THE EFFECT OF BIOACTIVE CONSTITUENTS ON PLANT OIL STABILITY

REEM ABUZAYTOUN

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The Effect of Bioactive Constituents on Plant Oil Stability

By

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ABSTRACT

Algal oils and oils rich in alpha-linolenic acid (ALA), and gamma-linolenic acid (GLA) have been used for the treatment of a wide range of nutritional and clinical disorders. The algal oils of interest were arachidonic acid single cell oil (ARASCO), docosahexaenoic acid single cell oil (DHASCO), and a single cell oil rich in docosahexaenoic acid (DHA) and docosapentaenoic acid (n-6 DPA) (OMEGA-GOLD oil). Flax oil contains ALA, and hemp oil is rich in both ALA and GLA. Little information is available about the oxidative stability of these nutritional oils and their bioactive minor constituents. Therefore, this research was initiated to evaluate the oxidative stability of flax, hemp, ARASCO, DHASCO, and OMEGA-GOLD oil, 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 these oils. Oil extracts were also investigated for their radical scavenging activity towards the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by a spectrophotometric method and measuring their total phenolic contents.

The results indicate that bioactive constituents of these nutritional oils play a major role in their oxidative stability in the dark as well as in the light. However, the fatty acid composition of the oil and the total tocopherols as well as the type of pigments present contribute to their stability. It was found that non-stripped flax and hemp were more stable than their corresponding stripped counterparts. However, non-stripped hemp oil had a higher oxidative stability than non-stripped flax oil as indicated in radical scavenging activity towards DPPH radical and total phenolic contents. On the other hand,

DHASCO contains a significant level of natural radical scavengers and phenolic compounds that contribute to its higher stability compared to ARASCO and the OMEGA-GOLD oil. The results demonstrate the importance of minor oil components on their oxidative stability, which will reflect on their food and supplement value as well as shelf life.

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LIST OF ABBREVIATIONS

AOCS	American Oil Chemists' Society
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CD	Conjugated dienes
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EPUFA	Essential polyunsaturated fatty acids
FAME	Fatty acid methyl esters
FID	Flame ionization detection
FO	Flax oil
GAE	Gallic acid equivalent
GLA	Gamma-linolenic acid
HO	Hemp oil
HPLC	High performance liquid chromatography
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
MA	Malonaldehyde
NFO	Non-stripped flax oil
NHO	Non-stripped hemp oil
N-ARASCO	Non-stripped ARASCO
N-DHASCO	Non-stripped DHASCO
N-OMEGA-GOLD oil	Non-stripped OMEGA-GOLD oil
OG	OMEGA-GOLD oil

OS	Oxidative stability
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PL	Phospholipids
PS	Phosphatidylserine
PV	Peroxide value
SF	Stripped flax
SH	Stripped hemp
S-ARASCO	Stripped ARASCO
S-DHASCO	Stripped DHASCO
S-OMEGA-GOLD oil	Stripped OMEGA-GOLD oil
TAG	Triacylglycerol
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert-Butylhydroquinone
UV	Ultraviolet

CHAPTER 1
INTRODUCTION

It is well recognized that dietary factors influence human health and life quality. Cold-pressed seed oils, including those of flax and hemp oils, as well as algal oils such as arachidonic acid single-cell oil (ARASCO), docosahexaenoic acid single-cell oil (DHASCO), and a single cell oil rich in docosahexaenoic acid (DHA) and docosapentaenoic acid (n-6 DPA) (OMEGA-GOLD oil) are commercially available. The oils from seeds of flax (*Linum usitatissimum L.,*), and hemp (*Cannabis sativa*) are rich in α- linolenic acid (ALA) and linoleic acid (LA). Moreover, hemp oil has a reasonable amount of GLA. Meanwhile, among commercially available algal oils ARASCO, DHASCO, and OMEGA-GOLD oil are highly concentrated with long-chain omega-3 and omega-6 fatty acids, such as DHA, DPA and arachidonic acid (AA). Findings on possible beneficial health effects of polyunsaturated fatty acids (PUFAs) such as ALA, GLA, DHA and EPA have led to major commercial developments of these essential polyunsaturated fatty acids for use in a variety of products for human consumption, animal feed and cosmetics (Syed Rahmatullah *et al.*, 1994; Bhatty, 1995; Arterburn *et al.*, 2000b).

The stability of oils depends on various factors, but mainly on the oil's fatty acid composition, the content of natural antioxidants, presence of oxygen as well as different storage and packaging conditions (Wagner and Elmadfa, 2000).

Edible oils consist mainly of triacylglycerols (TAG, 95%). Non-triacylglecerols (also known as minor components or unsaponifiable matter) make up the remaining 5%.

These minor components are naturally-occurring compounds with antioxidative properties that give these oils the ability to protect themselves and play an important role in the oxidative stability of vegetable oils (Shahidi and Shukla, 1996; Espin *et al.,* 2000). The minor components of vegetable oils are primarily composed of phospholipids, tocopherols, tocotrienols, flavonoids such as flavones and other phenolic compounds, pigments (carotenoids, chlorophylls), sterols, and free fatty acids, as well as mono- and diacylglycerols (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).

Several chemical, instrumental and sensory techniques are commonly used to monitor oxidation of foods and predict their shelf life stability. These techniques can be used to evaluate the effectiveness of antioxidants in different lipid systems (King *et al.*, 1995). While sensory methods are most accurate in predicting the stability of lipids, they are cumbersome and do not lend themselves to routine analysis (Wanasundara *et al.,* 1995).

Peroxide value (PV) and conjugated dienes (CD) are frequently used to measure primary oxidation products, lipid hydroperoxides. Meanwhile, thiobarbituric acid-reactive substances (TBARS) and headspace volatiles are employed as indicators for monitoring secondary products of oxidation such as aldehydes and other carbonyl compounds (Wong, 1989; Shahidi and Wanasundara, 1998).

Cold-pressed and algal oils contain beneficial components such as natural anioxidants. Antioxidants are well recognized for their potential in health promotion and

prevention of aging-associated diseases, including cancer and heart disease (Yu *et al.*, 2002a; Yu *et al.*, 2002b). Several chemical mechanisms have been proposed to explain the beneficial effects of antioxidants. These mechanisms include direct reaction of antioxidant for quenching of free radicals, forming chelation complexes with transition metals, reduction of peroxides, and stimulation of antioxidative defense enzymes (Parker *et al.*, 2003).

Little is known about the oxidative stability (OS) of flax, hemp and algal oils, and virtually nothing is known about the OS of these oils when stripped of their minor components. Hypotheses considered were: (1) although it is generally accepted that the OS of edible and algal oils is dictated by their degree of unsaturation, minor components might play an important role in their stability characteristics and (2) the higher the OS of these oils, the higher is the level of natural free radical scavengers and phenolic compounds in them.

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 non-stripped and stripped oils that are rich in ALA, such as flax and hemp oils, and algal oils that are rich in DHA and EPA, such as ARASCO, DHASCO, and OMEGA-GOLD oil, (2) determine some of the minor components in non-stripped and stripped oils such as pigments (carotenoids and chlorophylls), and antioxidants (tocopherols and phenolics) and (3) evaluate the free radical scavenging properties of extracts from oils tested.

CHAPTER 2
LITERATURE REVIEW

2.1 Nutritional and medicinal oils

A large variety of vegetable and algal oils are used in food and for medicinal purposes (Hammond, 1995; Arterburn *et al.*, 2000b). Vegetable, marine and algal oils are frequently used to correct nutritional deficiencies and treat different clinical disorders (Tenwolde *et al.,* 1997; Wang *et al.*, 1997; Arterburn *et al.*, 2000b). The effects of dietary oils rich in lauric, myristic, 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, 1996). Dietary supplementation for six weeks with 10 g per day of fish oil led to a significant improvement in schizophrenic symptoms. For patients requiring a special diet, information on the fatty acid composition of food is useful. For example, γ-linolenic acid (GLA) is an essential fatty acid for humans with Δ-6 desaturase deficiency (Goffman and Galetti, 2001).

