borage oils. The main PUFA in both oils was linoleic acid, which was more than 70 % in stripped and non-stripped evening primrose oil, but only 36 % in stripped and non-stripped borage oils. Borage oil had up to 22 % of γ-linolenic acid (18:3 ω 6) while evening primrose oil had only 9 %. Thus, borage oil serves as a richer source of this

essential fatty acid than evening primrose oil. Stripped and non-stripped borage oil had higher amounts ($p< 0.05$) of oleic acid (18:1) than stripped and non-stripped evening primrose oil. Furthermore, low quantities of eicosenoic or gondoic (20:1), docosenoic or erucic (22:1) as well as tetracosenoic or nervonic (24:1) acids were detected in borage oil.

4.1.3 Neutral and phospholipid composition

Thin layer chromatography-flame ionization detection (TLC-FID) combines the efficacy of TLC separation with quantification by FID. It has been applied to quantify marine lipids (Parrish and Ackman, 1983), phsospholipids in edible oils (Du Plessis and Pretorius, 1984), other lipids and pollutants in marine and environmental samples (Volkman and Nichols, 1991) as well as different lipid classes in vegetable oils (Imbas and Pham ,1997). Results in Table 4.3 demonstrates that NEPO has more ($p<0.05$) free fatty acids than NBO, while the latter contained more ($p<0.05$) mono- and diacylglycerols than the former. The main phospholipid sub-classes in NBO were phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, while the main phospholipid subclasses in NEPO were phosphatidic acid and phosphatidylcholine.

4.2 Oxidative stability of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions stored under Schaal oven conditions at 60 C

Several accelerated oxidation tests are used to examine the oxidative stability of edible oils and thus predict their shelf life. In the Schaal oven test, the method used in this study,

Table 4.2 Fatty acid composition (area percent) of non-stripped and stripped borage (NBO, SBO) and evening primrose (NEPO, SEPO) oils.

Fatty acid	Oil Samples			
	NBO	SBO	NEPO	SEPO
16:0	11.31 ± 0.09^{b}	11.00 ± 0.77^{b}	6.77 ± 0.33^{a}	6.54 ± 0.29^{a}
18:0	4.00 ± 0.21^{b}	4.28 ± 0.13^{b}	1.84 ± 0.11^{a}	1.80 ± 0.01^{a}
18:1	16.93 ± 0.42^{b}	16.54 ± 0.13^{b}	8.67 ± 0.28^{a}	8.31 ± 0.71^{a}
18:2	36.34 ± 1.33^{a}	36.96 ± 0.11^{a}	73.55 ± 1.81^{b}	75.75 ± 0.27^{b}
18:3 ω-6	22.05 ± 0.92^{c}	22.28 ± 1.26^{c}	9.16 ± 0.58^{b}	7.60 ± 0.75^{a}
20:1	4.58 ± 0.03^{a}	4.68 ± 0.02^{a}	-	-
22:1	2.85 ± 0.05^{a}	2.59 ± 0.01^{a}	-	-
24:1	1.85 ± 0.07^{a}	1.67 ± 0.01^{a}	-	-
PUFA	58.39 ± 0.29^{a}	59.24 ± 0.81^{a}	82.71 ± 0.39^{b}	83.35 ± 0.81^{b}

Values are means of three determinations ± standard deviations. Values followed by different superscripts in each row are different ($p < 0.05$) from one another.

Table 4.3 Neutral and phospholipids composition (g/100g) of non-stripped borage (NBO) and evening primrose (NEPO) oils.

Lipid classes	NBO	NEPO
Steryl esters	0.29± 0.06[a]	0.58± 0.08[b]
Triacylglycerols	98.01± 0.05[a]	97.75± 0.08[a]
Free fatty acids	0.30± 0.01[a]	0.64 ± 0.05[b]
Diacylglycerols	0.47± 0.06[b]	0.33± 0.08[a]
Monoacylglycerols	0.67± 0.05[b]	0.39± 0.01[a]
Phosphatidic acid	20.5± 0.8[a]	61.42 ± 2.67[b]
Phosphatidylethanolamine and Phosphatidylserine	33.11± 1.0[b]	5.37 ± 1.20[a]
Phosphatidylinositol	5.21± 0.33[a]	11.18 ± 1.92[b]
Lysophosphatidylethanolamine	3.22 ± 1.44[b]	0.17 ± 0.08[a]
Phosphatidylcholine	35.18 ± 1.15[b]	11.81 ± 1.49[a]
Lysophosphatidylcholine	0.78 ± 0.05[a]	10.04 ± 0.81[b]

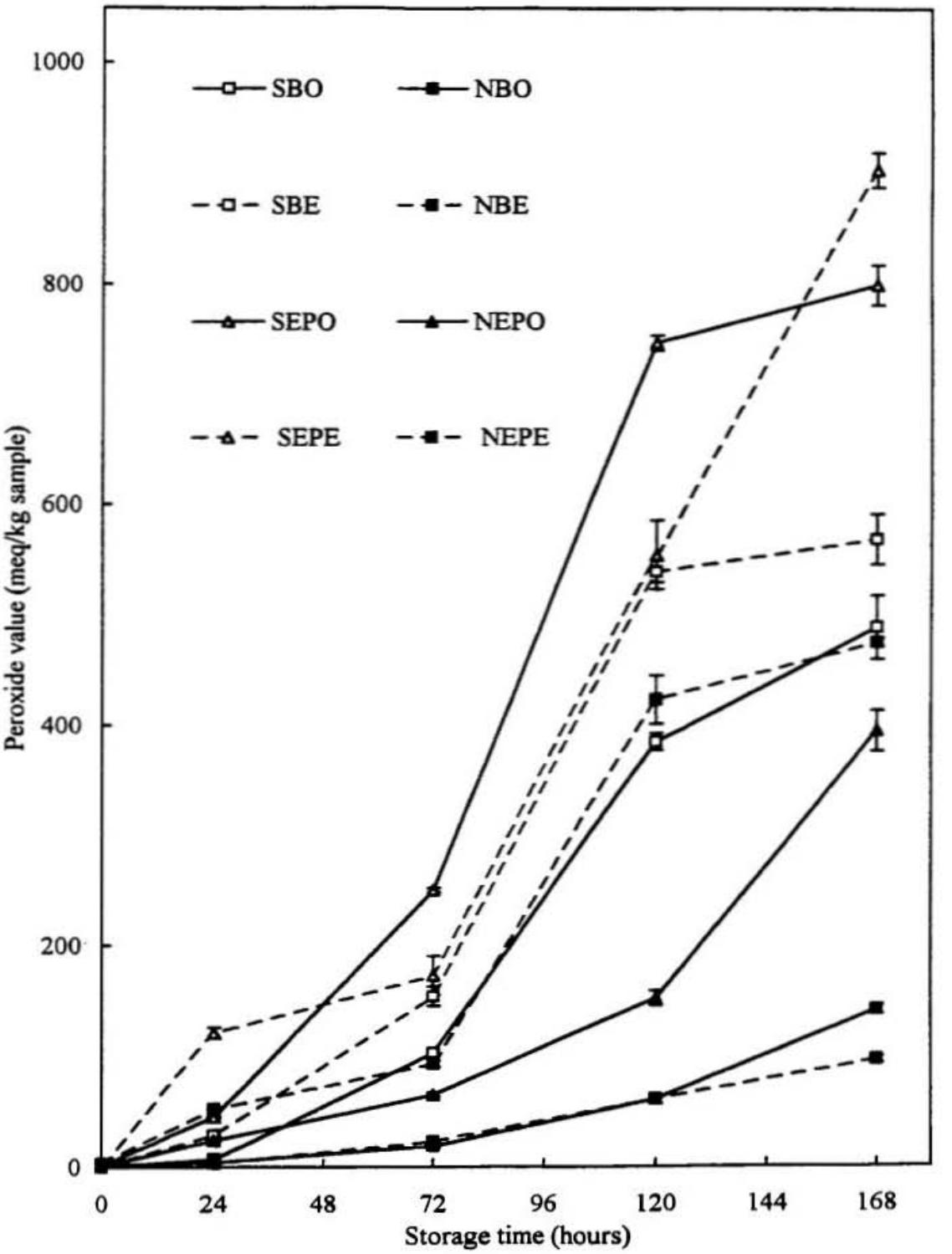
Values are means of three determinations ± standard deviations. Values followed by different superscripts in each row are different ($p<0.05$) from one another.

the lipid samples are placed in a forced air oven and the temperature is maintained between 60 and 70 C (Frankel, 1993b) or 60-65 C (Malcomson et al, 1994). It has been observed that 1 day storage under Schaal oven conditions at 65 C is equivalent to 1 month storage at room temperature (25 C) (Abou-Gharbia et al, 1996). Moreover, flavour scores of edible oils at 60 C for 4 days agreed with those stored at ambient temperature for 4 months (Warner et al, 1989).

4.2.1 Primary oxidation products

Based on PV obtained during the oxidation in the dark (Figure 4.1), NBO and non-stripped borage oil emulsions (NBE) were more ($p<0.05$) stable than their corresponding SBO and stripped borage oil emulsions (SBE). Similarly, NEPO and non-stripped evening primrose oil emulsions (NEPE) were more stable ($p<0.05$) than their corresponding SEPO and stripped evening primrose emulsions (SEPE). Nonetheless, non-stripped and stripped borage oils and emulsions were more stable ($p<0.05$) than non-stripped and stripped evening primrose oils. The higher oxidative stability of non-stripped borage and evening primrose oils and emulsions compared to their corresponding stripped samples can be partially attributed to the presence of minor components in both oils such as tocopherols and phospholipids. It is well known that tocopherols contribute to the oxidative stability of edible oils (Jung and Min, 1990; Kaitaranta,1992; Baldioli et al, 1996). Similarly, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) have been shown to be effective antioxidants in edible oils (King et al, 1992).

Figure 4.1 Peroxide values of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions stored under Schaal oven conditions at 60 C.


SBO
NBO
SBE
NBE
SEPO
NEPO
SEPE
NEPE
Peroxide value (meq/kg sample)
1000
800
600
400
200
0
0
24
48
72
96
120
144
168
Storage time (hours)

Meanwhile, non-stripped borage oil and emulsions were more stable than non-stripped evening primrose oils and emulsions due to the presence of higher amounts ($p<0.05$) of tocopherols as well as PC and PE.

Free radical chain reactions occur during autoxidation of oil and these proceed via three steps, namely initiation, propagation and termination. Tocopherols can interrupt these reactions and protect oils and emulsions from oxidation either by scavenging and thus removing the dissolved oxygen in the system or by reacting with the generated free radicals and terminating the propagation step (Rossel, 1994; Porter et al, 1995). In both cases, tocopherols are gradually consumed during the induction period. Thereafter, oils oxidize more rapidly and the end of the induction period can be determined by a dramatic increase in PV (Rossel, 1994). This might explain the sudden increase of PV of all samples examined.

Oil-in-water emulsions were physically stable during oxidation at 60 C for 168 h. No creaming, flocculation, coalescence or oil separation was observed in the emulsions examined. These criteria can be used as indicators of emulsion stability (Roedig-Penman and Gordon, 1997). Peroxide values in Figure (4.1) demonstrate that NBE was 0.05) more stable ($p<0.05$) than NBO during the storage, except after 72 hours, where NBO was more stable ($p<0.05$). No differences ($p>0.05$) were observed between the two samples after 120 h. This fluctuation in emulsion stability might be attributed to the change of pH values during the storage as no buffer was used in the emulsions prepared in this study. Huang et al (1996a) have reported that the antioxidant activity of tocopherols in emulsions is affected by the pH of the system. The higher oxidative stability of the NBE

at certain storage points can be explained by the so called "polar paradox" or "interfacial phenomenon" theory proposed and tested by Porter et al (1989) and supported by Frankel (1994). According to this theory, the non-polar lipophilic antioxidants such as tocopherols are sufficiently surface-active to be oriented at the oil-water interface in oil-in-water emulsions and thus form a protective membrane. Therefore, free radicals, which are generated in the aqueous phase, will be prevented from crossing the emulsion droplet membrane and oxidizing the oil in the interior of the droplet (Coupland and Mc Clements, 1996a). Meanwhile, the polar hydrophilic antioxidants such as ascorbic and carnosoic acids, both water soluble natural antioxidants, will be markedly diluted by moving to the water phase and, therefore, less effective in preventing oxidation (Frnakel, 1996a; Huang et al, 1996b). The opposite trend has been observed in bulk oil systems. In this dry system, the lipophilic antioxidants will remain in the oil, while the hydrophilic antioxidants will be oriented in air-oil interface and thus being more effective in reducing or preventing oxygen accessibility for oil oxidation (Frankel, 1996a).

The PV of NEPE were higher ($p< 0.05$) than NEPO. These observations are in sharp contrast with the "polar paradox" theory described above. The precise mechanisms that can explain this phenomenon are not fully understood. However, Miyashita et al (1995) observed that emulsified soybean triacylglycrols containing 127.5 ppm of α-tocopherol did not exhibit any improved oxidative stability. Although increasing the tocopherol content to 456.7 ppm improved the oxidative stability of the emulsion, it was still less stable than that of the soybean triacylglycerols. Therefore, the authors suggested that the globules of soybean triacylglycerols may have formed a structure susceptible to

oxidation in the aqueous system. Furthermore, polar phenolic antioxidants such as caffeic and ferulic acids were more effective than α-tocopherol in bulk, refined olive oil (Satue et al, 1995) which can be explained by the polar paradox theory. However, recently Lee and Min (1998) have reported that both ferulic and caffeic acids are more effective antioxidants than α-tocopherol in a linoleic acid emulsion and they suggested their use to improve the oxidative stability of lipids in aqueous foods. Therefore, stripped and non-stripped edible oil-in-water emulsions prepared under well controlled conditions should be examined in order to clarify the validity of the polar paradox theory.

SBE and SEPE were less stable than their corresponding SBO and SEPO. These results are in agreement with those obtained by Frankel et al (1994) where stripped corn oil-in-water emulsion was less stable than the stripped corn oil. However, Miyashita et al (1997) have reported that oxidative stability of sodium linoleate (LA) in aqueous phase, prepared with Tween 20, was improved slightly, while the stability of sodium docosahexaenoate (DHA) was dramatically increased in the same system. The authors suggested that DHA was protected from free radicals and/or oxygen attack by forming a closely and tightly packed micellar conformation with Tween 20.

Oxidation of methylene-interrupted lipid dienes such as linoleic acid or polyenes such as linolenic acid causes shifting in the double bond positions which can be reflected by the formation of conjugated dienes (Abou-Gharbia et al, 1997). Figure 4.2 shows that non-stripped borage and evening primrose oils and their emulsions were more stable than their corresponding stripped oil and emulsions. Moreover, NBE was more stable ($p<0.05$) than NBO after certain storage times, while NEPE was less stable than NEPO. These

results follow a similar trend when compared to PV, and thus a strong correlation ($r > 0.7$, $p < 0.01$) existed between the PV and CD of each sample (Table 4.4). This suggests that CD can be used, in addition to or in place of PV, to assess the oxidative stability of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions.

4.2.2 Secondary oxidation products

Secondary oxidation products of stripped and non-stripped borage and evening primrose oils were determined by examining TBARS and head space volatiles, mainly hexanal. The determination of TBARS is based on colour intensity of the reaction between TBA and secondary oxidation products of polyunsaturated fatty acids (PUFA), mainly malonaldehyde (MA). The TBARS expressed as μmol per g of NBO and NEPO as well as their emulsions, NBE and NEPE, respectively, were lower than those of their corresponding stripped samples (Figure 4.4), perhaps due to the presence of minor components such as tocopherols and phospholipids. Phospholipids effectively inhibited the formation of TBARS in a salmon oil model system (King et al, 1992). However, TBARS values of stripped and non-stripped borage oil and emulsions were higher ($p < 0.05$) than those reported for stripped and non-stripped evening primrose oils. These results are similar to those obtained by others (Wanasundara et al, 1995).

The most likely explanation is that borage oil contained higher amounts ($p < 0.05$) of linolenic acid than evening primrose oil. It has been suggested that TBARS are formed in substantial amounts from PUFA containing three or more double bonds (see Figure 4.3),

Figure 4.2 Conjugated dienes of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions stored under Schaal oven conditions at 60 C.