Over the last two decades, understanding of the role and nutritional importance of PUFAs in vegetable and algal oils has improved. Therefore, this section will focus on flax and hemp oils as sources of different PUFAs, the latter containing (GLA), while both oils contain alpha-linolenic acid (ALA), and linoleic acid (LA). The focus is also on ARASCO, DHASCO and OMEGA-GOLD oil as algal oils that are rich in long-chain

fatty acids, particularly docosahexaenoic acid (DHA) as well as docosapentaenoic acid (n-6 DPA) in the latter sample.

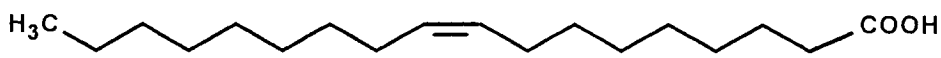
2.1.1 Essential polyunsaturated fatty acids (EPUFAs)

EPUFAs are nutrients that must be provided through the diet because they cannot be synthesized in the human body (Deferne and Pat, 1996). These EPUFAs serve as raw materials for structural lipids and as precursors of biochemicals which regulate many body functions (Riemersma, 2001).

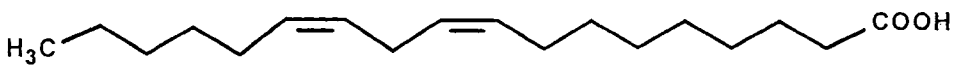
Polyunsaturated fatty acids (PUFAs) contain two or more double bonds (Figure 2.1). They are biosynthesized via extension of the saturated fatty acid pathway, in which stearate is converted to oleate (18:1n-9) and then linoleate, which is the central precursor for the n-6 and n-3 series (Figure 2.2; Gill and Rao, 1997; Horrobin, 1992).

The elongase and desaturase enzymes required for the *de novo* production of PUFA are present in 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 Δ4- desaturase, and thus can not synthesize linoleic acid (LA) and alpha-linolenic acids (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 Δ6-desaturase can be decreased or blocked in humans by several factors, such as stress,

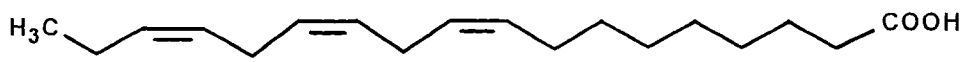
Figure 2.1 Chemical structure of oleic, linoleic, α-linolenic, and γ-linolenic acids.



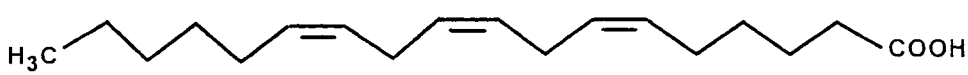
Oleic acid (18:1 n-9)



Linoleic acid (18:2 n-6)

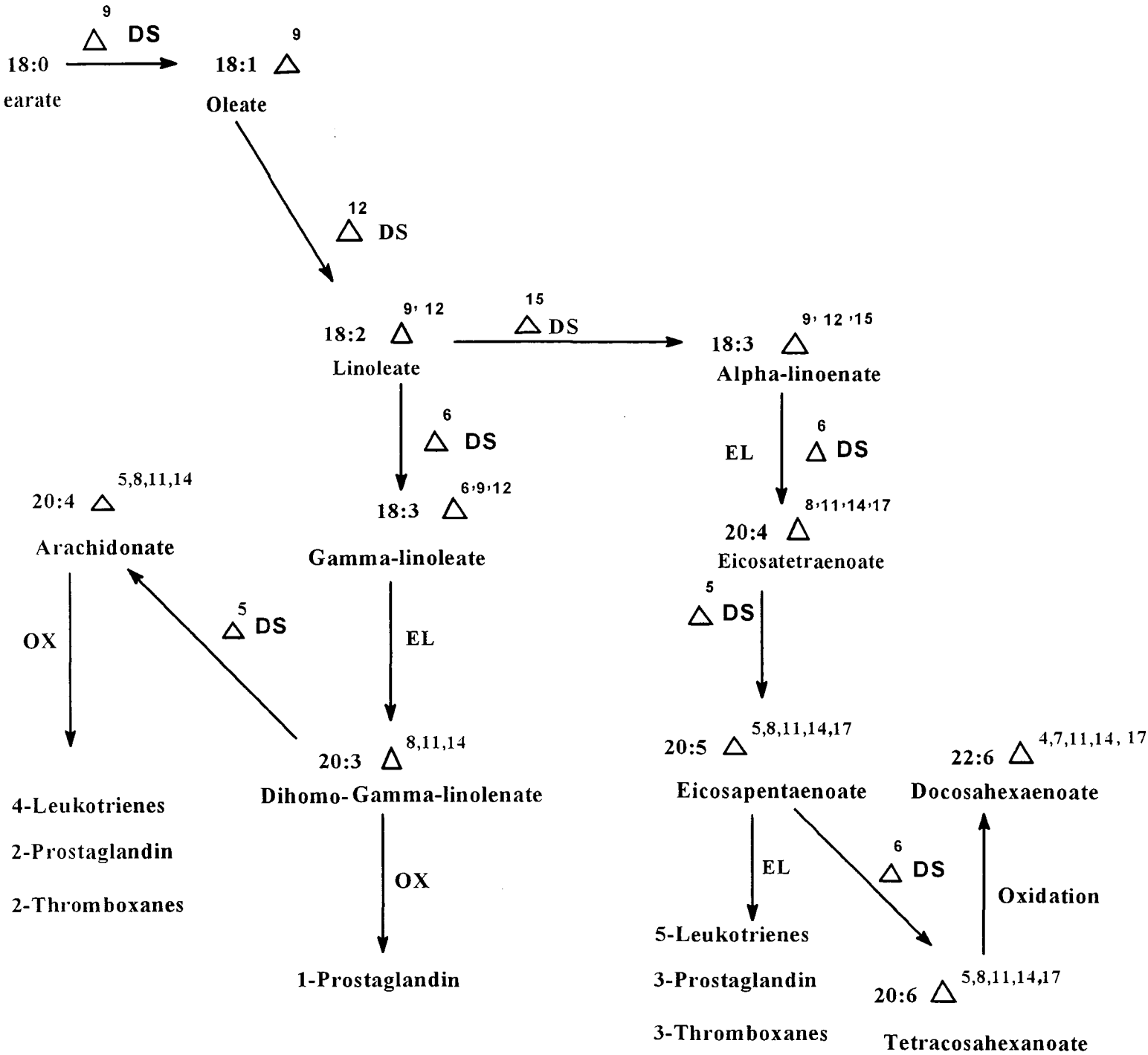


α-Linolenic acid (18:3 n-3)



γ -Linolenic acid (18:3 n-6)

Figure 2.2 Biosynthesis and interconversion of polyunsaturated fatty acids.



Abrreviation are: DS, desaturase; EL, elongase; and OX, oxidase enzymes

diabetes, obesity and aging (Haro and Rio, 1998). Supplementation of the diet with GLA and ALA or vegetable oils rich in them, as well as DHA and EPA, has reversed and corrected the health disorders related to the deficiency of the essential fatty acids and prostaglandins. Table 2.1 summarizes some applications of essential fatty acids.

2.2 Oilseeds

Oilseeds, such as flax, hemp, borage, evening primrose, sunflower, and soybean, are considered to be rich sources of essential fatty acids, phenolics and other natural antioxidants. Soybean is a good source of antioxidant compounds (Schuler, 1990). Canola oil contains considerable amounts of phenolic compounds compared with other oilseeds (Krygier *et al.,* 1982). Oilseeds are commercially available for human consumption as rich sources of essential fatty acids such ALA and GLA (Syed Rahmatullah *et al.*, 1994).

2.2.1 Flax oil (*Linum usitatissimum L.)*

Flax oil is produced by cracking the seeds, flaking them between rollers, and pressing them using expellers. The oil is cold-pressed under conditions that limit the maximum temperature during processing to 35°C. The oil produced is preserved in opaque bottles.

The oil is low in saturated fatty acids (approximately 9%), contains moderate amounts of monounsaturated fatty acids (approximately 18%), and is high in polyunsaturated fatty acids (approximately 73%). Almost 16% of PUFA in flax oil

Table 2.1 Nutritional and clinical applications of gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA).

Material	Application	References
GLA	management of impaired nerve function in diabetes treatment of rheumatoid arthritis anti-infective activity	Horrobin, 1997 Zurier *et al.*, 1996 Giamarellos-Bourboulis *et al.*, 1995
ALA	reduction in the risk of non-fatal coronary heart disease	Ascherio *et al.*, 1996
DHA	in human development and functioning including normal brain function and retina development Prevention of coronary artery disease	Holub, 2001 Cornor and Cornor, 1997
EPA,DHA	in cardiovascular disease, immune and renal disorders, allergies, diabetes, inflammation, and cancer	Haumann, 1997; Illingworth and Ullmann, 1990

belong to the omega-6 family, primarily LA, whereas the remaining 57% belong to the omega-3 family, primarily ALA.

Flaxseed oil contains three times more omega-3 fatty acids than omega-6 fatty acids; the omega-6 to omega-3 ratio of flaxseed oil is therefore 0.3:1 (Bhatty, 1995). When compared to other common plant oils, corn oil has an omega-6 to omega-3 ratio of 58:1, soybean oil 7:1, and canola oil 2:1 (Vaisey-Genser and Morris, 1997).

Flaxseed oil clearly provides all the EFAs, is healthy and has an optimal omega-6 to omega-3 ratio; hence it is considered to be a good source of omega-3 fatty acids (ALA) with a lower level of LA. It is recommended to consume 2ml (½ tea spoon) of oil on a daily basis. Using flax oil in frying is not recommended, since once the oil is extracted from the seed, the polyunsaturated fatty acids might undergo thermal oxidation when exposed to the high temperatures used in food frying (Wanasundara and Shahidi, 1998).

2.2.2 Hempseed oil (*Cannabis sativa*)

Hempseed oil is prepared by crushing the seeds of the hemp plant (*Cannabis sativa*). The oil is green or golden in colour, and comprises 35% of the total seed weight. Hempseed oil has up to 8% saturated fatty acids, and up to 80% unsaturated essential fatty acids. Hempseed oil contains about 55% omega-6 fatty acids (gamma-linolenic acid and linoleic acid), and 25% omega-3 fatty acids (alpha-linolenic acid). Only flax oil has more alpha-linolenic acid (58%), but hemp seed oil has the highest total essential fatty acids. The ratio of essential FA's required by the human body of omega-6 to omega-3 ratio is about 3:1 which makes this oil one of the optimal healthy oils that might be used

as a regular part of diet in products, such as dips, salad dressing and mayonnaise. Some prefer to consume this oil on a daily basis by taking a tea spoon of it as a nutritional supplement.

2.3 Algal oils

Algal oils, namely ARASCO, DHASCO and OMEGA-GOLD oil, provide a good source of long-chain fatty acids that serve as fuel and energy for the body. These oils also provide essential fatty acids such as AA, DHA, EPA and DPA.

2.3.1 Arachidonic acid single cell oil (ARASCO)

Arachidonic acid single-cell oil (ARASCO) is a concentrated source of arachidonic acid (40-50%) and is produced by the microfungus *Mortierella alpina* via fermentation technology. ARASCO contains small levels of other polyunsaturated fatty acids. Oleic acid (14.0 %) and linoleic acid (7.1%) are other major fatty acids present in ARASCO. Furthermore, the oil also contains saturated fatty acids such as stearic (10.5 %) and palmitic (7.8 %) acids (Arterburn *et al.*, 2000b).

2.3.2 Docosahexaenoic acid single-cell oil (DHASCO)

Fish and fish oils offer a good source of DHA. However, fish reserves are limited and intensive aquaculture practices are producing fish with lesser amounts of DHA. DHASCO oil from microalgae has recently been produced by a single cell alga *Crypthecodinium cohnii* (a member of the Dinophyta, a phylum of unicellular eucaryotic

microalgae); which grows under strictly controlled fermentation conditions. DHASCO is the first concentrated, well-defined, vegetarian source of DHA for infant formulas and food fortification. DHASCO contains 40-50 % DHA, but no EPA or other long-chain polyunsaturated fatty acids (Arterburn *et al.*, 2000b). DHASCO is a yellow-orange oil with > 95 % triacylglycerols with some diacylglycerols and unsaponifiable matter (about 1.5 % by weight). Myher *et al.* (1996) reported that about 45% of the DHA found in DHASCO was located in the sn-2 position. Because positional distribution of DHA in DHASCO triacylglycerols is almost the same as that of human milk, DHA digestion and absorption from DHASCO should be the same as that from human milk.

2.3.2.1 Safety studies of DHASCO

DHASCO is non-toxic and of much interest for use in infant formulas. Over the last ten years, many safety investigations have been carried out and these have included *in vitro* assays of mutagenicity and genotoxicity, traditional rat studies, including acute, subchronic, development, and multigenerational reproductive studies (Kyle, 2001). Arterburn *et al.* (2000a) reported that DHASCO and ARASCO did not have any genotoxic potential in three different *in vitro* mutagenesis assays (Ames reverse mutation, mouse lymphoma TK $^{+/-}$ forward mutation, and chromosomal aberration assays). In another study, Arterburn *et al.* (2000b) showed that DHASCO and ARASCO did not have any unfavourable developmental effects on pregnant rats. When DHASCO and ARASCO were administered orally at a dose of up to 1.25 and 2.5 g /kg body weight,

respectively, both oils did not result in any fatal malformation such as soft tissues and skeletal variations.

To further establish the safety of DHASCO, a 90-day subchronic investigation was conducted in pregnant rats using 0.5 and 1.25 g/kg body weight/day of DHASCO. The following parameters were investigated: haematology, clinical chemistry, pathology, and neurotoxicity. There were no unfavourable impacts on all parameters examined upon the administration of the test material. DHASCO did not lead to any mutation in developing fetus at doses that represented a 100-fold safety factor higher than the expected use levels (Aretburn *et al.*, 2000c).

2.3.2.2 Clinical studies using DHASCO

Many studies on feeding of DHASCO supplemented formulas to infants for various periods of time have been reported. All of these studies showed that DHASCO-supplemented formulas increased infants DHA status compared to that of breast-fed infants (Zeller *et al.*, 2001). Two studies using DHASCO-supplemented formulas, which also include ARASCO, reported considerable improvements in the growth rates of the babies fed the supplemented formulas compared to their regular counterparts (Carlson *et al.*, 1999). When infants were fed DHASCO-supplemented formulas, there was a significant improvement in their visual and mental acuity (Birch *et al.*, 1998).

2.3.3 Single-cell oil rich in DHA and n-6 DPA (OMEGA-GOLD oil)

OMEGA-GOLD oil is a commercial oil produced by the microalgae *Schizochytrium sp.* via a fermentation process. OMEGA-GOLD oil is a rich source of

DHA (41 %) and DPA (docosapentaenoic acid, the omega-6 isomer), which accounts for 18 % of the crude oil fraction. Myristic acid (9%) and palmitic acid (22%) are the other major fatty acids in OMEGA-GOLD crude oil fraction. The crude oil from algae *Schizochytrium sp* comprises triacylglycerols (90-92%), polar lipids (5%), minor amounts of sterol esters (0.4 %), diacylglycerols (1%), free sterols (1%) and free fatty acids (0.1%) (Zeller *et al.*, 2001).

2.4 Minor components of oils

Edible vegetable and algal oils consist mainly (95%) of triacylglycerols. Non-triacyglycerols (also known as minor components) make up the remaining 5%. The minor components of these oils, in general, are primarily composed of phospholipids, tocopherols, tocotrienols, flavonoids, other pigments (carotenoids, chlorophylls), sterols as well as free fatty acids and mono-and diacylglycerols (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).

2.4.1 Preparation of stripped oils

Several chromatographic techniques have been developed and used to remove (strip) different 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 and 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 oils. The column, used in our study was attached to a water pump and packed with activated silicic acid (top and bottom layers) and charcoal (middle layer). The column was conditioned with hexane and the oil was dissolved in an equal volume of hexane 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 (maximum 2 h). The solvent was then removed from the eluted oil using a rotary evaporator at 37°C; the product so obtained is defined as stripped oil. Khan and Shahidi (2001) employed this technique (using a mixture of charcoal and celite as well as sugar and celite in the middle layers) and successfully removed the minor components from borage and evening primrose oils. This technique was also successfully applied to remove minor components from soybean, virgin olive, sunflower and rapeseed as well as butter oils (Lampi *et al.*, 1992) and refined olive oil (Blekas, 1995).