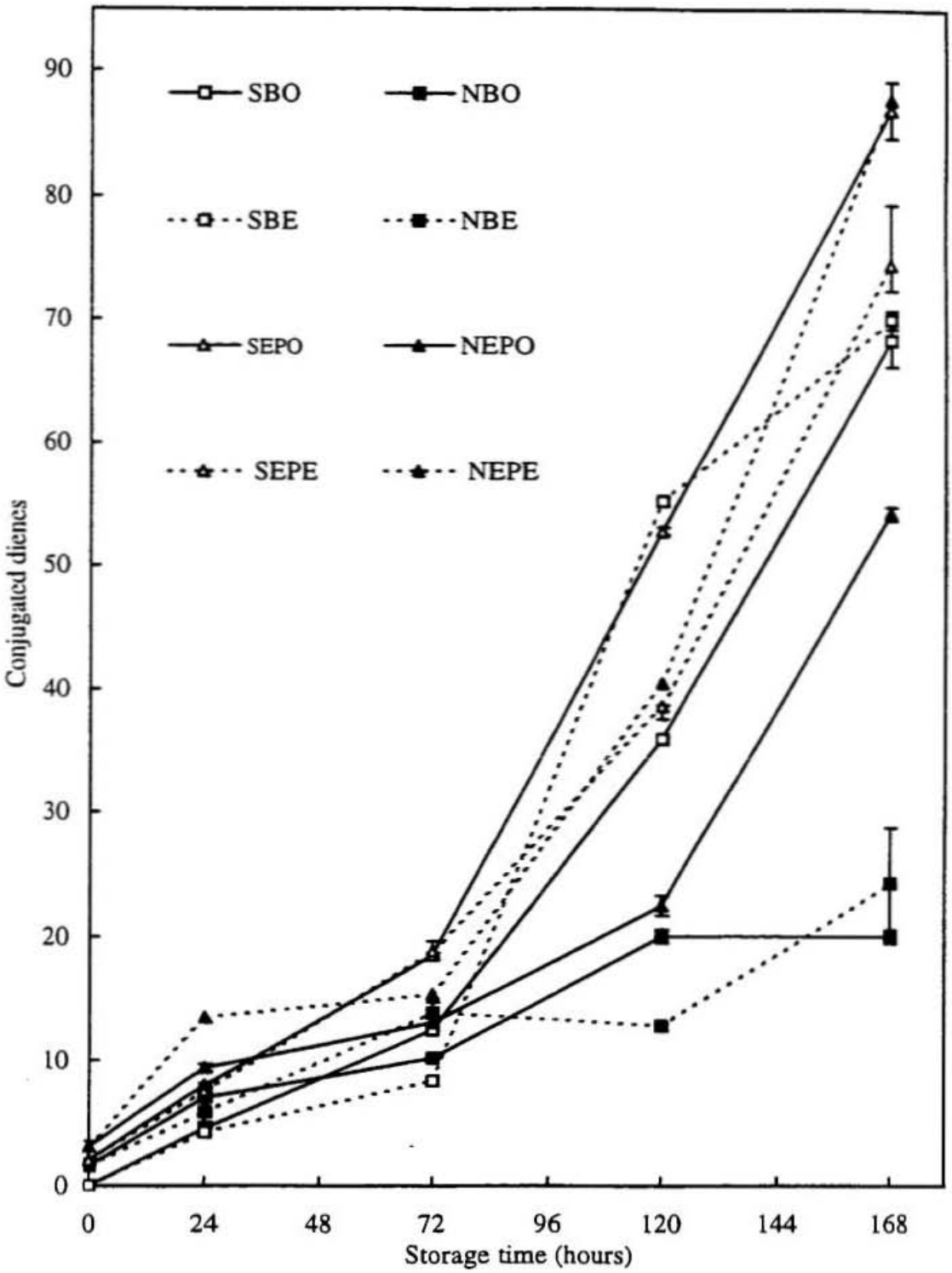

SBO
NBO
SBE
NBE
SEPO
NEPO
SEPE
NEPE
Conjugated dienes
0
10
20
30
40
50
60
70
80
90
0
24
48
72
96
120
144
168
Storage time (hours)

Table 4.4 Linear regression analysis of peroxide values (PV) and conjugated dienes (CD) of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions stored under Schaal oven conditions at 60 C.

Sample	r^2	r	No. of observations	Level of significance
SBO	0.970	0.984	15	p<0.01
NBO	0.860	0.927	15	p<0.01
SBE	0.952	0.976	15	p<0.01
NBE	0.667	0.817	15	p<0.01
SEPO	0.911	0.955	15	p<0.01
NEPO	0.906	0.952	15	p<0.01
SEPE	0.973	0.987	15	p<0.01
NEPE	0.855	0.925	15	p<0.01

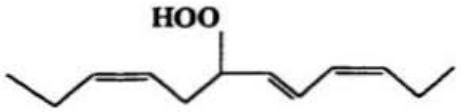
Abbreviations are: SBO, stripped borage oil; NBO, non-stripped borage oil ; SBE, stripped borage oil emulsion; NBE, non-stripped borage oil emulsion; SEPO, stripped evening primrose oil; NEPO; non- stripped evening primrose oil; SEPE, stripped evening primrose oil emulsions; NEPE, non- stripped evening primrose oil emulsion.

although it is possible that small amounts are formed from methyl linoleate (Hoyland and Taylor, 1991). The sudden decrease in TBARS of SEPO after 72 hours can not be easily explained.

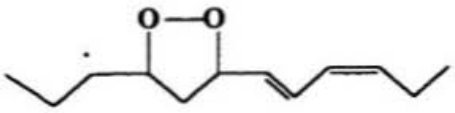
Determination of headspace volatiles, although time consuming and labour intensive, is a very effective method for evaluating the oxidative stability of edible oils. The gas chromatographic analysis can provide useful information regarding the origin of flavour and odour volatiles and their precursors and, thus is the most suitable method for comparison with the results of sensory analysis (Frankel, 1993) which is the most useful method used to predict consumer acceptability and the shelf life of lipid products. However, these tests are not used for routine analysis (Wanasundara et al, 1995).

The major volatile that has been observed during the autoxidation of stripped and non-stripped borage (Figure A.2a & A.2b) and evening primrose (Figure A.3a & A.3b) oils and their oil-in-water emulsions was hexanal. Meanwhile, 2,4-heptadienal and pentanal were detected in relatively small amounts. Hexanal is the major volatile produced from the oxidation of omega-6 (ω-6) polyunsaturated fatty acids (Frankel, 1993; Shahidi et al, 1997). Hexanal may possibly be formed via autoxidation of linoleic acid (Figure 4.5). The autoxidation of linoleate proceeds via hydrogen abstraction from carbon-11 only to produce a delocalized pentadienyl moiety which is in turn attacked by oxygen to produce an equal mixture of conjugated 9- and 13-hydroperoxide isomers. The decomposition of these hydroperoxides occurs via homolytic cleavage of the oxygen-oxygen bond to yield alkoxy and hydroxyl radicals, which in turn decompose by

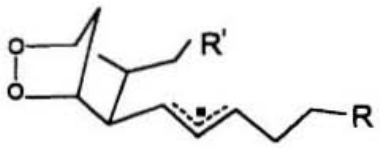
Figure 4.3 Mechanism of formation of malonaldehyde from linolenic acid.

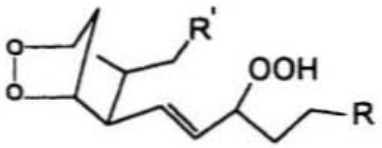


13 - Hydroperoxide of linolenic acid



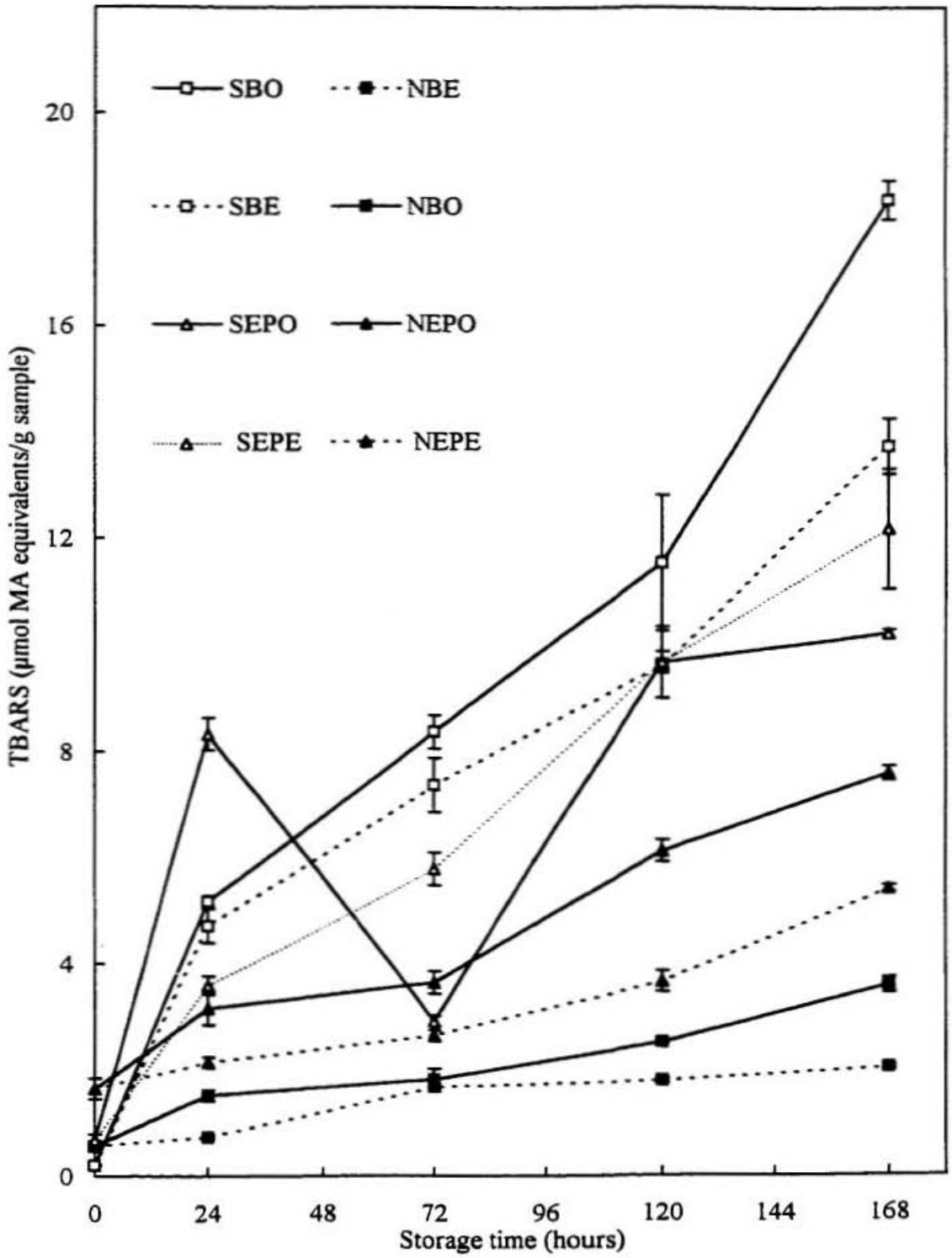
1,3-Cyclic peroxide





Malonaldehyde

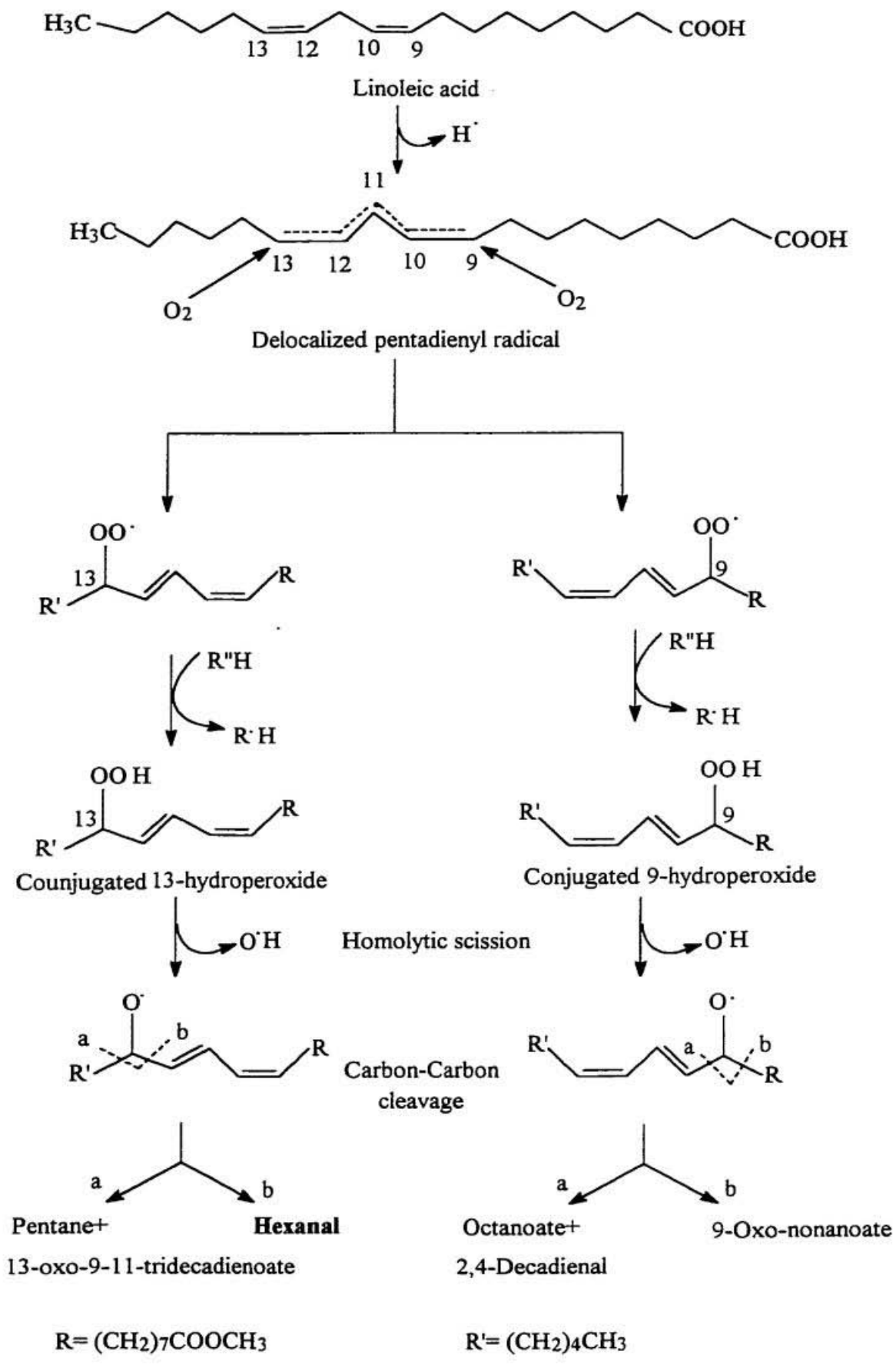
Figure 4.4 Thiobarbituric acid reactive substances (TBARS) of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions stored under Schaal oven conditions at 60 C.


SBO
NBE
SBE
NBO
SEPO
NEPO
SEPE
NEPE
TBARS (µmol MA equivalents/g sample)
0
4
8
12
16
20
0
24
48
72
96
120
144
168
Storage time (hours)

carbon-carbon cleavage to yield hexanal and pentane from the 13-hydroperoxide and methyl octanoate and 2,4-decadienal from 9-hydroperoxides (Frankel, 1984). The formation of pentanal from ω-6 PUFA can be explained by thermal decomposition of hexanal or by loss of formaldehyde (Frankel, 1993a). Meanwhile, 2,4-heptadienal may possibly be formed form autoxoidation of linolenic acid, which proceeds similar to the oxidation of linoleic acid. The abstraction of hydrogen from carbons -11 and -14 in the autoxidation of linolenate produces a mixture of 9-,12-,13- and 16-conjugated diene-triene hydroperoxide isomers. Decomposition of 12-hydroperoxide yields 2,4-heptadienal. Hexanal production increased gradually in all samples throughout the storage period (Figure 4.6).

Stripped and non-stripped borage and evening primrose oils as well as their oil-in-water emulsions were more stable ($p<0.05$) than their corresponding stripped oils and emulsions. This can be explained, in part, by the presence of tocopherols in both oils. Hunag et al (1994) have reported that individual tocopherols as well as their mixtures inhibited the formation of hexanal in stripped corn oil. Moreover, α-tocopherol was a very good inhibitor of hexanal formation when used at 100 and 500 ppm in refined, bleached and deodorized olive oil (Satue et al, 1995). Recently, Shahidi et al (1997) have reported that the loss of endogenous antioxidants in sesame oil, such as sesamin, sesamolin and γ-tocopherol during processing and storage paralleled an increase in their hexanal content. Meanwhile, the hexanal content in stripped and non-stripped evening primrose oil was higher ($p<0.05$) than that stripped and non-stripped borage oil. This

Figure 4.5 Mechanism of linoleic acid autoxidation and formation of hexanal.


H3C
COOH
13 12 10 9
Linoleic acid
H·
11
H3C
COOH
13 12 10 9
O2
O2
Delocalized pentadienyl radical
OO·
13
R'
R
R"H
R·H
OO H
13
R'
R
Counjugated 13-hydroperoxide
OO·
R'
9
R
R"H
R·H
OO H
R'
9
R
Conjugated 9-hydroperoxide
O·H
Homolytic scission
O·H
O·
a
b
R'
R
Carbon-Carbon
cleavage
O·
R'
a
b
R
a
b
Pentane+
Hexanal
13-oxo-9-11-tridecadienoate
a
b
Octanoate+
9-Oxo-nonanoate
2,4-Decadienal
R= (CH2)7COOCH3
R'= (CH2)4CH3

might be explained by the presence of higher amounts of linoleic acids in evening primrose oil as compared to that borage oil.