Yoshida *et al.* (1993) used a column packed with aluminum oxide to prepare stripped rapeseed, soybean and palm oils. 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 be obtained. Meanwhile, stripped corn oil may be acquired from commercial sources.

2.4.2 Determination of minor components

The minor components in 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.

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 examine 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 chlorophylls and carotenoids, can be determined qualitatively by measuring the absorbance of the oils at 430-460 nm for carotenoids and 550-710nm for chlorophylls (Blekas *et al.*, 1995). Moreover, pigments can also be determined quantitatively according to Hall and Cuppett (1993).

The efficiency of the stripping procedure employed may also be checked by HPLC using an ultraviolet (UV)-visible detector (Neff *et al.*, 1993). The minor components and their maximum absorption wavelengths used for the purity checks in soybean oil were: chlorophylls (670 nm), carotenes and xanthophylls (436 nm), tocopherols (298 nm),

squalene (254 nm), TAG hydroperoxides (232 nm), phospholipids, sterols, mono- and diacylglycerols and free fatty acids (215 nm).

2.4.3 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 present in edible oils via proper processing steps. Alternatively, it is 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 has been used to compare the antioxidant and prooxidant properties of green tea (He and Shahidi, 1997). Meanwhile, stripped sunflower oil has been used to study the antioxidant properties of myricetin and quercetin (Roedig-Penman 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).

2.5 Lipid Oxidation

Oxidation of lipids is a common and undesirable chemical change that may impact flavour, aroma, nutritional quality and texture of the food, and may lead to the production of toxic compounds. 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 pigments 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 (Aruoma, 1998). Proper understanding of lipid oxidation mechanisms and measurement techniques as well as use of 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 acids. The susceptibility to oxidation depends largely on the degree of unsaturation; thus the rate of oxidation of linolenic acid (3 double bonds) is 25 times

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

Target organ	Clinical disorders
Brain	Parkinson's disease
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

higher than that of oleic acid and twice as fast as that of linoleic acid (Labuza, 1971). Autoxidation and photooxidation, two mechanisms responsible for oxidation of fats and oils will be addressed in this thesis.

2.5.1 Autoxidation

Autoxidation is a natural process that takes place between molecular oxygen and unsaturated fatty acids. 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, to produce a free radical $R^{\bullet}$. 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).

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

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

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).

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

$ROO^{\bullet} + RH \xrightarrow{\text{catalyst}} ROOH + R^{\bullet}$ (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 non-radical dimers, trimers and polymers (eq. 2.5 and 2.6) or by antioxidants which react competitively with alkyl free radicals and/ or peroxyl radicals and remove them from the system (King *et al.*, 1995).

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

$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$ (eq.2.6)

Hydroperoxides (or primary oxidation products) formed during autoxidation of oleate, linoleate and linolenate (Table 2.3) have been reported by Frankel (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

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/ ethane	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.

decomposition of these hydroperoxides. The decomposition of hydroperoxides occurs via homolytic cleavage of oxygen-oxygen bonds to yield a hydroxyl and an alkoxyl 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 (Frankel *et al.*, 1993a; 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).

2.5.2 Photooxidation

Photooxidation is the most common pathway of oxidation. It occurs in the presence of photosenitizers and visible light. Natural pigments in edible oils include chlorophylls, 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 peroxyl radicals and eventually hydroperoxides. This reaction is known as the "ene" reaction (Hall and Cuppet, 1993; Hamilton, 1994).

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

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

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, 1984). Thermal breakdown products of pure hydroperoxides from photosensitized oxidized methyl oleate, linoleate and linolenate are summarized in Table 2.4. It has been observed that the most abundant volatile produced

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

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	Me10-Oxo-8-decanoate	14
	12-OOH	18	Hexanal	17
	13-OOH	33	Pentanal	4.3
Linolenic	9-OOH	21	Me octanoate	15
	10-OOH	13	Me10-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.

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).

2.5.3 Impact of lipid oxidation on human health

Lipid oxidation reduces the nutritional value and shelf life of edible oils. Furthermore, primary and secondary oxidation products are known 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). There is no direct evidence that lipid hydroperoxides are involved in human disease, but animal studies involving lipid hydroperoxides or oxidized fats have shown the potential health risk of these compounds (Cortesi and Privett, 1972). It is unlikely, however, that the dietary hydroperoxides, as such, pose a health problem because they readily decompose in the presence of heat. The major potentially toxic compounds such as malonaldehyde and 4-hydroxynonenal are formed due to the decomposition of lipid hydroperoxides (Dahle *et al.*, 1962; Pryor *et al.*, 1976).

2.5.3.1 Effects of lipid oxidation on proteins

Lipid oxidation products can react with proteins and amino acids to cause damage in living tissues (Gardner, 1979). Andrews *et al.* (1965) showed that the reaction between autoxidizing linoleate and two different proteins, namely insulin and gelatin, in model systems resulted in modifications of the proteins. Chio and Tappel (1969) characterized compounds formed by the reaction of leucine, valine and glycine with malonaldehyde. They reported that two moles of amino acids react with one mole of malonaldahyde to form one mole of conjugated Schiff base.

2.5.3.2 Effects of lipid oxidation on DNA

The presence of malonaldehyde in foods and other biological systems has promoted investigation into its possible harmful effects in animals. Crawford *et al.* (1965) reported that malonaldehyde reacts with DNA through cross linking with the amino groups of guanine and cytosine bases of DNA molecules. Reiss *et al.* (1972) have reported that structural changes in DNA, caused by malonaldehyde, eventually leads to the loss of template activity of DNA. Malonaldehyde has been shown to cause mutagenesis in some strains of *Salmonella.* However, according to Marnett and Tuttle (1980), the mutagenic activity of earlier malonaldehyde preparations was generally due to contaminants formed during the generation of malonaldehyde from tetraethoxypropane. These authors have isolated two intermediates, namely β-alkoxyacrolein and 3,3-dialkoxypropionaldehyde, formed during the hydrolysis of tetraethoxypropane, and shown that they were more mutagenic than malonaldehyde itself.

2.5.3.3 Effects of lipid oxidation on low-density lipoprotein (LDL)

Atherosclerosis vascular disease is the leading cause of death among individuals with a western life style. Although an elevated plasma level of low-density lipoprotein (LDL) is clearly a major risk factor for cardiovascular disease, the mechanisms involved are unclear (Esterbaur, 1992). Several lines of evidence attribute oxidative damage to LDL as an atherogenic agent. One such important evidence is the immunohistochemical detection of malonaldehyde in human and animal atherosclerotic lesions (Esterbauer, 1992). Lipoprotein oxidation is unlikely to occur in plasma because of the presence of high concentrations of antioxidants and proteins that chelate metal ions (Henning and Chow, 1988), but may occur in an environment where antioxidants can become depleted and lipoproteins are exposed to oxidative stress.

2.5.4 Effects of lipid oxidation on food quality

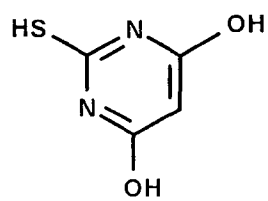
Several authors have reported the presence of various aldehydes, ketones, alcohols and hydrocarbons in oxidized meat (Buttery *et al.,* 1977; Hayashi *et al.,* 1986; Shahidi, 1989; Shahidi and Pegg, 1994; Tang *et al.,* 1983), milk (Chang *et al.,* 1966; Langler and Day, 1964; Shibamoto *et al.,* 1980), bulk oil (Krishnamurthy and Chang, 1967; Snyder *et al.,* 1985) and emulsions (Mick and Schreier, 1984). These secondary oxidation products might impart off flavours to these types of foods.

2.6 Methodology for assessing lipid oxidation

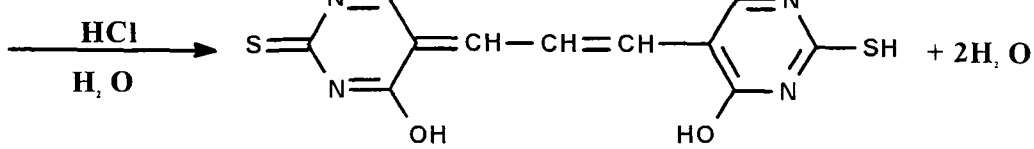
Lipid oxidation is a set of complicated catalytic processes. In order to determine the oxidative state and quality of edible oils, a number of stability tests are generally

employed. Methods reported in the literature include chemical, instrumental and sensory techniques that are used to monitor the oxidation of foods and thus predict their shelf life (Hawrysh, 1990). 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 for predicting the stability of lipids, but these are not used for routine analysis (Wanasundara *et al.*, 1995). Since hydroperoxides are the primary products of lipid oxidation, their content (i.e. peroxide value, PV) is often used as an indicator for the studies of initial stages of oxidation (Gray, 1978). However, hydroperoxides decompose rapidly during storage and heating. Therefore, PV may not necessarily be indicative of the actual extent of lipid oxidation (Sherwin, 1978). On the other hand, measurement of conjugated dienes (at 234nm) is a quick physical method which may also be employed to access the oxidative stability of oils (Lampi *et al.*, 1997). Secondary products are estimated by a classical method using the 2-thiobarbituric acid (TBA) test (Gary, 1978). This test measures the concentration of malonaldehyde and other TBA reactive substances (TBARS) as shown in Figure 2.3. Similarly, the volatiles, from the decomposition of hydroperoxides as well as those present before heating, collected in the headspace above the oil, can be analyzed using gas chromatography. Gas chromatographic analyses of edible oils have revealed that hexanal and pentanal are the major volatiles of oxidation of n-6 PUFA, while propanal is the predominant volatile derived from n-3 PUFA (Frankel, 1993a; Shahidi and Wanasundara 1998). Moreover, King *et al.* (1995) have reported that

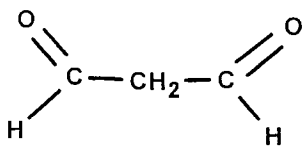
Figure 2.3 The formation of malonaldehyde-thiobarbituric acid chromogen.

2 

Thiobarbituric acid (TBA)

\+ 

TBA-MA complex



Malonaldehyde (MA)

an excellent correlation between GC data and sensory scores in photooxidized soybean oil, while pentanal and hexanal correlated well with flavour scores of autoxidized soybean oil. Table 2.5 summarizes some methods which are routinely used to monitor the oxidation of edible oils.

2.7 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 the 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, 1993b; 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).

2.8 Accelerated oxidation tests

In order to estimate the stability and susceptibility of fats and oils towards oxidation, and thus their keeping quality and shelf life, a number of accelerated oxidation tests are used, including the Schaal oven test, the Sylvester test and its automated form

Table 2.5 Recent applications of different 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+ Headspace	Evaluating the antioxidants properties of catechins and green tea extracts in sunflower oil-in-water emulsion	Roedig-Penman and Gordon, 1997

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

using Oxidograph equipment, and the Swift test and its automated forms using the Rancimat and Oxidative Stability Instrument (OSI) (Rossel, 1994). Heating is an effective and commonly used means of accelerating oxidation, although fluorescent light can also be used to study the role of sensitizers on the stability of edible oils (Frankel, 1984). The Schaal oven test (60-70°C), employed in this study, 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).

2.9 Controlling lipid oxidation

Food antioxidants can be classified into two broad categories, synthetic and natural. The oxidation of edible oils and fats can be controlled by proper application of these two categories 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 concentrations, absence of undesirable odour, flavour and colour, resistance to high temperatures 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 a 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, and 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.

2.9.1 Synthetic antioxidants

Phenolic antioxidants 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. 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, 1994).

BHA and BHT are highly soluble in animal fats and vegetable oils. However, they are more effective antioxidants for animal fats. Meanwhile, TBHQ 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)

2.9.2 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 provide protection or improve the health status of humans since many diseases are developed due to oxidation reactions in the body (Ramarathnam *et al.*, 1995; Wanasundara *et al.*, 1997; Ramadan *et al.*, 2003). This protection can be explained by the

capacity of these antioxidants to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids (Aruoma, 1998).

Most of the natural antioxidants are derived from plant sources (Table 2.6). 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 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, 1996). 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). 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.*, 1997). 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 reported (Nielsen *et al.*, 1996). Flavour deterioration of soybean oil

Table 2.6 Different lipid oxidation inhibitors found in plants and seeds.

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

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 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 *et al.*, 1997).

2.9.3 Tocopherols

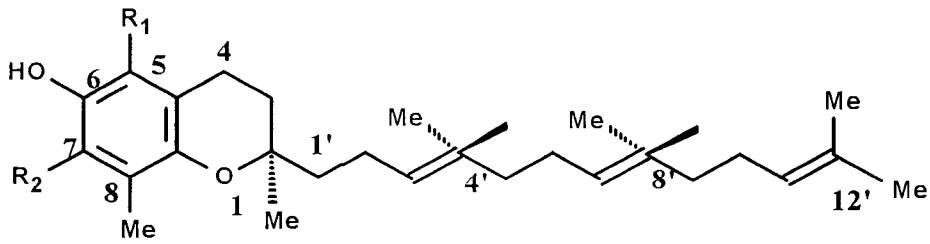
The main biochemical function of tocopherols is believed to be protection of PUFA against peroxidation. The correlations between fatty acid composition and tocopherol levels in 14 vegetable oils have been reported. Principal component and linear regression analyses showed positive correlation between linoleic acid and α-tocopherol as well as linolenic acid and γ-tocopherol (Kamal-Eldin and Andersson, 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 a phytyl tail (Kamal-Eldin and Appelqvist, 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

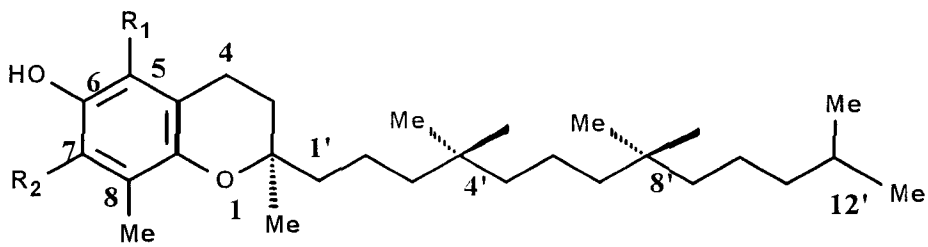
only in the number of methyl substituents and the pattern of substitution in the phenolic ring (Figure 2.4). The antioxidant activity of tocopherols is mainly due to their ability to donate phenolic hydrogens, similar to the previously described phenolic antioxidants, to lipid free radicals generated during free radical chain reactions (Yamauchi *et al.*, 1995). 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 proposed 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, 1990).

Tocopherols might act as antioxidants or prooxidants based on their concentration.

Figure 2.4 Chemical structures of tocotrienols and tocopherols.



Tocotrienols



Tocopherols

Tocopherol/ Tocotrienol	R_1	R_2
α	Me	Me
β	Me	H
γ	H	Me
δ	H	H

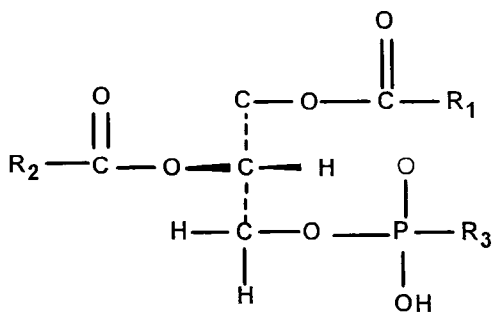
The effects of different concentrations (0, 100, 250 and 500 ppm) of various tocopherols (α, γ and δ) on the oxidative stability of stripped soybean oil have been reported (Jung and Min, 1990). Tocopherols above 500 ppm exhibited prooxidant activity. Similarly, Yoshida *et al.* (1993) have evaluated 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 and although α-tocopherol acted as an antioxidant at all levels, at 100 ppm it exhibited the best effect.

2.9.4 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 2.5) (Du Plessis and Pretorius, 1984; Wanasundara *et al.*, 1997; Imbs and Pham, 1995). 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 *et al.*, 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

Figure 2.5 Chemical structures of main phospholipid subclasses in edible oils.