Table 4.5 summarizes the linear relationships between hexanal content and TBARS. The results in this table demonstrate strong correlation ($r>0.7$, $p<0.01$) between hexanal content and TBARS of SBO, SPO, SBE, NBE and NEPE. Moreover, good correlations ($r>0.6$, $p<0.02$) existed between these two methods for SEPO and NEPO. However, no correlation ($r<0.5$, $p>0.05$) existed between these two indicators for NBO. Therefore, TBARS can be used in addition to or in place of headspace volatiles to monitor the oxidation of previous samples except NBO.

4.3 Oxidative stability of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions illuminated under fluorescent light at 27 C

4.3.1 Primary oxidation products

The peroxide values (PV) obtained during the photooxidation of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions are presented in Figure 4.7. Photooxidation of NBO and NBE, in the initial stages, progressed rapidly compared to SBO and SBE. Thereafter, the oxidation, as demonstrated by PV, progressed gradually for NBO, while the PV of NBE decreased gradually after 48 h. Meanwhile, the PV of SBO and SBE were lower ($p<0.05$) than their corresponding non-stripped counterparts. Therefore, NBO and NBE are more susceptible to photooxidation than SBO and SBE. This may be explained when considering the presence of pigments, mainly chlorophyll, in non-stripped borage oil. The visible spectra of pigments in non-stripped and stripped olive,

Figure 4.6 Hexanal content of stripped and non-stripped borage and evening primrose oil and their oil-in-water emulsions stored under Schaal oven conditions at 60 C.

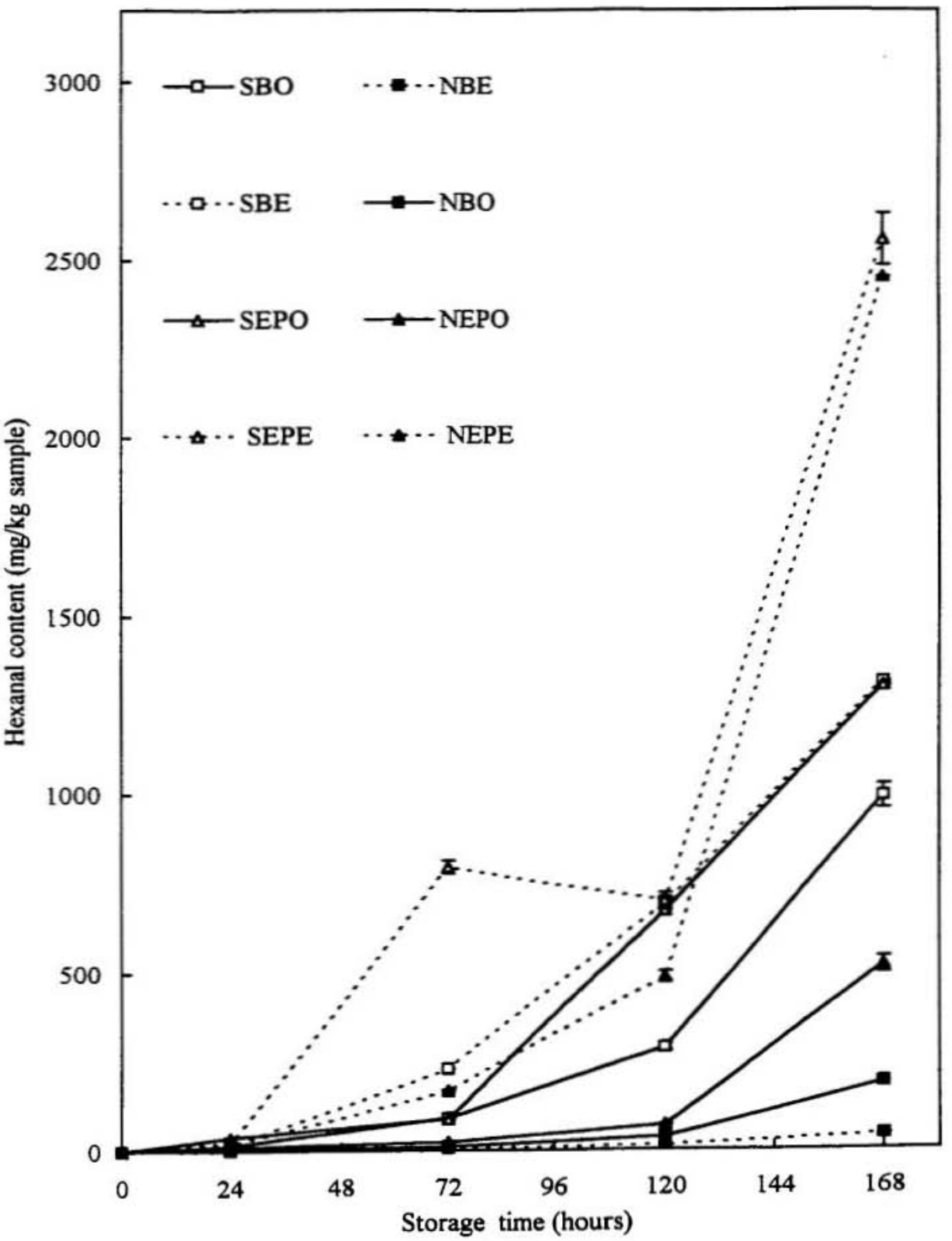

SBO
NBE
SBE
NBO
SEPO
NEPO
SEPE
NEPE
Hexanal content (mg/kg sample)
3000
2500
2000
1500
1000
500
0
0
24
48
72
96
120
144
168
Storage time (hours)

Table 4.5 Linear regression analysis of hexanal content and thiobarbituric acid reactive substances (TBARS) of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions stored under Schaal oven conditions at 60 C.

Sample	r^2	r	No. of observations	Level of significance
SBO	0.705	0.839	15	p<0.01
NBO	0.207	0.455	15	p>0.05
SBE	0.566	0.752	15	p<0.01
NBE	0.615	0.784	15	p<0.01
SEPO	0.643	0.802	15	p<0.01
NEPO	0.443	0.660	15	p<0.02
SEPE	0.550	0.742	15	p<0.01
NEPE	0.606	0.778	15	p<0.01

SBO, stripped borage oil; NBO, non-stripped borage oil ; SBE, stripped borage oil emulsion; NBE, non-stripped borage oil emulsion; SEPO, stripped evening primrose oil; NEPO; non-stripped evening primrose oil; SEPE, stripped evening primrose oil emulsions; NEPE, non- stripped evening primrose oil emulsion.

borage and evening primrose oils are presented in Figure 4.8. It is well documented that olive oil contains naturally-occurring photosensitizers such as chlorophyll and pheophytin as well as carotenoids (Kiritsakis and Dugan, 1985; Rahmani and Csallany, 1991). The characteristic visible absorbance peaks occurring below 500 nm corresponds to carotenoids in olive oil, while absorption at 605, 647 and 667 nmcorrespond to different types of chlorophyll. These peaks qualitatively confirm the presence of chlorophyll in non-stripped olive, borage and evening primrose oils, while no carotenoids were detected in borage or evening primrose oils. Meanwhile, NEPO contained more pigments than NBO. It has been reported that edible oils containing natural pigments such as chlorophyll and pheophytin are highly susceptible to light-induced oxidation or photooxidation (Hall and Cuppett, 1993; Lee and Kim, 1997). These pigments or photosensitizers may absorb light and transform to an excited state, which in turn might convert to a less stable triplet state sensitizer. The sensitizer can transfer its energy to the most stable triplet state oxygen, thus converting it to a higher energy level singlet oxygen which would in turn attack the double bonds in unsaturated fatty acids. This will generate free peroxy radicals which can initiate free radical chain reactions as in autoxidation and eventually leads to the formation of hydroperoxides, which in turn decompose to volatiles causing rancidity or oxidative deterioration of edible oils (Warner and Frankel, 1987; Hall and Cuppett, 1993).

The role of photosensitizers in light-induced oxidation has also been observed in non-stripped evening primrose oil and its oil-in-water emulsions. Both NEPO and NEPE were more ($p<0.05$) susceptible to photooxidation than SEPO and SEPE.

Figure 4.7 Peroxide values of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions illuminated with fluorescent light at 27 C.

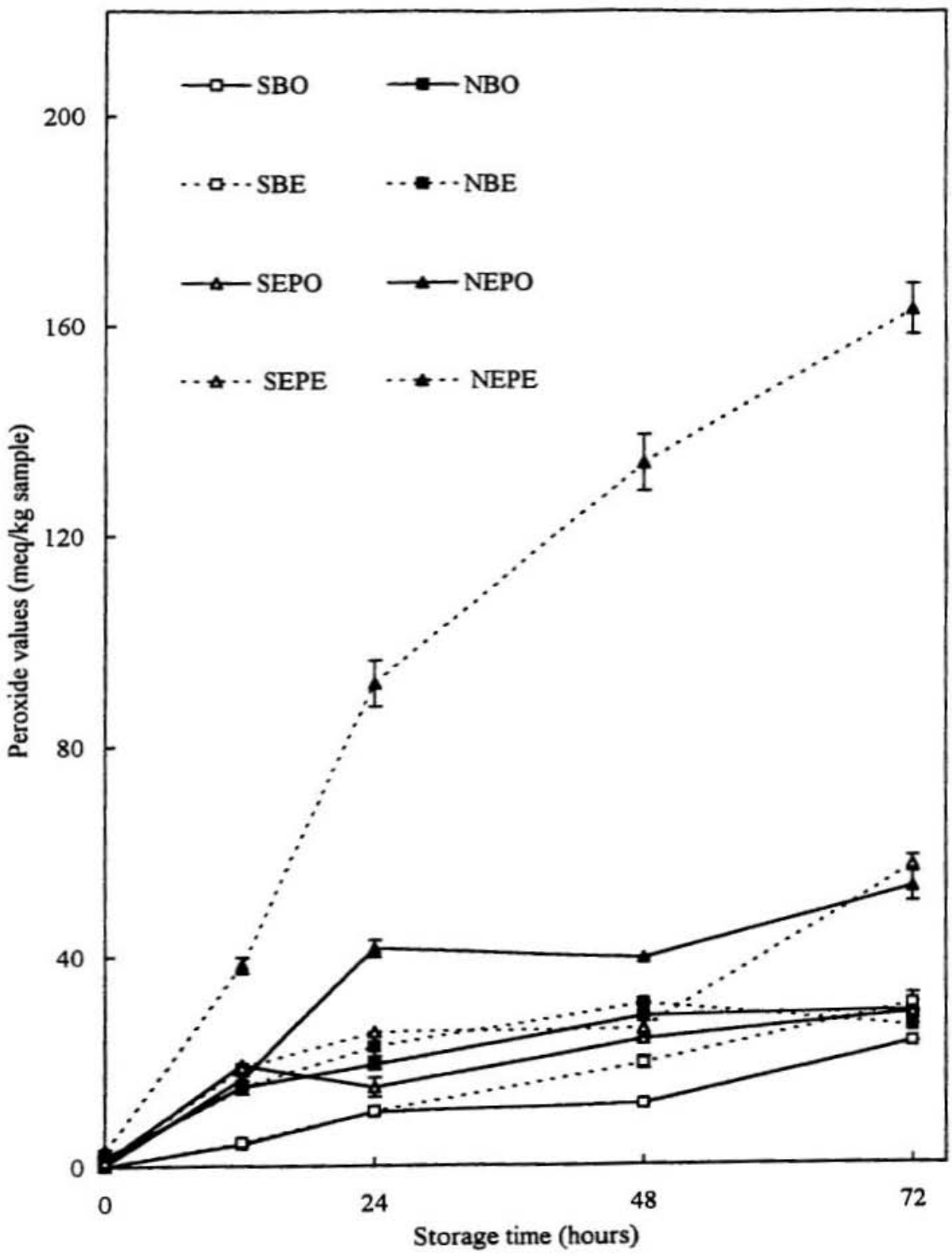
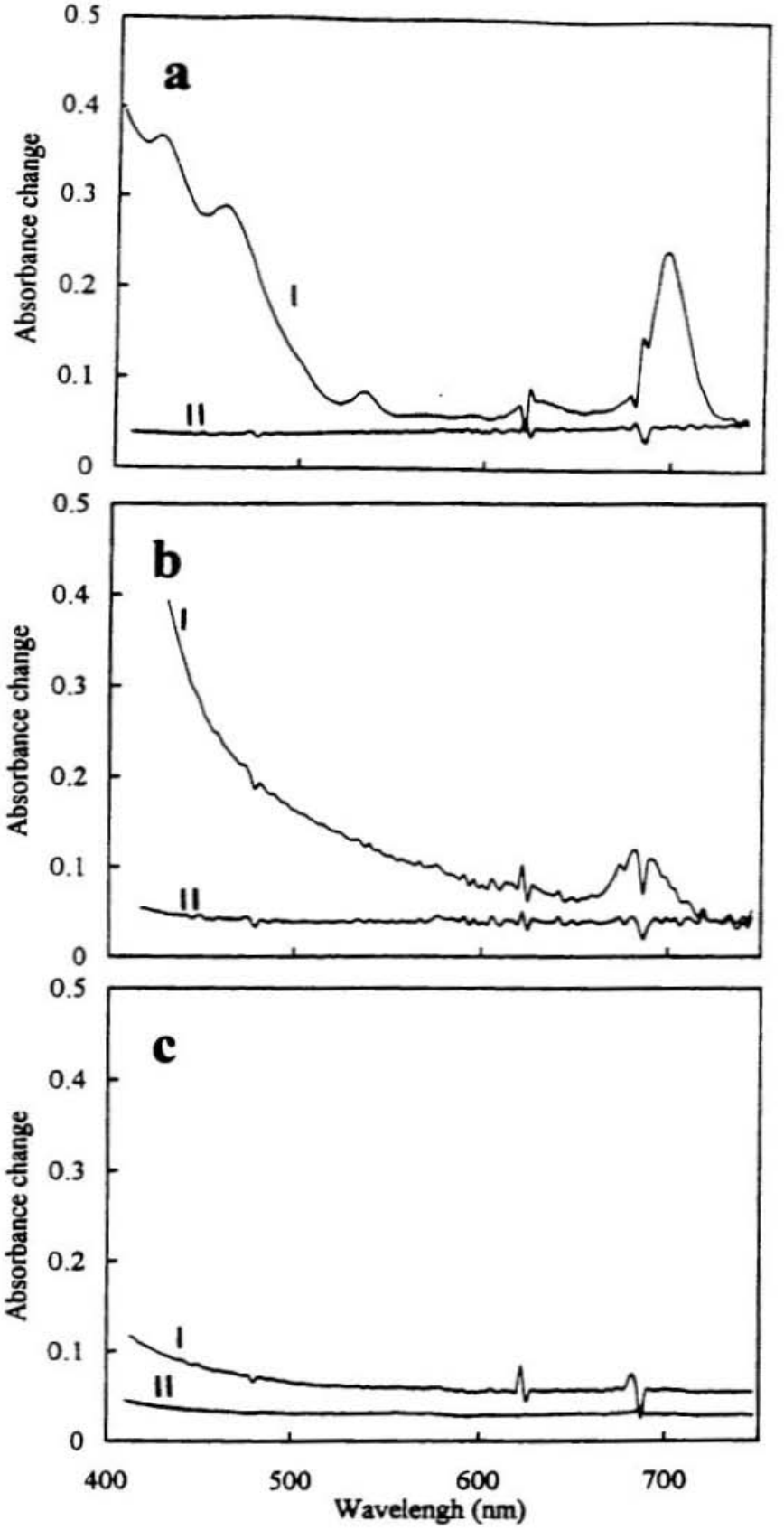

SBO
NBO
SBE
NBE
SEPO
NEPO
SEPE
NEPE
200
160
120
80
40
0
Peroxide values (meq/kg sample)
0
24
48
72
Storage time (hours)

Figure 4.8 Visible spectra of pigments in oil/hexane (1:1, v/v) of non-stripped (I) and stripped (II) olive, (a); evening primrose, (b); and borage, (c) oils.


a
b
c
Absorbance change
Absorbance change
Absorbance change
0.5
0.4
0.3
0.2
0.1
0
I
II
400
500
600
700
Wavelengh (nm)

Meanwhile, the PV of NEPO and NEPE were higher ($p<0.05$) than those of NBO and NBE. This may be due to the presence of higher levels of cholorophyll and linoleic acid as well as lower amounts of tocopherols in the evening primrose oil as compared to those of borage oil. NEPO contained only 16 ppm of α- and 335 ppm of γ-tocopherols compared to 52 ppm of δ- and 659 ppm of γ- tocopherols in NBO. Jung and Min (1991) have reported that α-, δ-, and γ- tocopherols reduce the photooxidation of soybean oil. Moreover, Kamal-Eldin and Appelqvist (1996) have reported that the quenching efficiency of the tocopherols for singlet oxygen are in decreasing order of α- > β- > γ- > δ-. However, γ- and δ- tocopherols may prove to be more effective than α-tocopherol at the later stages of photooxidation due to their higher resistance to destruction by singlet oxygen.