R_3 =	**Phosphlipid subclasses**
$-OH$	**Phosphatidic acid (PA)**
$-O-CH_2-CH(NH_2)-COOH$	**Phosphatidylserine (PS)**
$-O-CH_2-CH_2-NH_2$	**Phosphatidylethanolamine (PE)**
$-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$	**Phosphatidylcholine (PC)**

fractions (PL) extracted from bluefish have been examined using a salmon oil model system (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) 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).

2.10 Extraction of minor components

A variety of techniques for the extraction of antioxidants from plant materials have been employed. The simplest procedure involves the heating of the fresh or dry plant parts in water followed by filtration or centrifugation. This procedure was used for green and black tea. Organic solvents such as methanol, ethanol, acetone, hexane, ethyl ether and diethyl sulphoxide as well as the aqueous solutions of polar solvents have been used for the extraction of antioxidants from different plant parts. Inatani *et al.* (1982), for example, have extracted rosemary antioxidants with hexane. Mizuno *et al.* (1987) have used methanol and hexane to extract phenolic compounds from seeds of *Coptis japonica*

var. *Dissecta* while Jaworski and Lee (1987) used absolute ethanol to extract phenolics from crushed grapes. Acetone and absolute ethanol were used to extract antioxidants from licorice roots (Gordon and An, 1995). Wanasundara *et al.* (1994) have extracted antioxidants of canola meal into 95% (v/v) aqueous ethanol and Shahidi *et al.* (1994) have used the same medium to extract antioxidants from low-pungency mustard flour. Aqueous methanol (75%, v/v) has been used to extract antioxidants from wild rice hulls (Asamarai *et al.,* 1996) and fruit and vegetable juices (Chambers *et al.,* 1996). Absolute methanol was used by Oomah *et al.* (1995) to extract phenolic acids from flaxseed. Both methanol and ether were employed to extract phenolic compounds from lipophilized avocado powder (Torel *et al.,* 1986). Extracts from borage using aqueous solutions of ethanol, methanol and acetone were investigated for their antioxidative capacity (Wettassinghe and Shahidi, 1999). Acetone was employed for extraction of antioxidants present in canola and rapeseed hulls (Amarowicz et al., 2000), and almond extract was prepared by ethanolic extraction and evaluated for their antioxidative capacity (Siriwardhana and Shahidi, 2002).

The extraction temperatures and time courses have been reported to be among the other important parameters involved in determination of the efficacy of extraction. Unfortunately, most authors have not provided a justification as to why a specific set of conditions has been employed. For example, Duh and Yen (1995) used methanol at room temperature for 12 h to extract antioxidants from irradiated peanuts hulls. Phenolics from barley and malt were extracted by Maillard *et al.* (1996) using methanol at room

temperature for 80 min. Extraction times as short as 20-30 min heve also been reported (Oomah *et al.*, 1995).

2.10.1 Measuring antioxidant activity

The tests used for measuring antioxidant capacity can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions (Ramadan, 2003). However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods (Schwarz *et al.*, 2000). The extracts prepared by using different solvents might be evaluated for their antioxidant activity. Free radical, hydroxyl radical, hydrogen peroxide and superoxide radical scavenging activity of the extracts can be evaluated.

The oxygen radical absorbance capacity (ORAC) method is a widely used method for assessing antioxidant capacity in biological samples and food. This method is based on the inhibition of the peroxyl radical-induced oxidation initiated by thermal decomposition of azo-compounds, such as 2,2-azobis(2-amidino-propane) dihydrochloride (AAPH). Recently, this method has been adapted to use fluorescent as the fluorescent probe (Prior *et al.*, 2003). However, scavenging of the stable radical 2,2-diphenyl-picrylhydrazyl (DPPH) radical assay is based on the measurement of scavenging ability of antioxidants towards the stable DPPH radical. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors (Contreras-Guzman and Strong, 1982). The DPPH radical assay is considered a valid and easy assay

to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical scavenging assays (Sanchez-Moreno *et al.,* 1998).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Cold-pressed borage oil was obtained from Bioriginal Food & Science Co. (Saskatoon, SK). Evening primrose oil was aquired from Scotia Pharmaceuticals (Kentvill, NS). Cold-pressed flax oil was obtained from Herbal Select (Guelph, ON). The cold-pressed hemp oil was obtained from Hemp Oil Canada Inc.(Ste. Agathe, MB). Algal oils, namely docosahexaenoic acid (DHA) single cell oil (DHASCO) containing 47.4% DHA and arachidonic acid (ARA) single-cell oil (ARASCO) containing 40-50% ARA were obtained from Martek Bioscience Corporation (Columbia, MD). OMEGA-GOLD oil is the commercial name for another oil from single-cell microalgae, containing 41% DHA and 18% docosapentaenoic acid (n-6 DPA), and was a product from Monsanto (St. Louis, MO). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, 2-thiobabituric acid (2-TBA), gallic acid (3,4,5-trihydroxybenzoic acid), silicic acid powder (mesh size:100-200, acid-wash), and α-tocopherol, were obtained from Sigma Chemical Co. (St. Louis, MO). Activated charcoal was acquired from BDH Inc. (Toronto, ON). Compressed air, hydrogen and ultra-high purity (UHP) helium were obtained from Canadian Liquid Air Ltd. (St. John's, NL). Hexane, methanol, sulphuric acid, isooctane and 1-butanol were purchased from Fisher Scientific Company (Nepean, ON).

3.2 Methods

3.2.1 Preparation of minor-component stripped oils

Flax, hemp; ARASCO, DHASCO, and OMEGA-GOLD oil were stripped of their minor components essentially according to the method of Khan and Shahidi (2001) with minor modifications. A chromatographic column (3.4 cm internal diameter x 40 cm height) was connected to aspirator vacuum pump and packed sequentially with two adsorbents. The first layer in the column consisted of 50 g of activated silicic acid, the second layer was 50g activated charcoal, and the top layer was another 50g activated silicic acid. All adsorbents were suspended in n-hexane. The silicic acid (100g) was first, before introduction to the solvent, activated as described by Min (1973) by washing 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 (50 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. The volume of oil recovered from 50 g was approximately 20 g. Stripped oils (stripped hemp oil; SHO, stripped flax oil; SFO, stripped OMEGA-GOLD oil; SO, stripped DHASCO; SD and stripped ARASCO; SA) were obtained and transferred into a 10 mL bottle, flushed with nitrogen and kept at -70°C for subsequent studies.

3.2.1.1 Preparation of samples for accelerated oxidation tests

Stripped and non-stripped oil samples (0.5 g in 2mL vials) were used to study their oxidative stability in the dark upon heating, and in the light (photooxidation). For accelerated oxidation at 60°C, the sample containers were placed in a forced air 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 Watts 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 samples were removed from the oven after 1,3,5, and 7 days, and from the light box after 4, 8, 12 and 24 hours, flushed with nitrogen, covered with parafilm and kept at -70°C for oxidative stability tests.

3.2.1.2 Oxidative stability tests

The oxidative stability of stripped and non-stripped oils was evaluated by determining conjugated dienes (CD), 2-thiobarbituric acid-reactive substances (TBARS) and headspace volatiles.

3.2.1.2.1 Determination of conjugated dienes (CD)

Conjugated dienes (CD) of the oil samples were determined by the method of IUPAC (1987). Oil samples (0.02-0.04 g) were weighed into 25 mL volumetric flasks, dissolved in isooctane (2,2,4-trimethylpentane) and made up to the mark 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 (Agilent, Palo Alto, CA). Pure isooctane was used as the blank. Conjugated dienes were calculated according to the following equation

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

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 in cm.

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

Oil samples (0.05 - 0.20 g) were analyzed for their contents of TBARS according to the AOCS (1990) method. The samples were accurately weighed into 25 mL volumetric flasks and dissolved in a small volume of 1-butanol and made up to the mark with the same solvent. Five millilitres of this mixture were transferred into a dry test tube, then 5 mL of fresh 2-thiobarbituric acid (TBA) reagent (200 mg TBA in 100 mL 1-butanol) were added to it. 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 0.415 determined from a standard line prepared using 1,1,3,3-tetramethoxypropane as precursor of malonaldehyde (Khan and Shahidi, 2001; He and Shahidi, 1997).