The pigments that act as initiators of photooxidation in edible oils may be destroyed by light (Usuki et al, 1984; Kiritsakis and Dugan, 1985), and this might explain the reduction of the PV of NBE during the latter stage of photooxidation. The pigments were substantially removed from the stripped borage oil and therefore, the oxidation was not affected by fluorescent light. The gradual increase in PV of SBO and SBE can be attributed to free radical chain reactions which can occur at room temperature. Meanwhile, residual pigments in NEPO and NEPE may explain the rapid increase in PV during the earlier stages.

The emulsions prepared and kept at 27 C under fluorescent light for 72 h were physically stable. The peroxide values of NBO and NEPO were lower ($p<0.05$) than

those of NBE and NEPE. Therefore, NBO and NEPO are more stable than their corresponding oil-in-water emulsions. These results are in contrast with the polar paradox theory proposed by Porter et al (1989) and supported by Frankel et al (1994), which has been critically discussed in the previous section (4.2.1) The most likely explanation is that the droplets of the emulsions have taken a configuration that is more susceptible to photooxidation than the oil (Miyashita et al, 1995).

Although photooxidation of lipids proceeds differently from their autoxidation, CD are formed in both mechanisms (Frankel, 1984), and thus the photooxidation can be followed by determination of CD (Kiritsakis and Dugan, 1985). However, formation of conjugated dienes may not necessarily occur under photooxidation conditions. Based on CD, NBO, NBE, NEPO and NEPE were more ($p<0.05$) photooxidizable than their corresponding SBO, SBE, SEPO and SEPE (Figure 4.9). Furthermore, NEPO and NEPE were more ($p<0.05$) susceptible to oxidation than NBO and NBE. Meanwhile, a strong positive correlation ($r>0.7$, $p<0.01$) existed between PV and CD of all samples (Table 4.6). Therefore, CD can be used in addition to or in place of PV to evaluate the oxidative stability of stripped and non-stripped evening primrose oils and their oil-in-water emulsions under florescent light

4.3.2 Secondary oxidation products

Figure 4.10 shows the TBARS values of samples stored under fluorescent light at 27 C for 72 hours. The TBARS of stripped and non-stripped evening primrose oils and emulsions were higher ($p<0.05$) than stripped and non-stripped borage. oil and

emulsions. This might be due to higher contents of linoleic acid in the former samples. Photooxidation of linolenic acid in stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions can lead to the formation of 9-, 10-, 12-, 13-, 15- and 16-hydroperoxides. The 12- and 13- hydroperoxides may undergo cyclization to form 1,3- mono endoperoxide, which finally forms malonaldehyde (Frankel, 1984). Mono endoperoxides have been observed in the photooxidation of linoleate, and thus it is expected that linoleate contributes markedly to the formation of malonaldehyde (Neff et al, 1983). Thiobarbtiuric acid (TBA) reacts with malonaldehyde (MA) to give a pink-coloured complex (TBA-MA) with a maximum absorbance at 532 nm (Shahidi and Wanasundara, 1998). Based on their TBARS values, NEPO, NEPE, NBO and NEPO were more ($p<0.05$) susceptible to oxidation than their corresponding SEPO, SEPE, SBO and SBE.

The primary hydroperoxides generated in photooxidation of unsaturated fatty acids are decomposed to volatiles, which in turn have detrimental effects on the flavour stability of edible oils (Abou-Gharbia et al, 1997). The major volatile detected, using headspace gas chromatographic analysis of photooxidized stripped and non-stripped borage (Figure A4a & A4b), and evening primrose (Figure A5a & A5b), oils and emulsions, was hexanal which has been shown to cause undesirable flavours in lipids (Shahidi et al, 1997). Heptenal was also detected as another major volatile in non-stripped evening primrose oil and its emulsions. Hexanal and 2-heptenal may be formed via photooxidation of linoleic acid, which gives 4 isomers; 2 conjugated 9- and 13- diene hydroperoxides

Figure 4.9 Conjugated dienes of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions illuminated with fluorescent light at 27C.

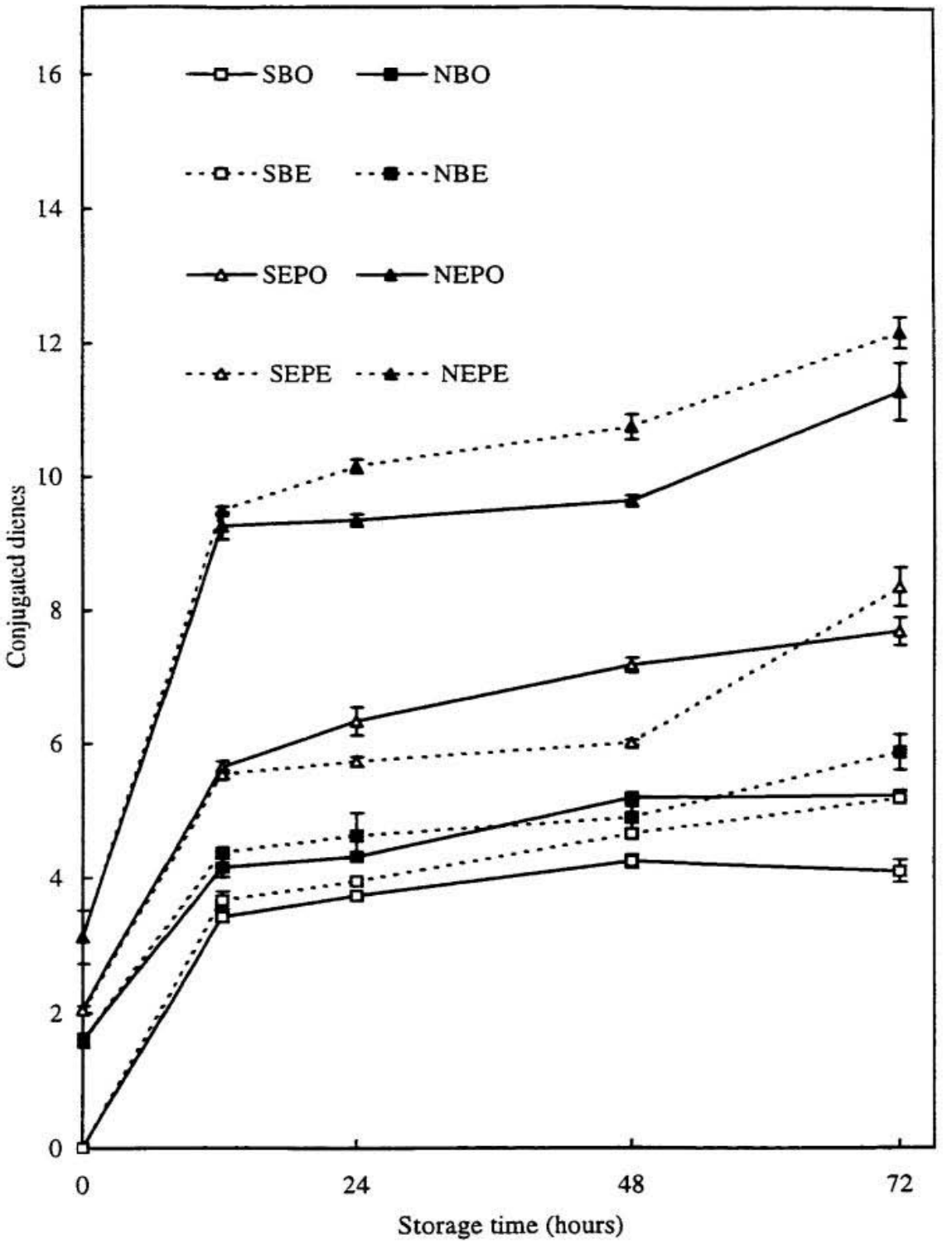
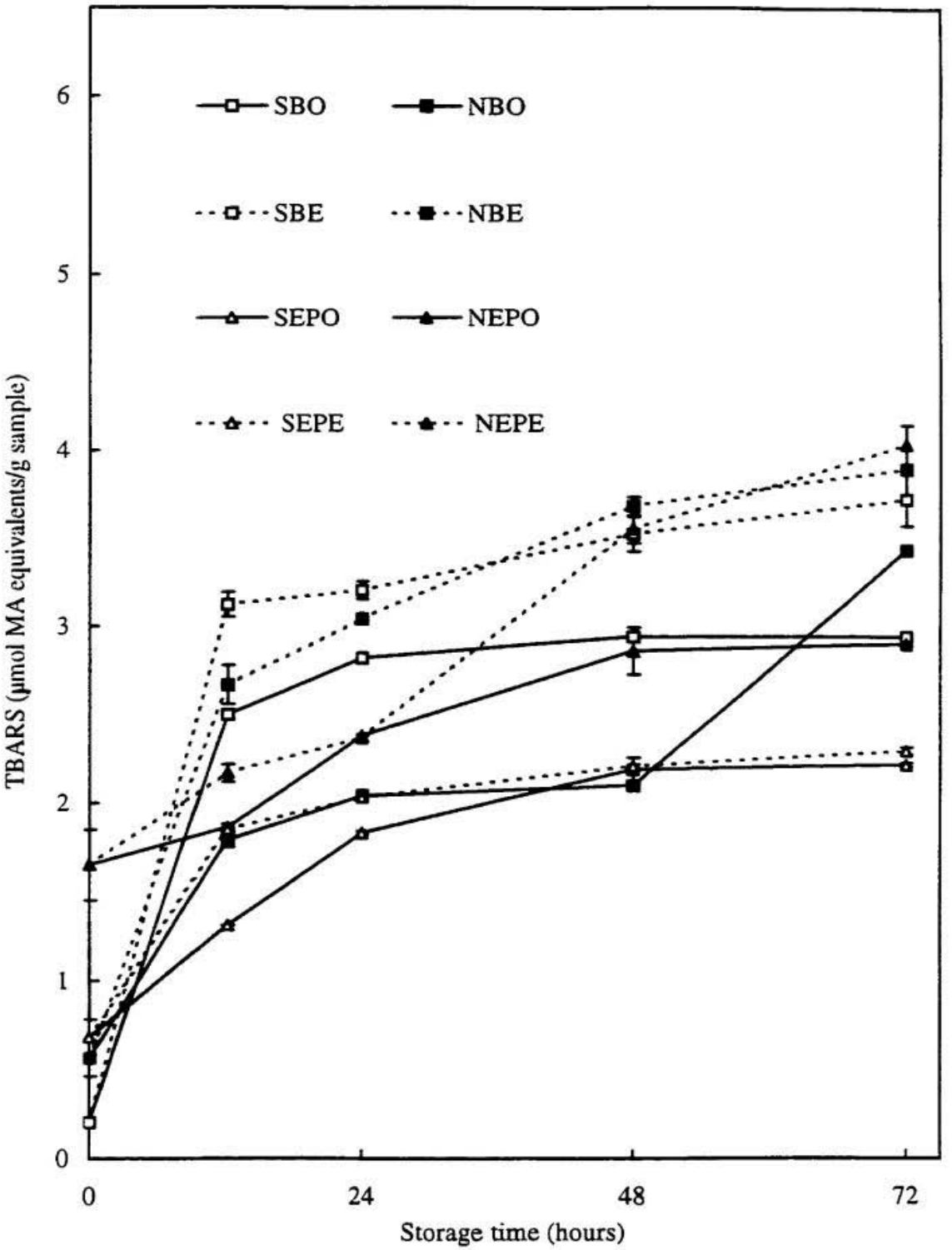

SBO
NBO
SBE
NBE
SEPO
NEPO
SEPE
NEPE
Conjugated dienes
0
2
4
6
8
10
12
14
16
0
24
48
72
Storage time (hours)

Table 4.6 Linear regression analysis of peroxide values (PV) and conjugated dienes (CD) of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions illuminated under fluorescent light at 27 C.

Sample	r^2	r	No. of observations	Level of significance
SBO	0.540	0.735	15	$p<0.01$
NBO	0.892	0.944	15	$p<0.01$
SBE	0.607	0.778	15	$p<0.01$
NBE	0.819	0.905	15	$p<0.01$
SEPO	0.889	0.943	15	$p<0.01$
NEPO	0.607	0.779	15	$p<0.01$
SEPE	0.793	0.891	15	$p<0.01$
NEPE	0.659	0.812	15	$p<0.01$

Abbreviations are: SBO, stripped borage oil; NBO, non-stripped borage oil; SBE, stripped borage oil emulsion; NBE, non-stripped borage oil emulsion; SEPO, stripped evening primrose oil; NEPO; non- stripped evening primrose oil; SEPE, stripped evening primrose oil emulsions; NEPE, non- stripped evening primrose oil emulsion.

Figure 4.10 Thiobarbituric acid reactive substances (TBARS) of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions illuminated with fluorescent light at 27 C.


SBO
NBO
SBE
NBE
SEPO
NEPO
SEPE
NEPE
TBARS (μmol MA equivalents/g sample)
0
1
2
3
4
5
6
0
24
48
72
Storage time (hours)

and 2 unconjugated 10- and 12- diene hydroperoxides which in turn may be converted to alkoxy radicals. Carbon- carbon cleavage on either side of this radical can produce different types of aldehydes (Figure 4.11) (Frankel, 1984).

The hexanal content for all samples kept under light increased gradually throughout the storage period (72 h). Based on hexanal contents (Figure 4.12), NEPO, NEPE, NBO and NBE were more ($p<0.05$) susceptible to oxidation than SEPO, SEPE, SBO and SBE. Meanwhile, NEPO and NEPE were more ($p<0.05$) susceptible to photooxidation than NBO and NBE. This trend is similar to that obtained when considering TBARS values. Therefore, a strong correlation ($r> 0.7$, $p<0.01$) existed between TBARS values and hexanal contents of NBO, SEPO, NEPO, and NEPE, but such a correlation was weak ($r>0.5$, $p<0.05$) between NBE and SEPE. However, no correlation have been ($r<0.5$, $p>0.05$) observed between these two indicators for the remaining samples (Table 4.7). Therefore, TBARS may generally be used in addition to or in place of headspace volatile determination to follow the photooxidation of all samples except SBO and SBE.

4.4 Effects of minor components on the oxidative stability of stripped borage and evening primrose oils

Minor components detected in borage oil, mainly tocopherols (TOC) and phospholipids (PL), were added at different levels to stripped borage and evening primrose oils, in order to examine the effects of these components on the oxidative stability of the stripped oils stored under Schaal oven conditions at 60 C. The additions of these components was within the permissible levels established for edible oil industry (Yosihda

Figure 4.11 Mechanism of linoleic acid photooxidation and formation of hexanal.

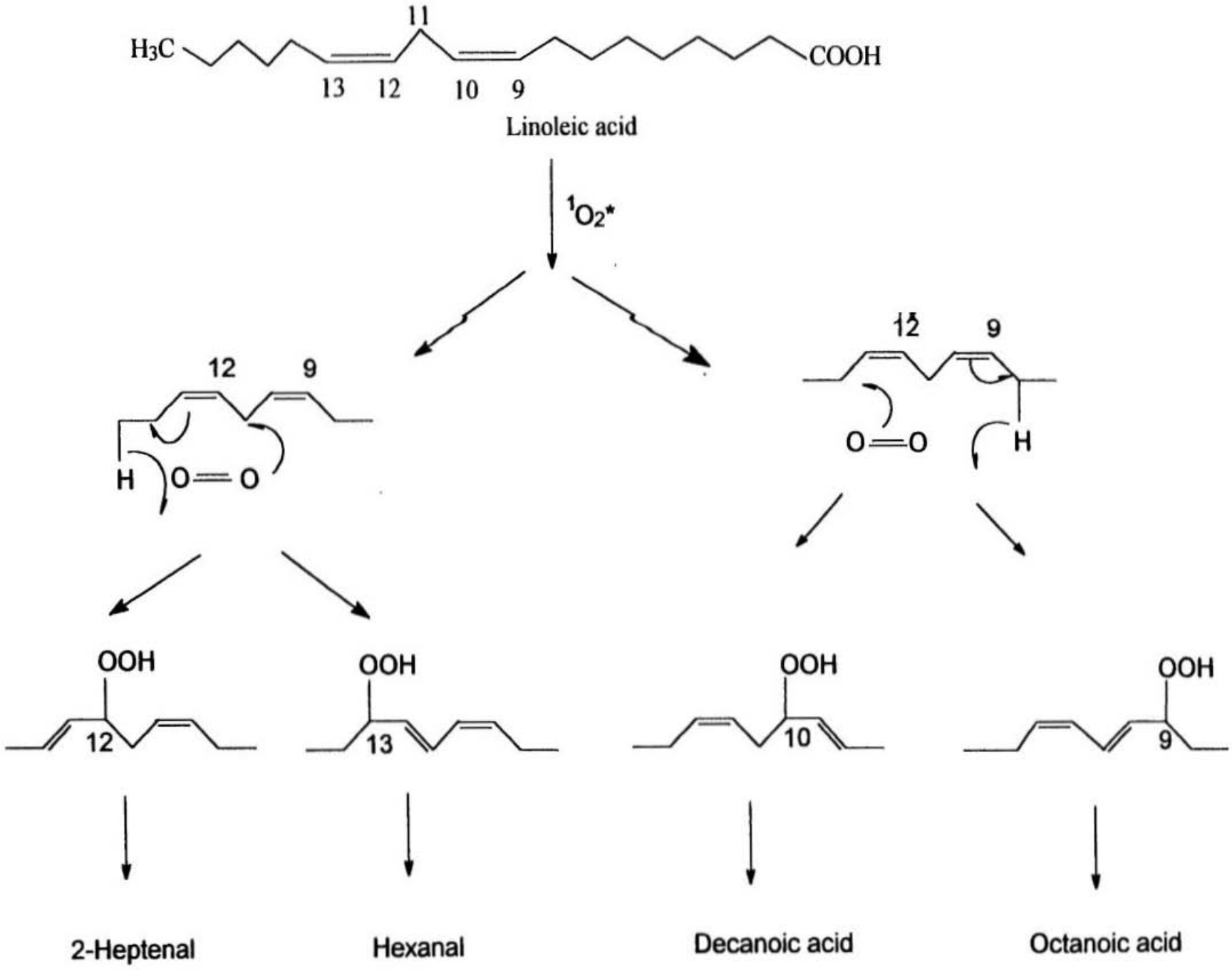

H3C
11
13
12
10
9
COOH
Linoleic acid
1O2*
12
9
H
O=O
12
9
O=O
H
OOH
12
OOH
13
OOH
10
OOH
9
2-Heptenal
Hexanal
Decanoic acid
Octanoic acid

Figure 4.12 Hexanal content of stripped and non-stripped borage and evening primrose oil and their oil-in-water emulsions illuminated with fluorescent light at 27C.