3.2.1.2.3 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 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 samples (0.2 g) were transferred into special glass vials which were capped with special butyl septa, crimped, and analyzed. Vials were 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, hexanal, and propanal was accomplished using 1% 2-heptanone (in stripped corn oil) as an internal standard. Formation of several volatiles (pentanal, hexanal, 2-heptanone) was monitored as a measure of the oxidation of the oil samples.

3.2.2 Preparation of antioxidant extract

A measured amount of oil sample (20g) was diluted with hexane (1:10, w/v), and extracted with methanol (10:2, v/v, hexane/methanol) three times at room temperature. The methanol extract was washed with hexane (1:1, v/v), and the methanol was completely removed under vacuum. The extract (2g) was re-dissolved in 10mL methanol, flushed with nitrogen and kept at -70°C for further analysis.

3.2.2.1 Evaluation of antioxidative activity of extracts

3.2.2.1.1 Determination of total phenolic content

The total phenolic content was determined according to the procedure explained by Singleton and Rossi (1965) with minor modifications. One millilitre of Folin-Ciocalteu reagent was added to 50 mL centrifuge tubes containing 1 mL of the extracts (0.2g/mL) prepared previously. Contents were mixed thoroughly and 1.5 mL of 20% sodium carbonate were added. The final volume was made up to 10 mL with distilled water and mixed thoroughly. After two hours of reaction at room temperature, the absorbance of the mixture was measured at 765 nm and used to calculate the phenolic contents using a standard curve prepared with gallic acid (Figure A.2). Total extracted phenolics were expressed as mg of gallic acid equivalent, which is a common reference compound, per mL extract.

3.2.2.1.2 DPPH radical scavenging activity

One millilitre of freshly prepared (DPPH radical) DPPH$^{\bullet}$ solution (0.125mM) was added to 1mL of the extract (0.2g/mL) prepared in the previous section (3.2.2), and

mixed well to start the radical-antioxidant reaction. The absorbance at 517nm was determined against a blank of pure methanol after 0, 2, 4, 6, 8, 10, 15 and 20 min of reaction and used to estimate the remaining radical levels according to the standard curve (Figure A.1). The reference antioxidant used was α- tocopherol. The inhibition percent was calculated according to Lee *et al.* (2002) using the following equation:

% inhibition = [(absorbance of control-absorbance of test sample)/absorbance of control] ×100

3.2.3 Chemical and instrumental analysis

3.2.3.1 Analysis of fatty acid composition

Fatty acid composition of the oils was determined according to the method described by Wanasundara and Shahidi (1997). Oil samples (5-10 mg) were weighed into 6 mL conical vials. Subsequently, 2 mL of freshly prepared 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 the volume up to the mark with spectral-grade methanol and adding 15 mg of hydroquinone as an antioxidant. The mixture of fatty acids and the reagent was incubated at 60°C for 17 h. At the end of the incubation period, vials were removed and 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. Few crystals of hydroquinone were added to each vial before the extraction step to prevent oxidation of unsaturated fatty acids. Hexane layers were separated, combined and transferred to a clean dried tube. The combined hexane extracts were washed two times with distilled water. In the first

wash, the aqueous layer was removed. In the second wash, the hexane layer was separated and evaporated under a stream of nitrogen at room temperature.

3.2.3.2 Analysis of fatty acid methyl esters by gas chromatography

Fatty acid methyl esters, extracted in the previous section, were analyzed using a Hewlett Packard 5890 II gas chromatograpgh (Agilent, Palo Alto, CA) equipped with a 30 m x 0.25 mm, 0.25μm film thickness; 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 (GLC-461; Nu-Check). Results were presented as weight percentage.

3.2.3.3 Measurement of pigments

Pigments present in the stripped and non-stripped oil samples were determined qualitatively by measuring: (1) the absorbance at 430 - 460 nm for carotenoids and 550-710 for chlorophylls and their derivatives (Blekas *et al.*, 1995). The oil sample was mixed with hexane (1:1 for borage and evening primrose oils; 1:12 for hemp oil; 1:5 for flax oil, 1:6 for DHASCO, and 1:3 for ARASCO and OMEGA-GOLD oil) and transferred into Hellma glass cells and the absorbance was read using Specronic Genesys™ 2 and

Genesys™ 5 spectrophotometers and (2) by recording the absorption spectra between 430 and 710 nm.

3.2.3.4 Determination of tocopherols by high performance liquid chromatography

For γ- and δ- tocopherol analysis, a Shimadzu high performance liquid chromatograph (HPLC) system equipped with two LC-10AD pumps, a SPD-M10A diode array detector and a SCL AA system controller was employed. The conditions of separation were as follows: Pre-packed LUNA SILICA (2) column, Phenomenex (25cm, 4.6mm in diameter, 5μm particles) (Aschaffenburg, Germany); mobile phase 4% dioxane in hexane, flow rate 1.5 mL/ min, injection volume 20mL and the detector was set at 295 nm. A Shimadzu (HPLC) system was used for α-tocopherol analysis (LC 10AD pumps, RF-535 fluorescence detector, C-R4A Chromatopac). The conditions of separation were as follows: pre-packed LUNA SILICA (2) column, Phenomenex (25cm, 4.6mm in diameter, 5μm particles) (Aschaffenburg, Germany); mobile phase 0.5% isopropanol in hexane, flow rate 1mL/min, injection volume 20mL, and the detector was set at Ex 290nm, Em 330 nm .

One gram of stripped and non-stripped oil sample was dissolved in 10 mL of mobile phase, passed through o.45m filter and injected on the HPLC column (Holfman-La Roche Ltd., Basle, Switzerland).

3.2.4 Statistical analysis

All the experiments were performed in triplicate and the results reported as means ± standard deviation. Normality was examined using Sigmastat. Analysis of variance and Tukey's standardized test were performed at a level of $p<0.05$ to assess the significance of differences among mean values.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Analysis of non-stripped and stripped oil samples

4.1.1 Chemical characteristics of non-stripped and stripped flax, hemp, ARASCO, DHASCO, and OMEGA-GOLD oil

The chemical characteristics of non-stripped and stripped flax and hemp oils are summarized in Table 4.1. Data provided indicate that the original non-stripped hemp oil (NHO) contained more ($p<0.05$) primary oxidation products than the original non-stripped flax oil (NFO). Similarly, the original samples of non-stripped flax and hemp oils contained more ($p<0.05$) primary and secondary products than their corresponding stripped counterparts, namely stripped flax oil (SFO) and stripped hemp oil (SHO), respectively. Table 4.2 summarizes the chemical characteristics of non-stripped and stripped ARASCO, DHASCO and OMEGA-GOLD oil. The original samples of non-stripped ARASCO (N-ARASCO), non-stripped DHASCO (N-DHASCO) and non-stripped OMEGA-GOLD oil (N-OMEGA-GOLD oil) contained higher ($p<0.05$) amounts of primary and secondary oxidation products than their corresponding stripped ARASCO (S-ARASCO), stripped DHASCO (S-DHASCO) and stripped OMEGA-GOLD oil (S-OMEGA-GOLD oil). Similar results were obtained by Khan and Shahidi (2001) for borage and evening primrose oils. They found that stripped borage and evening primrose oils had good oxidative status compared to their non-stripped counterparts.