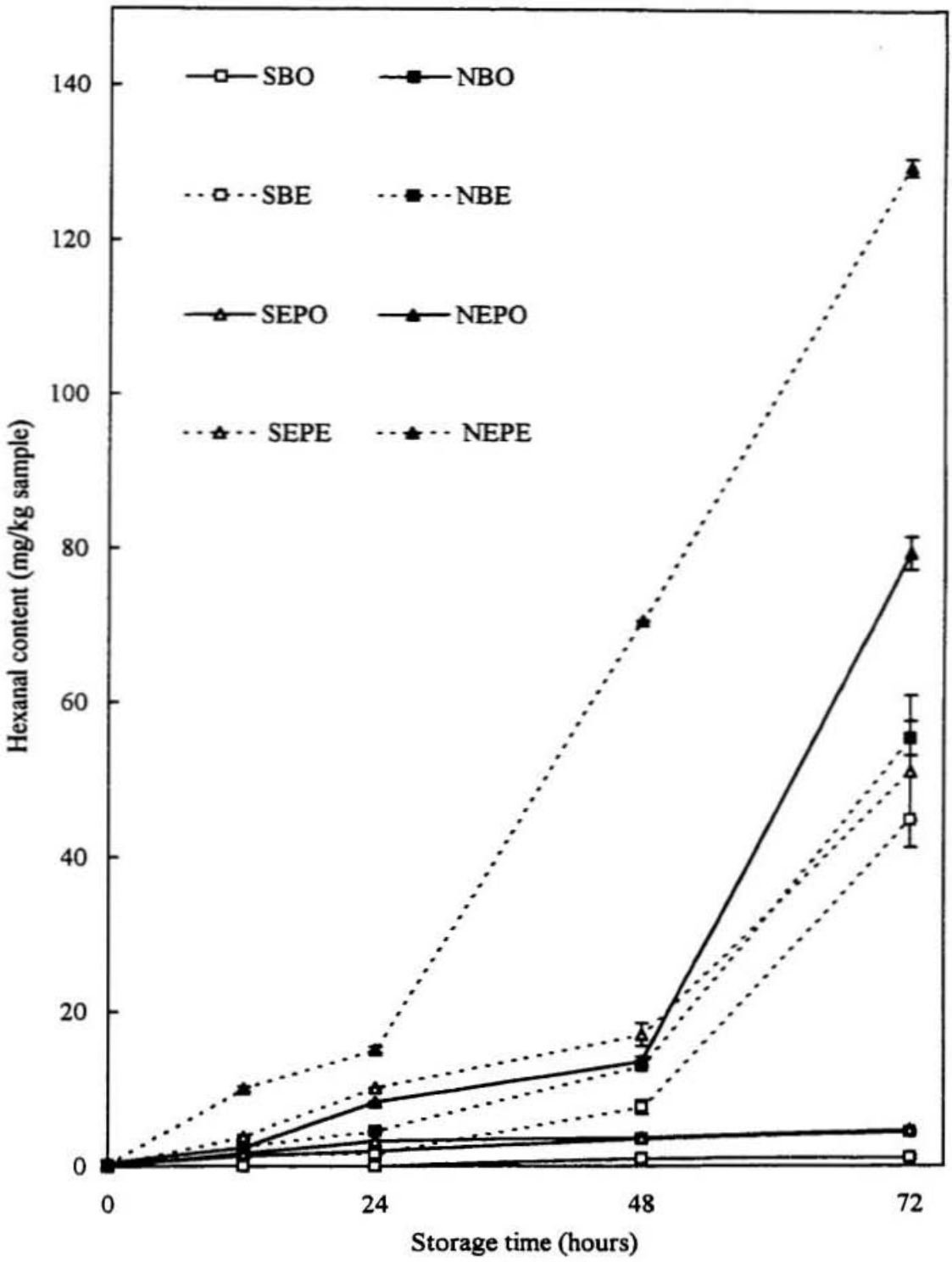

SBO
NBO
SBE
NBE
SEPO
NEPO
SEPE
NEPE
Hexanal content (mg/kg sample)
0
20
40
60
80
100
120
140
0
24
48
72
Storage time (hours)

Table 4.7 linear regression analysis of hexanal content and thiobarbituric acid reactive substances of stripped and non-stripped borage and evening primrose oils and their oil-in-water emulsions illuminated under fluorescent light at 27 C.

Sample	r^2	r	No. of observations	Level of significance
SBO	0.243	0.493	15	$p>0.05$
NBO	0.508	0.713	15	$p<0.01$
SBE	0.191	0.437	15	$p>0.05$
NBE	0.286	0.535	15	$p<0.05$
SEPO	0.567	0.753	15	$p<0.01$
NEPO	0.675	0.822	15	$p<0.01$
SEPE	0.284	0.533	15	$p<0.05$
NEPE	0.554	0.744	15	$p<0.01$

Abbreviations are: SBO, stripped borage oil; NBO, non-stripped borage oil ; SBE, stripped borage oil emulsion; NBE, non-stripped borage oil emulsion; SEPO, stripped evening primrose oil; NEPO; non- stripped evening primrose oil; SEPE, stripped evening primrose oil emulsions; NEPE, non- stripped evening primrose oil emulsion.

etal, 1993). The effects of additives were evaluated by measuring the formation of CD and TBARS content, respectively, of stripped borage and evening primrose oils as described in section 3.5.

4.4.1 The effects of tocopherols

Table 4.8 summarizes the effects of natural and synthetic antioxidants on conjugated dienes formation in stripped borage oil. α-, and δ-tocopherols, as well as Tenox GT-2 were more effective ($p<0.05$) at 500 ppm than at 200 ppm. The most effective antioxidant was Tenox GT-2 followed by δ-tocopherol and then α-tocopherol. Moreover, antioxidant activity of α-tocopherol at 200 ppm declined which coincided with an increase in CD after 120 h at 60 C. Similarly, a gradual decrease of Tenox GT-2 antioxidant activity after the same period was observed. Meanwhile, TBHQ was a more effective ($p<0.05$) antioxidant than BHA and BHT.

The use of TBARS to evaluate the oxidative stability and effectiveness of different antioxidants in vegetable oils has been frequently reported in the literature (Ganthavorn and Hughes, 1997; Duh et al, 1997). Based on TBARS values (Table 4.9), α- and δ-tocopherols as well as Tenox GT-2 were more effective ($p<0.05$) antioxidants in stripped borage oil at 500 ppm than at 200 ppm. Furthermore, TBHQ was the most effective synthetic antioxidant followed by BHA and BHT. These trends in antioxidant effectiveness are similar to those obtained based on CD. The effects of tocopherols, BHA, BHT and TBHQ on conjugated dienes in SEPO stored under Schaal oven conditions are

Table 4.8 The effects of tocopherols (TOC) and synthetic antioxidants on conjugated dienes in stripped borage oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (hours)			
		12	72	120	168
Control	0	4.9 ± 0.01^{e}	6.64 ± 0.4^{f}	13.06 ± 2.53^{f}	22.23 ± 0.08^{g}
α-TOC	200	4.2 ± 0.04^{cd}	5.89 ± 0.07^{e}	9.89 ± 0.04^{d}	21.92 ± 0.06^{g}
	500	4.18 ± 0.04^{e}	5.63 ± 0.04^{d}	10.31 ± 0.02^{e}	15.22 ± 0.34^{e}
δ-TOC	200	3.99 ± 0.09^{c}	6.19 ± 0.05^{e}	9.55 ± 0.02^{c}	15.73 ± 0.71^{e}
	500	4.16 ± 0.10^{c}	5.59 ± 0.14^{d}	10.41 ± 0.38^{e}	14.73 ± 0.13^{d}
Tenxo GT-2	200	4.08 ± 0.11^{c}	5.65 ± 0.10^{d}	9.03 ± 0.19^{b}	17.10 ± 0.34^{f}
	500	4.13 ± 0.06^{c}	4.86 ± 0.13^{c}	10.04 ± 1.52^{e}	9.86 ± 0.37^{c}
BHA	200	2.83 ± 0.01^{b}	2.94 ± 0.12^{b}	2.23 ± 0.09^{a}	4.57 ± 0.20^{b}
BHT	200	4.23 ± 0.02^{cd}	5.80 ± 0.14^{de}	11.13 ± 0.53^{e}	15.34 ± 0.40^{e}
TBHQ	200	2.45 ± 0.12^{a}	2.66 ± 0.07^{a}	2.31 ± 0.05^{a}	1.71 ± 0.08^{a}

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene and tert-butylhydroquinone, (TBHQ). Tenox GT-2 is a commercial natural antioxidant consist of 7.88 ppm of α-, 39.67 ppm of γ- and 21.77 of δ-tocopherol. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

Table 4.9 The effects of tocopherols (TOC) and synthetic antioxidants on the formation of thiobarbituric acid reactive substances (TBARS), (μmol of malonaldehyde equivalents per g oil) in stripped borage oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (days)			
		1	3	5	7
Control	0	3.49 ± 0.11^{h}	5.18 ± 0.02^{f}	5.39 ± 0.04^{f}	6.40 ± 0.47^{g}
α-TOC	200	2.74 ± 0.05^{b}	3.67 ± 0.02^{e}	3.73 ± 0.06^{c}	5.60 ± 0.05^{f}
	500	3.11 ± 0.02^{f}	3.19 ± 0.06^{d}	3.45 ± 0.10^{b}	4.31 ± 0.07^{d}
δ-TOC	200	3.22 ± 0.02^{g}	3.76 ± 0.02^{e}	3.94 ± 0.05^{e}	4.55 ± 0.02^{e}
	500	3.06 ± 0.04^{f}	3.26 ± 0.15^{d}	3.79 ± 0.02^{de}	4.22 ± 0.01^{d}
Tenox GT-2	200	3.04 ± 0.03^{f}	2.45 ± 0.66^{b}	3.79 ± 0.10^{de}	4.80 ± 0.20^{e}
	500	1.88 ± 0.01^{a}	2.95 ± 0.06^{c}	3.36 ± 0.06^{b}	3.42 ± 0.02^{b}
BHA	200	2.96 ± 0.06^{e}	3.16 ± 0.04^{d}	3.74 ± 0.06^{d}	3.88 ± 0.04^{c}
BHT	200	2.86 ± 0.02^{bc}	2.52 ± 0.06^{b}	3.39 ± 0.06^{b}	3.81 ± 0.12^{c}
TBHQ	200	1.92 ± 0.09^{a}	1.23 ± 0.02^{a}	2.50 ± 0.14^{a}	2.59 ± 0.09^{a}

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene and tert-butylhydroquinone, (TBHQ). Tenox GT-2 is a commercial natural antioxidant consist of 7.88 ppm of α-, 39.67 ppm of γ- and 21.77 of δ-tocopherol. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

presented in Table 4.10. The results indicated that α- and δ- tocopherols as well as Tenox GT-2 at both 200 and 500 ppm showed antioxidant activity. Nonetheless, α- and δ-tocopherols as well as Tenox GT-2 at 500 ppm were more effective ($p<0.05$) than at 200 ppm. The higher antioxidant activity was observed with Tenox GT-2 followed by δ- and α-tocopherols. Meanwhile, TBHQ was significantly more effective than BHT, which in turn was more effective ($p<0.05$) than BHA. A similar trend was observed for differences between antioxidant activities based on TBARS in SEPO (Table 4.11). The effectiveness of antioxidants decreased in the following order after 168 h at 60 C: TBHQ (200 ppm) > BHT (200 ppm) > BHA (200 ppm) = Tenox GT-2 (500 ppm) > δ-tocopherol (500 ppm) ≅ α-tocopherol (500 ppm) > Tenox GT-2 (200 ppm) > δ-tocopherol (200 ppm) > α-tocopherol (200ppm).

The following observations can be summarized from CD and TBARS values of SBO and SEPO stored under Schaal oven conditions at 60 C as affected by different additives: (1) Tocopherols were more effective antioxidants at 500 ppm than at 200 ppm. However, the effectiveness of α-tocopherol at 200 ppm declined at the later stages of storage, (2) the most effective natural antioxidant was Tenox GT-2 followed by δ- and α-tocopherols, while the most effective synthetic antioxidant was TBHQ followed by BHA or BHT, (3) the strongest antioxidant activity was exhibited by TBHQ among all other treatments in both SBO and SEPO. Minor deviations from these general trends may be observed. These observations suggest that antioxidant activities of tocopherols are concentration-dependent. This theory may be supported by variations in optimum

Table 4.10 The effects of tocopherols (TOC) and synthetic antioxidants on conjugated dienes in stripped evening primrose oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (hours)			
		12	72	120	168
Control	0	6.79 ± 0.02^{a}	16.96 ± 0.39^{f}	19.69 ± 0.39^{g}	35.59 ± 0.72^{h}
α-TOC	200	5.97 ± 0.07^{c}	10.52 ± 0.05^{e}	15.54 ± 0.18^{f}	31.25 ± 0.67^{g}
	500	6.22 ± 0.01^{c}	9.6 ± 0.04^{d}	13.11 ± 0.10^{d}	25.43 ± 0.12^{e}
δ-TOC	200	6.09 ± 0.04^{c}	10.00 ± 0.02^{e}	13.81 ± 0.16^{e}	28.28 ± 0.28^{f}
	500	6.24 ± 0.04^{c}	9.23 ± 0.02^{d}	12.31 ± 0.12^{c}	24.14 ± 0.17^{d}
Tenox GT-2	200	5.70 ± 0.13^{b}	9.51 ± 0.07^{d}	13.55 ± 0.06^{d}	27.68 ± 0.50^{f}
	500	6.79 ± 0.03^{d}	8.51 ± 0.08^{c}	11.14 ± 0.13^{b}	20.43 ± 0.52^{c}
BHA	200	5.78 ± 0.09^{b}	6.37 ± 0.09^{b}	13.29 ± 0.49^{d}	21.11 ± 0.69^{c}
BHT	200	5.53 ± 0.03^{b}	8.79 ± 0.22^{c}	10.76 ± 0.44^{a}	17.68 ± 0.19^{b}
TBHQ	200	4.41 ± 0.11^{a}	5.36 ± 0.24^{a}	12.28 ± 0.48^{c}	15.59 ± 0.62^{a}

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene and tert-butylhydroquinone, (TBHQ). Tenox GT-2 is a commercial natural antioxidant consist of 7.88 ppm of α-, 39.67 ppm of γ- and 21.77 of δ-tocopherol. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

Table 4.11 The effects of tocopherols (TOC) and synthetic antioxidants on the formation of thiobarbituric acid reactive substances (TBARS), (μmol of malonaldehyde equivalents per g oil) in stripped evening primrose oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (days)			
		1	3	5	7
Control	0	1.66 ± 0.02^{e}	2.40 ± 0.01^{f}	2.45 ± 0.14^{g}	4.21 ± 0.06^{f}
α-TOC	200	1.62 ± 0.01^{e}	1.83 ± 0.05^{d}	2.12 ± 0.01^{e}	3.21 ± 0.03^{e}
	500	1.47 ± 0.02^{d}	1.97 ± 0.02^{e}	2.10 ± 1.88^{e}	2.43 ± 1.04^{cd}
δ-TOC	200	1.58 ± 0.01^{e}	1.93 ± 0.05^{e}	2.21 ± 0.02^{f}	3.08 ± 0.04^{e}
	500	1.46 ± 0.04^{d}	1.86 ± 0.03^{d}	2.05 ± 0.04^{e}	2.32 ± 0.03^{d}
Tenox GT-2	200	1.51 ± 0.01^{e}	1.71 ± 0.02^{c}	2.2 ± 0.60^{e}	2.50 ± 0.04^{d}
	500	1.14 ± 0.01^{c}	1.61 ± 0.02^{b}	1.77 ± 0.05^{d}	2.05 ± 0.02^{c}
BHA	200	1.21 ± 0.02^{b}	1.68 ± 0.04^{b}	1.52 ± 0.06^{c}	2.04 ± 0.04^{c}
BHT	200	1.23 ± 0.01^{b}	1.04 ± 0.06^{a}	1.22 ± 0.03^{b}	1.42 ± 0.03^{b}
TBHQ	200	0.95 ± 0.04^{a}	1.04 ± 0.04^{a}	1.10 ± 0.03^{a}	1.00 ± 0.03^{a}

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene and tert-butylhydroquinone, (TBHQ). Tenox GT-2 is a commercial natural antioxidant consist of 7.88 ppm of α-, 39.67 ppm of γ- and 21.77 of δ-tocopherol. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

levels of different tocopherols required to stabilize vegetable oils. Optimum concentrations. However, the optimum concentration to increase the oxidative stability of stripped rapeseed, soybean and palm oils during microwave heating were 100, 150-200 and 500 ppm of α-, (β-, γ-) and δ-tocopherols, respectively (Yoshida et al, 1993). Moreover, maximum antioxidant activities of α- and γ-tocopherol mixtures and a natural mixture of α-, γ- and δ-tocopherols from soybean were 250 and 500 ppm in stripped corn oil (Huang et al, 1994). It has been reported that the optimum level of γ-tocopherol varies from 250 to 500 ppm in stripped corn oil (Lampi et al, 1997).

Tocopherols are considered to be primary or chain breaking antioxidants in free radical chain reactions which can convert lipid radicals to more stable products, thus extend the shelf life of edible oils (Gordon, 1990). In autoxidation of vegetable oils containing unsaturated fatty acids, free alkyl radicals are formed in the slow initiation step. These radicals react rapidly with atmospheric oxygen to produce peroxy radicals, which in turn can give hydroperoxides and new alkyl radicals. These radicals will propagate the oxidation reaction and cause a dramatic increase in the formation of primary and secondary oxidation products, (Porter et al, 1995). Tocopherols through their chromanol moiety can donate their phenolic hydrogens to a lipid peroxy radical to form a resonance stabilized chromanoxyl radical which in turn reacts with other radicals to form stable adducts and, therefore, terminates the free radical chain reactions (Kamal-Eldin and Appelqvist,1996). Meanwhile, the tocopherols can be consumed and oxidized during these processes, and thus their antioxidant activities gradually or rapidly decline depending on

their initial levels and temperature of the lipid system (Gordon and Kourimska,1995). This of α-, γ-, and δ-tocopherols to improve the oxidative stability of stripped soybean oil were 100, 250 and 500 ppm, respectively (Jung and Min, 1990).may explain, in part, the sudden increase of CD of SBO treated with 200 ppm of α-tocopherol.

The differences in the antioxidant activities within tocopherols in different lipid systems are not yet fully understood nor can these be explained solely by structural differences. Therefore, it has been proposed that the antioxidant activity of tocopherols is dependent on concentration, temperature, light, type of substrate and solvent as well as synergists and other chemical species acting as prooxidants (Kamal-Eldin and Appleqvist,1996).

The chemical structures of tocopherols suggest that α-tocopherol is more potent as a hydrogen donor than β-,γ- and δ-tocopherols because of the absence of one or two ortho-methyl groups. The presence of these electron releasing groups in the ortho and or para positions increases the hydrogen donating ability of substituted phenols (Kamal-Eldin and Appelqvist, 1996). Although it has been reported that at physiological conditions around 37 C the antioxidant activity is in the order $\alpha > \beta > \gamma > \delta$, which supports the previous suggestion, the reverse trend $\delta > \gamma > \beta > \alpha$ has been observed at higher temperatures between 50 and 100 C (Madhavi et al,1996). Moreover, during microwave heating of stripped vegetable oil with an equimolar mixture of tocopherols added, the stability of tocopherols decreased in the order $\delta > \beta > \gamma > \alpha$ (Yoshida et al, 1991). Similarly, Burkow et al (1995) have examined the effectiveness of different antioxidants in

cod liver oil at 80 C by the Rancimat method. The antioxidant activity increased in the following order δ-tocopherol > Tenox GT-2 > α-tocopherol. This might explain the higher antioxidant activity of δ-tocopherol in SBO and SEPO compared to α-tocopherol. Meanwhile, the lower stability of α-tocopherol at higher temperatures was attributed to its higher reactivity. Therefore, when investigating the antioxidant effects of tocopherols in accelerated storage tests with vegetable oils, α-tocopherol was consumed first, followed by β- and γ-tocopherols. Finally, δ-tocopherol was more stable and consumed more slowly (Yoshida et al, 1993).

The most effective synthetic antioxidant in both stripped oils was TBHQ. Similar observations have been reported by others. TBHQ was the most effective antioxidant in soybean, hydrogenated soybean and peanut oils compared to Tenox GT-2 and α-tocopherol (Akoh,1994). Meanwhile, Gordon and Kourimska (1995) have examined the effects of different antioxidants on the oxidative stability of rapeseed oil during heating and the order of antioxidant activity was TBHQ > lecithin > ascorbyl palmitate > rosemary extract > BHT, BHA and δ-tocopherol. Similarly, TBHQ at 100 ppm was a more effective antioxidant than BHA at 200 ppm, which in turn was more effective than 200 ppm of BHT in capelin oil at 60 C (Kaitaranta, 1992). More recently, Sharma et al (1997) found that TBHQ was the most effective antioxidant in fried potato chips, banana chips and fried begalgram dhal followed by BHT and then BHA.

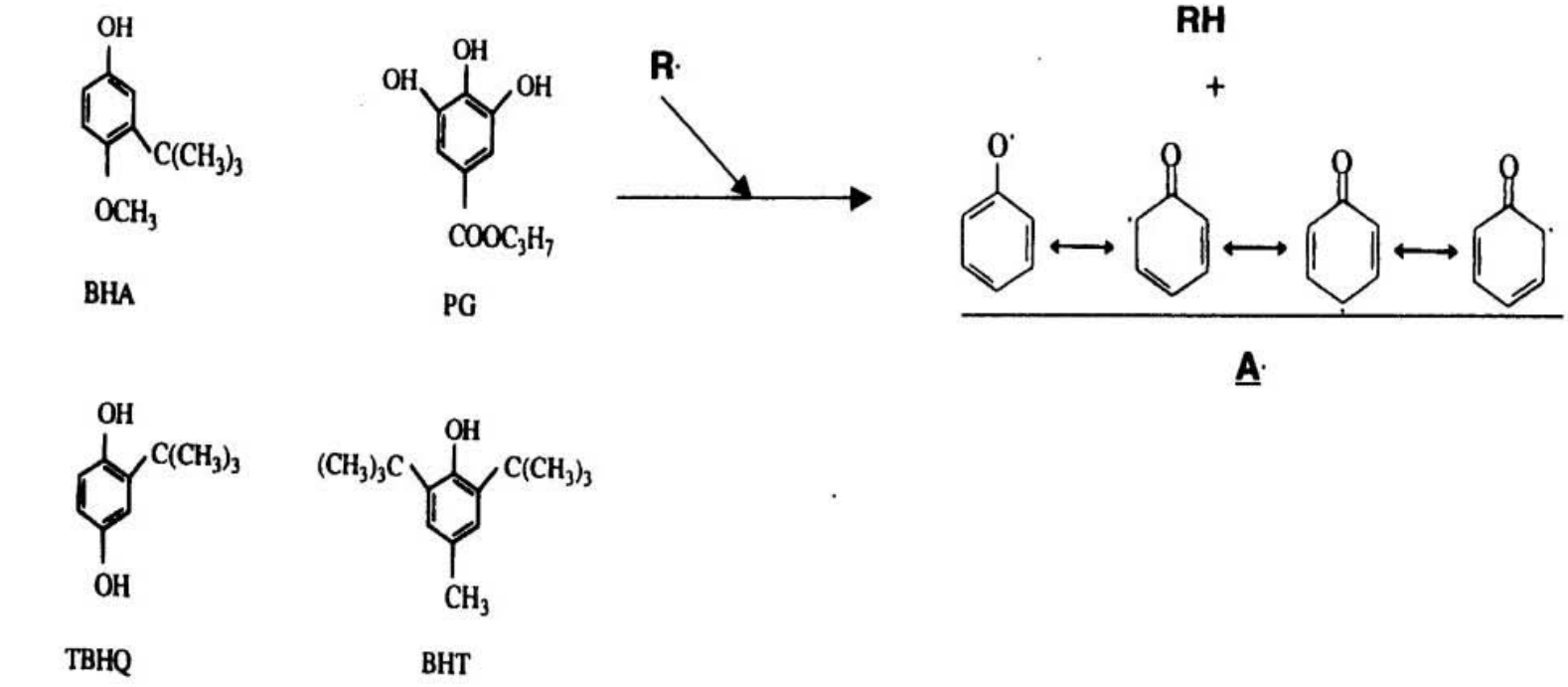
Synthetic phenolic antioxidants interrupt the free radical chain reaction in a similar manner to tocopherols by donating their phenolic hydrogen to fatty acid radicals, thus

terminating the reaction. Phenol itself does not act as an antioxidant, but substitution of alkyl groups into 2,4, and 6 positions increase the electron density on the hydroxyl group by an inductive effect and thus increases hydrogen donation ability (King et al,1995). The antioxidant activity of BHA is due to the strong electron donating potency of the methoxy substitutent. The phenoxy radical formed during this process is stabilized by delocalization of the unpaired electron around the aromatic ring (Figure 4.13). The stability of phenoxy radicals reduces the rate of propagation and reaction and thus increases the oxidative stability of lipids (Gordon,1990). The presence of bulky branched groups, as in BHT, will increase the stability of phenoxy radicals. However, it may also decrease the ability of these radicals to react with the fatty acid peroxy radicals (King et al,1995). This might explain, in part, the higher antioxidant activity of BHT in SEPO but not in SBO. The introduction of a second hydroxyl group into position 2 or 4 enhances the oxidative stability (Gordon,1990). Therefore, it has been proposed that the two para hydroxyl groups are responsible for the superior antioxidant activity of TBHQ in various edible oils (Madhavi et al, 1995).

4.4.2 Effects of phospholipids and phospholipids together with tocopherols.

Table (4.12) presents the effects of phospholipids, tocopherosls and their mixture on CD of stripped borage oil. Based on CD values, phosphatidylcholine (PC) was more effective ($p<0.05$) than phosphatidylethanolamine (PE). PC as well as PE acted synergistically with α- and δ- tocopherols. All combinations of tocopherols and phospholipids inhibited the formation of CD in SBO.

Figure 4.13 Mechanism of antioxidant activity of synthetic phenolic antioxidants.



$$RH \xrightarrow{\text{initiator}} R\cdot$$

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

$$AH + R\cdot \longrightarrow A\cdot + RH$$

$$A\cdot + O_2 \longrightarrow AOO\cdot$$

$$AOO\cdot + RH \longrightarrow AOOH + R\cdot$$

$$A\cdot + R\cdot \longrightarrow AR$$

Synergism between PC and α-tocopherol after 120 h was more pronounced ($p<0.05$) than that between PC and δ-tocopherol as determined by CD values. Similarly, PE with α-tocopherol was more effective than PE together with δ-tocopherols. The most effective treatments among the previous combinations was PC together with α-tocopherol. Similar trends of antioxidant effectiveness and synergism were observed based on TBARS values in stripped borage oil (Table 4.13).

Based on CD values (Table 4.14), PE was a more ($p<0.05$) effective antioxidant in SEPO than PC. Moreover, PC and PE acted synergistically with α- and δ- tocopherols to reduce CD formation. The most effective treatments were PE together with δ-tocopherol followed by PC with δ-tocopherol. Meanwhile, based on TBARS values (Table 4.15), PE was again more effective ($p<0.05$) antioxidant than PC in SEPO. Furthermore, PC and PE acted synergistically with α- and δ- tocopherols to inhibit the TBARS. The most effective combination was that of PE and α-tocopherol.

In summary, based on CD and TBARS formed in SBO and SEPO, the antioxidant effectiveness of PC was higher than PE in SBO, while the reverse trend was observed in SEPO. Meanwhile, synergism was observed between tocopherols and phospholipids in SBO and SEPO. The most effective combination in SBO was that PC and α-tocopherol, while the most effective treatment in SEPO was that of PE and α-tocopherol.

The most likely explanation for the results obtained is that phospholipids may vary in their antioxidant or prooxidant activities in lipid systems depending on the fatty acid composition and the functional groups attached to the phosphate group as well the

Table 4.12 Effects of phospholipids (PL), tocopherols (TOC) and PL together with TOC on conjugated dienes in stripped borage oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (hours)			
		12	72	120	168
Control	0	4.39 ± 0.10^{e}	6.64 ± 0.04^{f}	13.06 ± 2.53^{g}	22.23 ± 0.08^{h}
PC	500	4.19 ± 0.08^{c}	5.08 ± 0.02^{d}	9.09 ± 0.31^{d}	16.80 ± 0.18^{f}
PE	500	4.23 ± 0.02^{d}	5.52 ± 0.06^{e}	11.28 ± 0.33^{f}	19.41 ± 0.36^{g}
α-TOC	500	4.18 ± 0.04^{c}	5.63 ± 0.04^{e}	10.31 ± 0.01^{e}	15.22 ± 0.34^{e}
δ-TOC	500	4.16 ± 0.10^{c}	5.59 ± 0.14^{e}	10.41 ± 0.38^{e}	14.73 ± 0.13^{d}
PC+α-TOC	500+500	3.85 ± 0.06^{b}	4.05 ± 0.04^{c}	4.62 ± 0.12^{a}	7.53 ± 0.04^{a}
PC+δ-TOC	500+500	3.46 ± 0.05^{a}	3.28 ± 0.23^{a}	7.89 ± 0.19^{c}	7.44 ± 0.26^{a}
PE+α-TOC	500+500	4.01 ± 0.02^{c}	4.07 ± 0.05^{c}	5.18 ± 0.16^{b}	8.48 ± 0.03^{b}
PE+δ-TOC	500+500	3.53 ± 0.04^{a}	3.56 ± 0.02^{b}	9.03 ± 0.09^{d}	9.15 ± 0.07^{c}

PC, Phosphatidylcholine; and PE, Phosphatidylethanolamine. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

Table 4.13 Effects of phospholipids (PL), tocopherols (TOC) and PL together with TOC on thiobarbituric acid reactive substances (TBARS) (μmol of malonaldehyde equivalents per g oil) in stripped borage oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (hours)			
		12	72	120	168
Control	0	3.49 ± 0.11^{g}	5.18 ± 0.12^{g}	5.39 ± 0.04^{g}	6.40 ± 0.47^{g}
PC	500	2.78 ± 0.06^{d}	3.85 ± 0.05^{e}	3.60 ± 0.05^{e}	5.34 ± 0.13^{e}
PE	500	3.24 ± 0.02^{f}	4.56 ± 0.04^{f}	4.58 ± 0.03^{f}	5.96 ± 0.03^{f}
α-TOC	500	3.11 ± 0.02^{ef}	3.19 ± 0.06^{d}	3.45 ± 0.01^{d}	4.31 ± 0.07^{d}
δ-TOC	500	3.06 ± 0.04^{e}	3.26 ± 0.15^{d}	3.79 ± 0.02^{e}	4.22 ± 0.01^{d}
PC+α-TOC	500+500	1.14 ± 0.04^{a}	1.63 ± 0.05^{a}	2.11 ± 0.02^{a}	2.05 ± 0.05^{a}
PC+δ-TOC	500+500	1.34 ± 0.01^{b}	2.64 ± 0.04^{c}	3.14 ± 0.07^{c}	3.15 ± 0.08^{c}
PE+α-TOC	500+500	1.92 ± 0.01^{c}	2.16 ± 0.06^{b}	2.71 ± 0.06^{b}	2.30 ± 0.05^{b}
PE+δ-TOC	500+500	2.97 ± 0.04^{e}	2.56 ± 0.08^{c}	3.50 ± 0.08^{d}	4.38 ± 0.17^{d}

PC, Phosphatidylcholine; and PE, Phosphatidylethanolamine. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

chemical composition of the system and the oxidation conditions.This theory can be supported by the fact that phospholipids in edible oils have been shown to act as antioxidants, prooxidants, synergists, chelating agents and off flavour precursors (King et al, 1995; Shahidi and Shukla, 1996; Wanasundara et al, 1997). Koga and Terao (1994) have attributed the disagreement on the effectiveness of each lipid class to the differences of the oxidation conditions in the individual studies. Nowas et al (1997) have recently examined the antioxidant effectiveness of commercial PA, PE and PC varying in their degree of saturation on the oxidative stability of salmon and menhaden oils. The authors have reported the following observations: (1) PC was more effective than PA and PE in preventing the oxidation of salmon oil and protecting polyunsatuaterd fatty acids, (2) PL which contained 18 carbons or more, were better antioxidants than PL with short chain fatty acids, and (3) all the PL examined had little or no effect in menhaden oil while both soybean and egg lecithin increased the oxidative stability of cod liver oil (Burko et al, 1995).

Several mechanisms have been proposed to explain the existing differences in antioxidant effectiveness of different PL classes as well the synergism between PL and tocopherols (Koga and Terao, 1994; Nwosu et al, 1997). The antioxidant activity of PL was mainly attributed to their head groups and not to their fatty acid composition. Thus PC was more effective than PG in salmon oil, although both of them had similar fatty acid compositions (King et al,1992). Similarly, dipalmitoyl PC was a more effective antioxidant than dipalmitoyl PA in salmon oil (Nwosu et al,1997). However, the degree of fatty acid saturation in PL may contribute to their antioxidant effectiveness. Therefore, PC which

contained 6 % PUFA was a more effective antioxidant than PE in salmon oil which contained more than 21 % PUFA (Nwosu et al,1997). PE from soybean oil have higher amounts of unsaturated fatty acids than PC (Wang et al, 1997). This might explain, in part, the lower antioxidant effectiveness of PE in SBO.

The antioxidant activity of serine and ethanoalamine in the presence of a pair of free electrons from the nitrogen molecule of an amine ($-NH_2$) has been detected in autoxidation of methyl linoleate at pH 7.9 or 10.2. Furthermore, choline which includes a tertiary amine group [$(CH_3)_3N^+$)] was not effective in this system. This might explain in part the superior antioxidant activity of PE compared to PC in SEPO. Moreover, during the oxidation of SEPO, but not SBO, samples containing PE showed a bright yellow colour. This may be explained by reaction of the amino groups with aldehydes formed during the storage at 60 C. These coloured products may provide additional antioxidant activities (King et al, 1992). Although this observation is in contrast to that occurring in SBO, it is in agreement with the results obtained by others which indicated that phosphatidylinositol (PI) and PE were more effective than PC in enhancing the oxidative stability of soybean oil (Hildebrand,1984). Moreover, the OS of perilla oil was improved with the addition of PE and phosphatidylserine (PS), but not with PC Similarly, Chen and Nawar (1991) have reported that PE was more active than PC in inhibiting milk fat oxidation at both 50 and 90 C.

Recently, Saito and Ishihara (1997) have tested the antioxidant activity of various compounds which represent the major functional groups in PL, PC and PE in a sardine oil model system to determine the relationship between molecular structure and the

Table 4.14 Effects of phospholipids (PL), tocopherols (TOC) and PL together with TOC on conjugated dienes in stripped borage oil stored under Schaal oven conditions at 60 C.

Additives	Conc. (ppm)	Oxidation time (hours)			
		12	72	120	168
Control	0	6.79 ± 0.30^{f}	11.27 ± 0.37^{g}	16.96 ± 0.39^{g}	35.59 ± 0.72^{h}
PC	500	6.34 ± 0.03^{e}	5.65 ± 0.02^{c}	13.78 ± 0.35^{e}	33.7 ± 0.36^{g}
PE	500	5.66 ± 0.14^{d}	5.23 ± 0.03^{b}	12.25 ± 0.35^{d}	23.28 ± 0.56^{e}
α-TOC	500	6.22 ± 0.01^{e}	10.52 ± 0.05^{f}	15.54 ± 0.18^{f}	31.25 ± 0.07^{f}
δ-TOC	500	6.24 ± 0.04^{e}	9.23 ± 0.02^{e}	12.31 ± 0.12^{d}	24.14 ± 0.17^{e}
PC+α-TOC	500+500	4.65 ± 0.02^{c}	7.00 ± 0.12^{d}	7.94 ± 0.08^{c}	14.94 ± 0.47^{d}
PC+δ-TOC	500+500	4.04 ± 0.06^{b}	6.80 ± 0.23^{d}	7.19 ± 0.15^{b}	10.78 ± 0.29^{b}
PE+α-TOC	500+500	4.01 ± 0.04^{b}	5.10 ± 0.26^{b}	7.15 ± 0.05^{b}	12.60 ± 0.16^{c}
PE+δ-TOC	500+500	3.11 ± 0.10^{a}	4.12 ± 0.12^{a}	5.20 ± 0.11^{a}	9.69 ± 0.24^{a}

PC, Phosphatidylcholine; and PE, Phosphatidylethanolamine. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

Table 4.15 Effects of phospholipids (PL), tocopherols (TOC) and PL together with TOC on thiobarbituric acid reactive substances (TBARS) (μmoles of malonaldehyde equivalents per g) in stripped evening primrose oil stored under Schaal oven conditions at 60 C.

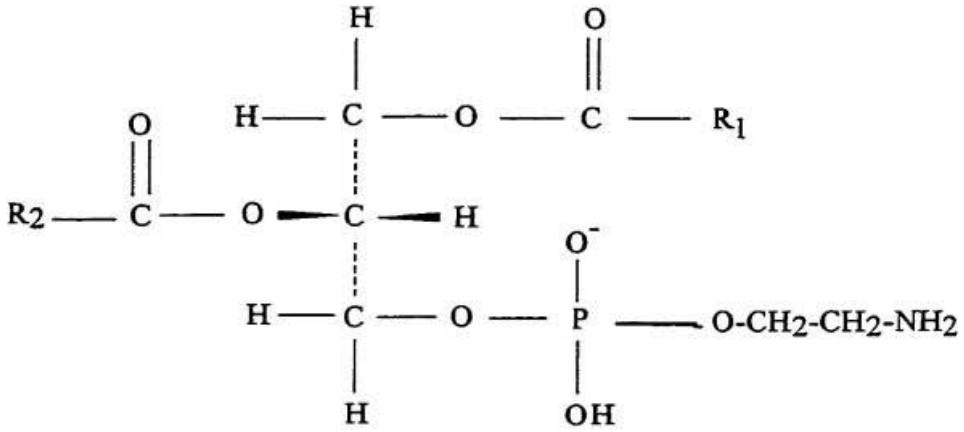
Additives	Conc. (ppm)	Oxidation time (hours)			
		12	72	120	168
Control	0	1.66 ± 0.02^{e}	2.45 ± 0.14^{g}	2.40 ± 0.01^{f}	4.21 ± 0.07^{e}
PC	500	1.51 ± 0.02^{d}	1.81 ± 0.04^{e}	2.14 ± 0.02^{e}	3.79 ± 0.04^{d}
PE	500	1.41 ± 0.06^{d}	1.50 ± 0.06^{c}	1.36 ± 0.06^{c}	2.60 ± 0.07^{c}
α-TOC	500	1.47 ± 0.02^{d}	1.97 ± 0.02^{f}	2.10 ± 0.01^{e}	2.43 ± 0.11^{b}
δ-TOC	500	1.46 ± 0.04^{d}	1.86 ± 0.03^{e}	2.05 ± 0.04^{e}	2.32 ± 0.03^{b}
PC+α-TOC	500+500	1.23 ± 0.03^{c}	1.63 ± 0.05^{d}	1.80 ± 0.06^{d}	2.88 ± 0.06^{d}
PC+δ-TOC	500+500	1.12 ± 0.03^{b}	1.25 ± 0.07^{b}	1.28 ± 0.07^{b}	2.76 ± 0.13^{c}
PE+α-TOC	500+500	1.44 ± 0.01^{d}	1.83 ± 0.06^{e}	1.54 ± 0.10^{c}	2.77 ± 0.09^{d}
PE+δ-TOC	500+500	1.08 ± 0.04^{a}	1.18 ± 0.06^{a}	1.14 ± 0.08^{a}	1.58 ± 0.04^{a}

PC, Phosphatidylcholine; and PE, Phosphatidylethanolamine. Values are means of three determinations ± SD. Values within each column with different superscripts are different ($p<0.05$) from one another.

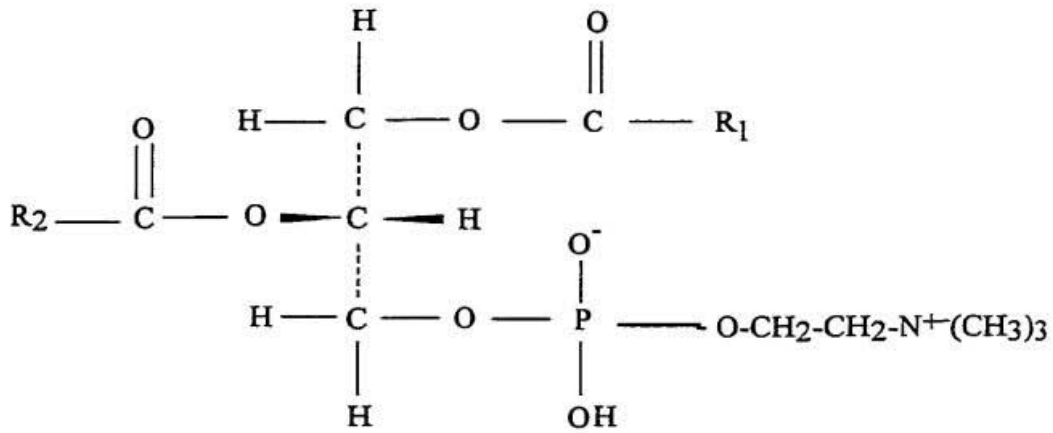
antioxidant activity. Side chain moieties in choline and ethanolamine (Figure 4.14),exhibited strong antioxidant activities, while phosphatidic acid derivatives as well as glycerol did not show any antioxidant activity. Moreover, the authors postulated that the intramolecular hydroxy group might possibly strengthen the nucleophilicity of the amines through intramolecular hydrogen bonding. Therefore, the nucleophilic amines may form bonds with oxygen by donating the electron pair to the electron-deficient oxygen. Homolytic cleavage of the oxygen-oxygen bond of hydroperoxide will produce alcohols.

Although the synergism between tocopherols and phospholipids is well known to increase the OS of edible oils, the mechanisms involved are still a subject of discussion (Koga and Terao, 1994; King et al, 1995). The lag phase in autoxidation of edible oils lasts until their tocopherol is consumed, thus resulting in accelerated lipid oxidation. Phospholipids can enhance the antioxidant effectiveness of tocopherols by delaying their irreversible oxidation to tocophenyl quinone (Hildebrand et al, 1984). The effect of PC to enhance the antioxidant activity of α-tocopherol, as it has been observed in SBO, can be explained, in part, by the fact that phospholipids increase the accessibility of α-tocopherol into the aqueous microenviroment where the initiation reaction takes place, because usually contain small amounts of water (Koga and Terao, 1995). Nonetheless, Lambelet et al (1994) have reported that PC has poor synergistic activity compared to PE with either natural or synthetic antioxidants. The authors have postulated that secondary amines can react with peroxy radicals to form an aminyl radical (eq.4.1), which in turn reacts

Figure 4.14 Chemical structure of phosphatidylethanolamine and phosphatidylcholine.



Phosphatidylethanolamine (PE)



Phosphatidylcholine (PC)

rapidly with oxygen to form, via an N-peroxy radical intermediate, the corresponding secondary nitroxides (eqs. 4.2 & 4.3).

$R_2NH + R'OO^{\cdot} \longrightarrow R_2N^{\cdot} + ROOH$ (eq 4.1)

$R_2N^{\cdot} + O_2 \longrightarrow R_2NOO^{\cdot}$ (eq 4.2)

$2\ R_2NOO^{\cdot} \longrightarrow 2\ R_2NO^{\cdot} + O_2$ (eq 4.3)

This mechanism can be extended to explain the synergism between tocopherols and PE, which possess a primary amine group. However, primary nitroxides are expected to be formed. This reaction may not occur in the case of PC as it contains a tertiary amine group.

CONCLUSIONS AND RECOMMENDATIONS

Column chromatographic techniques provide an effective means for stripping the minor components for vegetable oil triacylglycerols. However, some minor components may be retained in the stripped oils. The minor components in borage and evening primrose oils influenced their oxidative stability in the dark as well as in the light. Moreover, the behaviour of endogenous antioxidants in borage and evening primrose oil-in-water emulsions may or may not be explained by the so-called "polar paradox" theory. Meanwhile, CD and TBARS can be used to assess the oxidative stability of borage and evening primrose oils and their oil-in-water emulsions in addition to or in place of PV and headspace volatiles, with few exceptions.

δ-tocopherol was more effective than α-tocopherol in stabilizing stripped borage and evening primrose oils. However, Tenox GT-2 was the most effective natural antioxidant and TBHQ was the most effective synthetic antioxidant in both stripped oils. Phospholipids may act as antioxidants or prooxidants, depending on their fatty acid composition and the functional groups attached to their phosphate groups as well as the chemical composition of the assay system and the oxidation conditions. Moreover, PE and PC acted synergistically to enhance the oxidative stability of stripped borage and evening primrose oils.

The results presented in this thesis have (1) provided simple and reliable analytical methods to follow the oxidation of stripped and non-stripped borage and evening primrose oils, (2) enhanced our understanding of the parameters involved in the oxidation of

nutritional and medicinal oils and emulsions, and (3) shown improvement in oxidative stability of stripped borage and evening primrose oils through the use of natural and synthetic antioxidants.

Based on the results of this thesis, it is recommended that: (1) new and more effective techniques be developed to prepare stripped vegetable as well as marine oils and that the kinetics as well as the mechanisms involved in these processes be examined; (2) well controlled stripped and non-stripped vegetable and marine oil-in-water emulsions with precise chemical and physical characteristics be examined in order to clarify the validity of the "polar paradox" theory; (3) the flavour stability and volatiles generated during auto- and photoxoidation of borage and evening primrose oils and their oil-in-water emulsions be examined, and (4) optimization of the levels of natural antioxidants in borage and evening primrose oils be achieved via innovative refining processes as well as genetic manipulation of their seeds.

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APPENDIX

Figure A.1 Standard curve of 1,1,3,3-tetramethoxypropane (precursor of malonaldehyde)

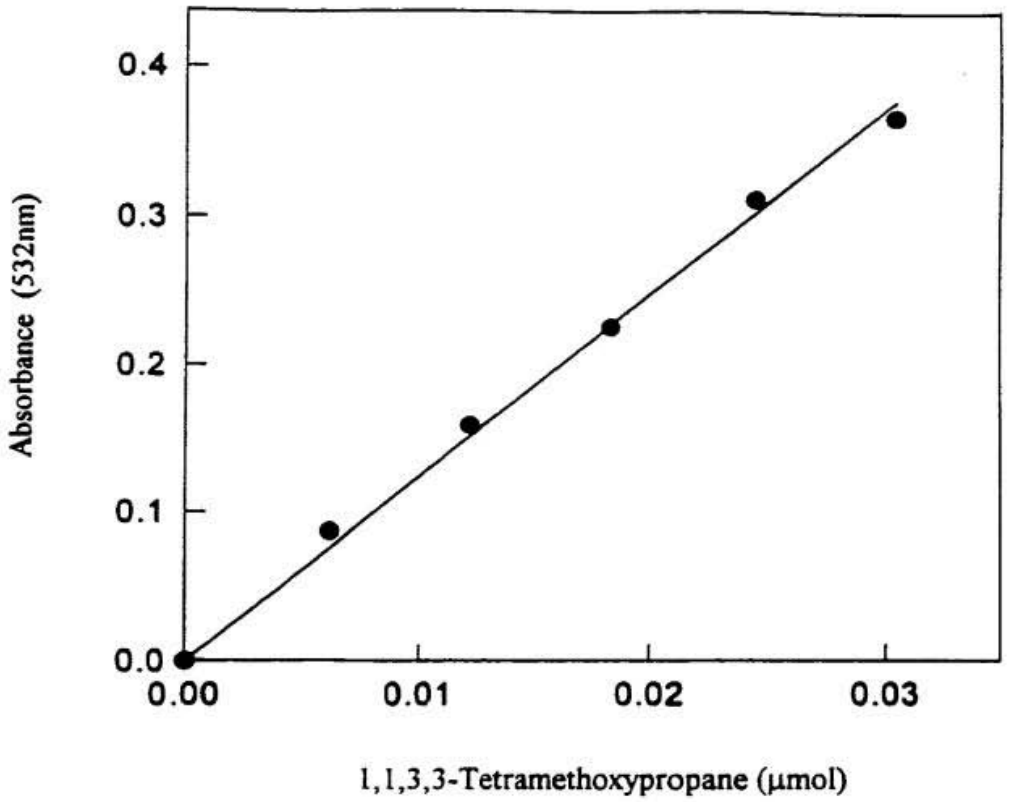


Figure A. 1 Standard line of 1,1,3,3-tetramethoxypropane (precursor of malonaldehyde).

Regression coefficient (r) =0.997
Equation of the line (y=ax+b) where,

y= absorbance
x= concentration of malonaldehyde (MA) in 5 mL solution, μmol (C)
a= 12.04
b= 0.0
$A_{532} = 12.04 \times C$

Therefore, $C = 0.083 \times A_{532}$

and since the wt g of oil is dissolved in 25 mL of solution, the MA concentration is

$C = (0.415 \times A_{532}) / wt$ (μmol of MA/g oil).

Figure A.2 Gas chromatogram of headspace volatiles of stripped (a) and non-stripped (b) borage oil stored under Schaal oven conditions at 60 C for 72 hours. Peaks: (1) pentanal; (2) hexanal; (3) 2,4-heptadienal.

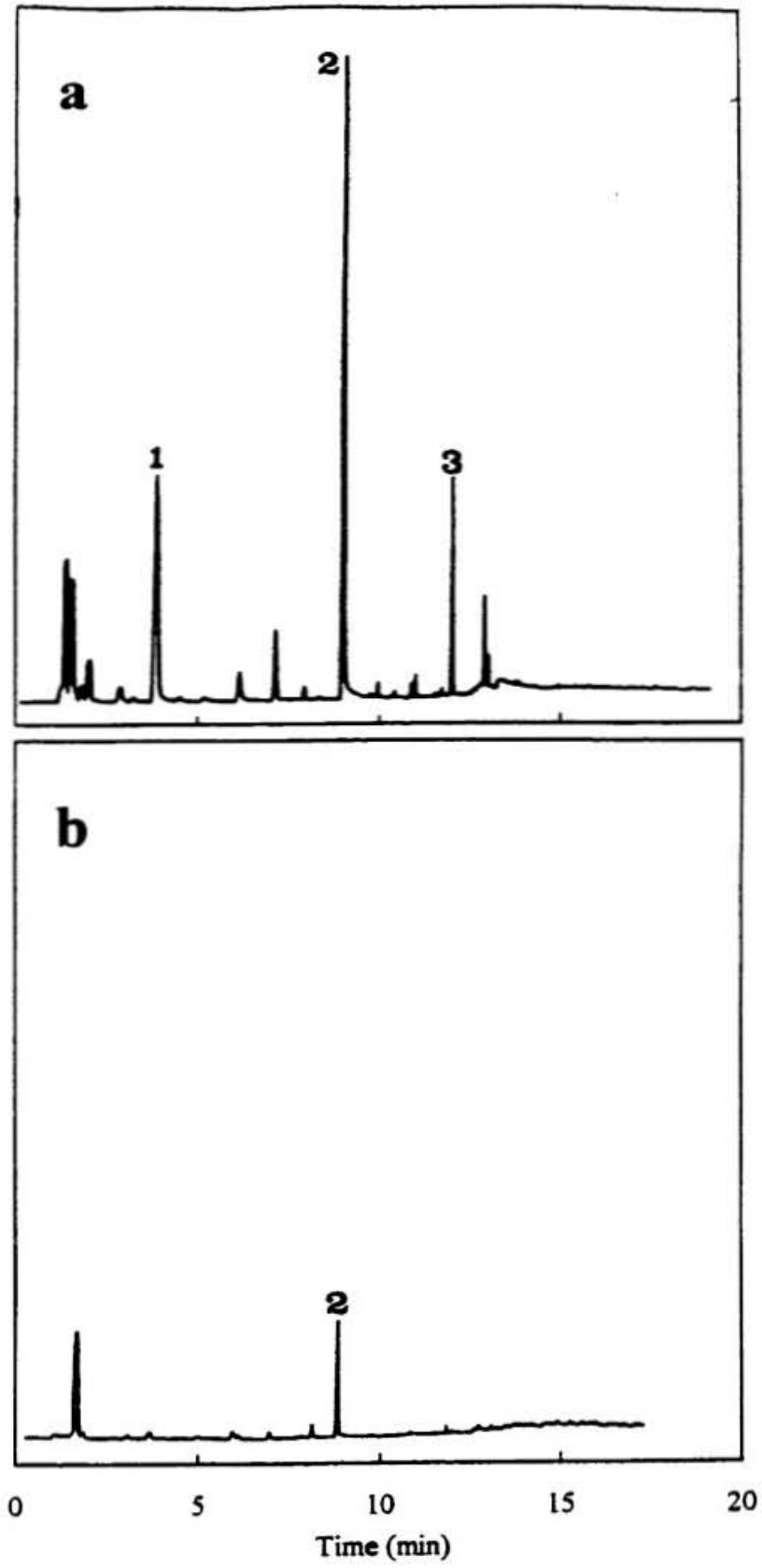

a
2
1
3
b
2
0
5
10
15
20
Time (min)

Figure A.3 Gas chromatogram of headspace volatiles of stripped (a) and non-stripped (b) evening primrose oil stored under Schaal oven conditions at 60 C for 72 hours. Peaks: (1) pentanal; (2) hexanal; (3) 2,4-heptadienal.

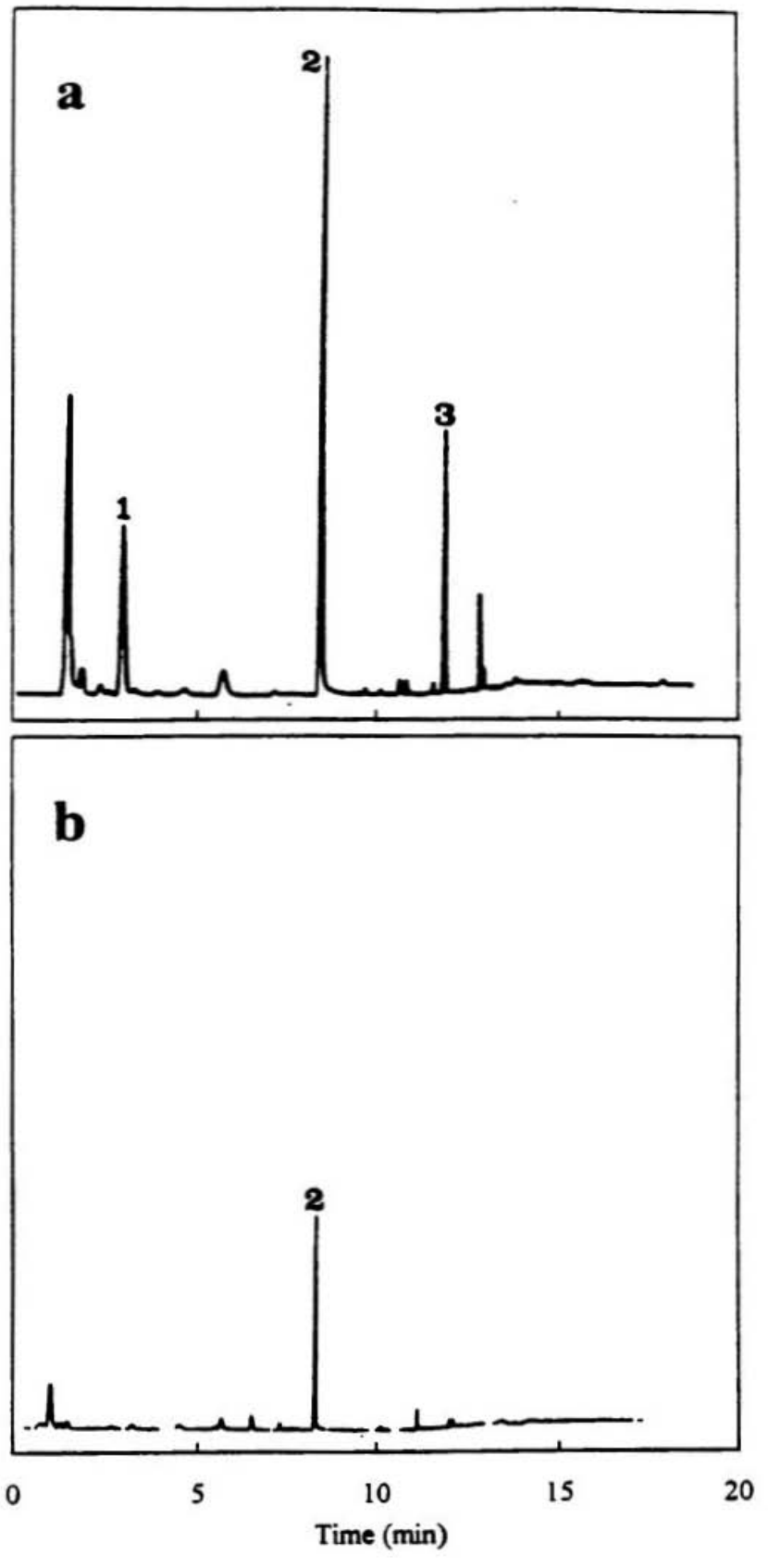

a
2
3
1
b
2
0
5
10
15
20
Time (min)

Figure A.4 Gas chromatogram of headspace volatiles of stripped (a) and non-stripped (b) borage oil illuminated with fluorescent light at 27 C for 72 hours. Peak: (1) hexanal.

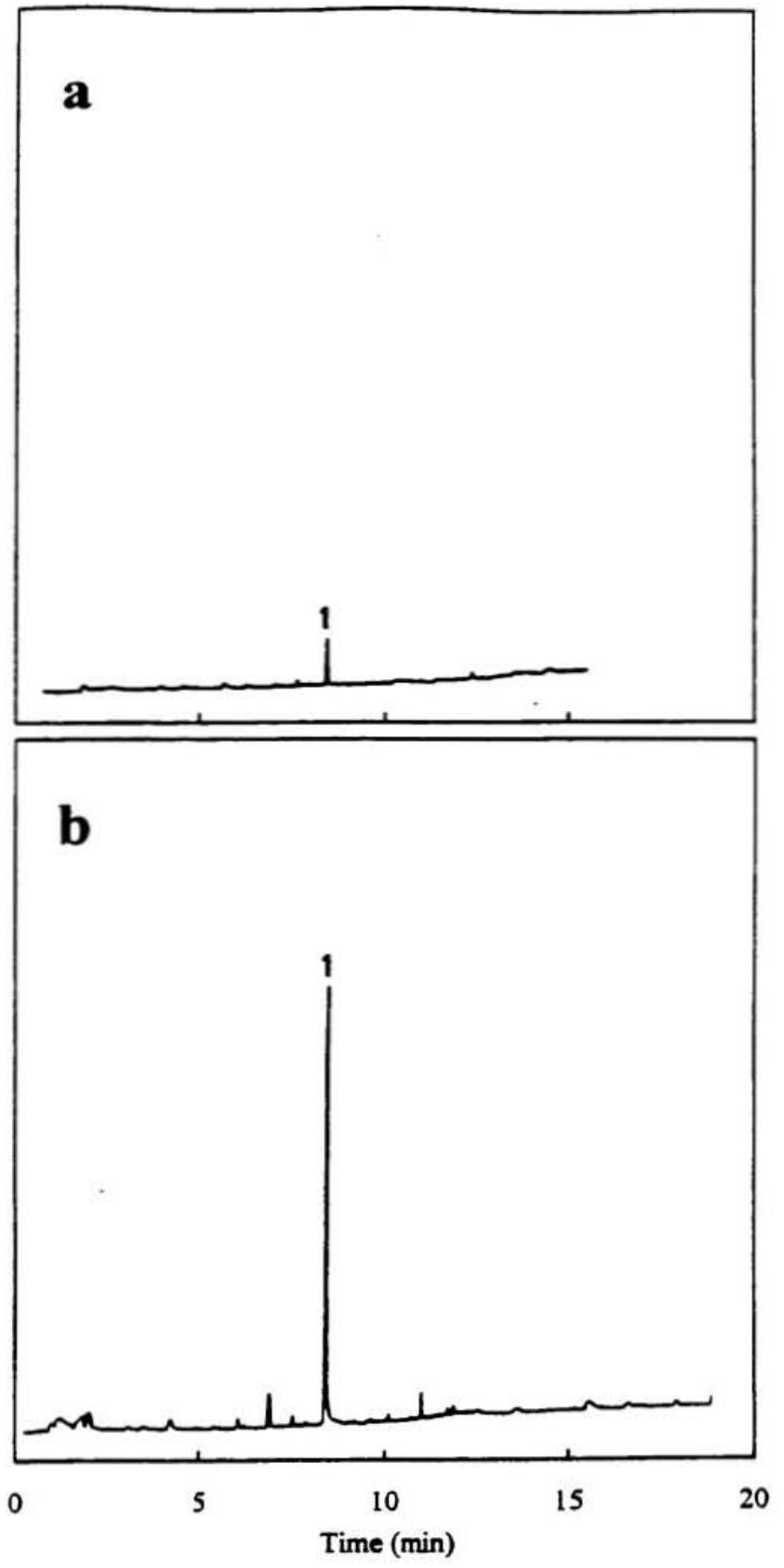
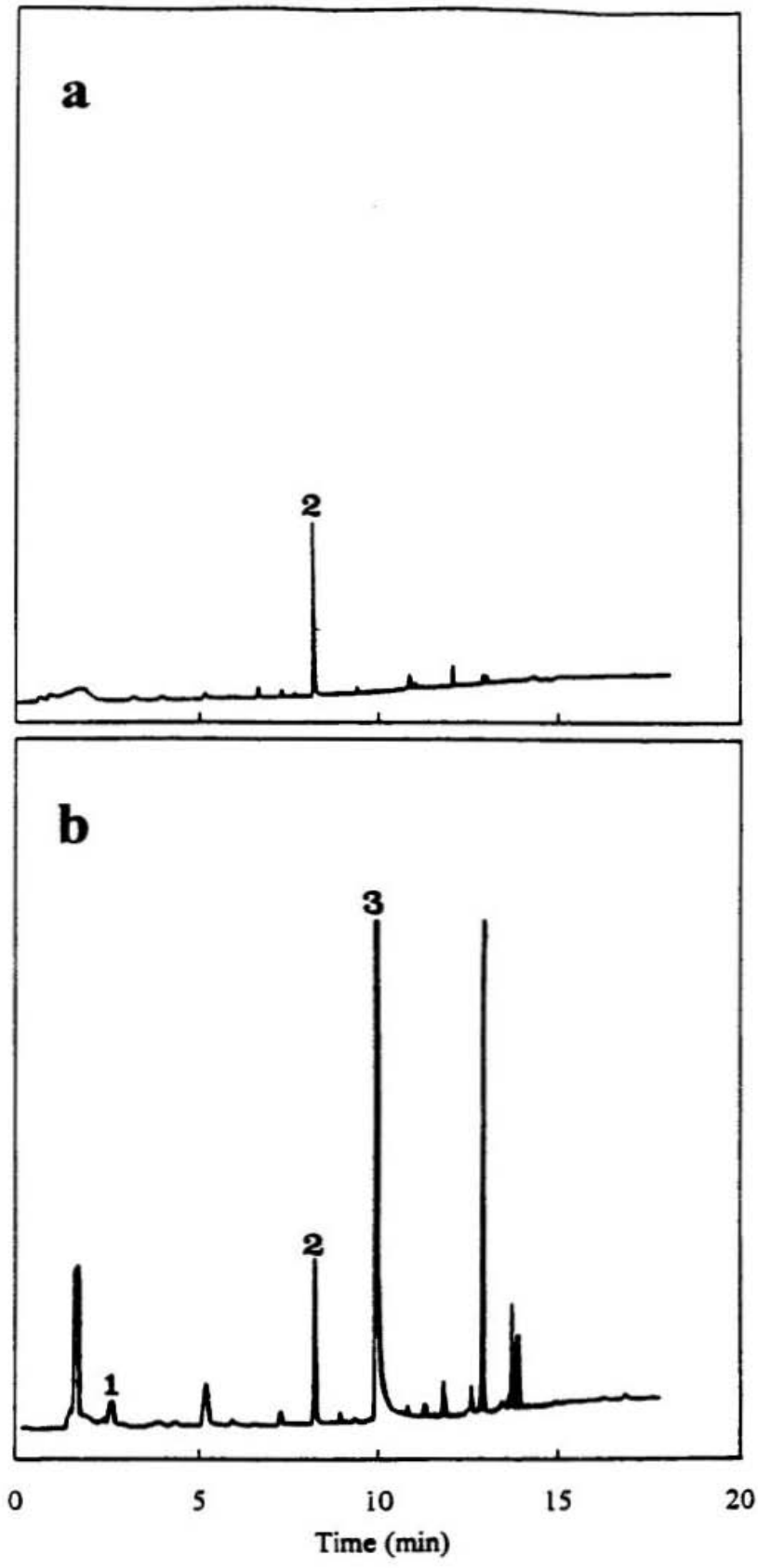

a
1
b
1
0
5
10
15
20
Time (min)

Figure A.5 Gas chromatogram of headspace volatiles of stripped (a) and non-stripped (b) evening primrose oil illuminated with fluorescent light at 27 C for 72 hours. Peaks: (1) pentanal; (2) hexanal; (3) 2-heptenal.


a
2
b
3
2
1
0
5
10
15
20
Time (min)