The main tocopherols, as determined by high performance liquid chromatography (HPLC), in NFO were α-tocopherol (40 ppm) and γ- tocopherol (800 ppm); no δ-

Table 4.1 Chemical characteristics of non- stripped and stripped flax and hemp oils

Characteristics		NFO	SFO	NHO	SHO
Oxidative status	Conjugated dienes	1.65 ± 0.00^{c}	1.09 ± 0.05^{ab}	1.95 ± 0.15^{d}	1.09 ± 0.02^{ba}
	TBARS (μmol/g)	6.01 ± 0.04^{cd}	4.54 ± 0.11^{b}	6.14 ± 0.05^{dc}	3.02 ± 0.04^{a}
Tocopherols	α	40^{a}	0	0	0
(mg/kg)	γ	800^{a}	0	900^{b}	0
	δ	0	0	70^{a}	0
	Total	840^{a}	0	970^{b}	0
Pigments*	430nm	0.94 ± 0.00^{c}	0.024 ± 0.00^{b}	0.67 ± 0.00^{d}	0.01 ± 0.00^{a}
(Absorbance)	460nm	0.81 ± 0.00^{d}	0^{ba}	0.35 ± 0.00^{c}	0^{ab}
	550nm	0.14 ± 0.00^{d}	0^{ba}	0.06 ± 0.00^{c}	0^{ab}
	620nm	0.11 ± 0.00^{d}	0^{ba}	0.07 ± 0.00^{c}	0^{ab}
	670nm	0.11 ± 0.00^{c}	0^{ab}	0.37 ± 0.01^{d}	0^{ab}

Values are means of three determinations ± standard deviations. Values with different superscripts in each row are different ($p< 0.05$) from one another. Abbreviations are: NFO, non-stripped flax oil; NHO, non-stripped hemp oil; SFO, stripped flax oil; SHO, stripped hemp oil.

*The ratio (oil: hexane (v:v) was (1:5) for flax oil and (1:12) for hemp oil.

*Absorbance between 430-460nm indicates presence of carotenoids, and between 550-710nm indicates presence of chlorophylls.

Table 4.2 Chemical characteristics of non- stripped and stripped ARASCO, DHASCO and OMEGA- GOLD oil.

Characteristics		N-ARASCO	S-ARASCO	N-DHASCO	S-DHASCO	N-OMEGA-GOLD	S-OMEGA-GOLD
Oxidative status	Conjugated dienes	3.87 ± 0.07^{b}	1.67 ± 0.25^{a}	10.27 ± 0.19^{ef}	8.83 ± 0.02^{d}	10.03 ± 0.11^{ef}	6.10 ± 0.06^{c}
	TBARS (μmol/g)	8.53 ± 0.06^{cb}	5.27 ± 0.02^{a}	13.88 ± 0.44^{e}	11.44 ± 0.31^{d}	14.05 ± 0.02^{f}	8.01 ± 0.07^{bc}
Tocopherols (mg/kg)	α	100^{b}	0	250^{c}	0	30^{a}	0
	γ	660^{b}	0	810^{c}	0	650^{a}	0
	δ	30^{a}	0	80^{c}	0	50^{b}	0
	Total	790^{b}	0	1140^{c}	0	730^{a}	0
Pigments* (Absorbance)	430nm	0.11 ± 0.00^{c}	0.02 ± 0.00^{b}	0.7 ± 0.00^{f}	0.38 ± 0.00^{e}	0.15 ± 0.01^{d}	0^{a}
	460nm	0.06 ± 0.00^{d}	0.01 ± 0.00^{ba}	0.4 ± 0.00^{c}	0.05 ± 0.00^{c}	0.1 ± 0.00^{e}	0.01 ± 0.00^{ab}
	550nm	0.01 ± 0.00^{cde}	0.01 ± 0.00^{dce}	0.01 ± 0.00^{ecd}	0^{ab}	0.03 ± 0.01^{f}	0^{ba}
	620nm	0.01 ± 0.00^{de}	0.01 ± 0.00^{ed}	0^{abc}	0^{bac}	0.03 ± 0.00^{f}	0^{cab}
	670nm	0.01 ± 0.00^{e}	0^{a}	0^{abcd}	0^{abcd}	0.03 ± 0.00^{f}	0^{abcd}

Values are means of three determinations ± standard deviations. Values with different superscripts in each row are different ($p< 0.05$) from one another. Abbreviations are: N-ARASCO, non-stripped ARASCO; N-DHASCO, non-stripped DHASCO; N-OMEGA-GOLD , non-stripped OMEGA-GOLD oil; S-ARASCO, stripped ARASCO; S-DHASCO, stripped DHASCO ; S-OMEGA-GOLD, stripped OMEGA- GOLD oil.

*The ratio (oil: hexane (v: v) was (1:3) for ARASCO and OMEGA-GOLD and (1:6) for DHASCO.

*Absorbance between 430-460nm indicates presence of carotenoids, and between 550-710nm indicates presence of chlorophylls.

tocopherol was detected. However, NHO contained 900 ppm of γ-, and 70 ppm of δ-tocopherols as shown in Table 4.1. Thus, NHO had higher amounts ($p<0.05$) of total tocopherols than NFO which might contribute to its oxidative stability. According to previously published data, flax oil may contain 82 ppm of α-tocopherol, and 589 ppm of γ-tocopherols (Wagner and Elmadfa, 2000). Furthermore, flax oil may contain δ-tocopherol in addition to α- and γ- tocopherols (Wagner and Elmadfa, 2000). Meanwhile, hemp oil has been reported to contain only 17.3 ppm of α-tocopherol, 228.1 ppm of γ- tocopherol and 8.1ppm of δ-tocopherol (Kriese *et al.,* 2004). Variation of tocopherols within the same type of vegetable oil may be attributed to the different analytical procedures employed as well as existing genetic differences within the same plant biotype and/or storage conditions (Brandle *et al.*, 1993). Tocopherols were not detected in SFO and SHO.

Referring to the data in Table 4.2, non-stripped DHASCO had the highest ($p<0.05$) total tocopherols compared to non-stripped ARASCO and non-stripped OMEGA-GOLD oil; high amounts of tocopherols might contribute to the superior stability of DHASCO. Two hundred fifty parts per millions of α-tocopherol, 810 ppm of γ-tocopherol, and 80 ppm of δ-tocopherol were detected in N-DHASCO, and 100 ppm of α-tocopherol, 660 ppm of γ- tocopherol and 30 ppm of δ-tocopherol were present in ARASCO. Meanwhile, OMEGA-GOLD oil contained 30 ppm of α-tocopherol, 650 ppm of γ- tocopherol and 50 ppm δ- tocopherol . Similarly, S-ARASCO, S-DHASCO, and S-OMEGA-GOLD oil had

no tocopherols, thus confirming that the stripping procedure was effective in removing tocopherols from all oil samples examined.

Pigments such as carotenoids, with absorbance between 430 and 460 nm (Blekas *et al.*, 1995) were present in high amounts in NFO and NHO (Table4.1). Meanwhile, chlorophylls, which absorb light between 550 and 710 nm (Blekas *et al.*, 1995), were present at a significantly ($p<0.05$) higher quantity in NHO than NFO (Table 4.1). On the other hand, NFO and NHO contained more ($p<0.05$) carotenoids and chlorophylls compared to their stripped counterparts (SFO) and (SHO), respectively. SFO and SHO retained traces of carotenoids (Table 4.1) which might interfere with their stability under light. On the other hand, the content of carotenoids in non-stripped DHASCO (N-DHASCO) was significantly ($p<0.05$) higher than those present in N-ARASCO and N-OMEGA-GOLD oil (Table 4.2), however, chlorophylls existed in higher levels in N-OMEGA-GOLD oil than N-ARASCO. No chlorophylls were found in N-DHASCO. By comparing pigments found in N-ARASCO, N-DHASCO and N-OMEGA-GOLD oil with their counterparts S-ARASCO, S-DHASCO and S-OMEGA-GOLD oil, it is clear that non-stripped oils had more ($p<0.05$) pigments than their stripped counterparts (Table 4.2) as indicated by the absorbance of pigments at different wavelengths. For example, the absorbance at 430nm for N-ARASCO was 0.11 which was higher $p<0.05$ than that of S-ARASCO (0.02). Similarly, the absorbance at 430nm for N-DHASCO was 0.7 which was also higher ($p<0.05$) than that of S-DHASCO (0.37). Meanwhile, stripping OMEGA-GOLD oil from its carotenoids was more effective compared to the stripping of the other

two algal oils (ARASCO and DHASCO) as indicated by no detectable absorbance for S-OMEGA-GOLD oil compared to 0.15 for N-OMEGA-GOLD oil at 430nm.

NHO was stripped from its minor components more effectively than NFO by using a modified multi-layer column chromatographic technique developed by Lampi *et al.* (1992). This procedure required only 2 hours to strip 50 g of flax and hemp oils. Less conjugated dienes and pigments were detected in stripped hemp oil (SHO) compared to its non-stripped counterpart (NHO). However, more secondary oxidation products were retained or produced in the stripped flax oil (SFO) compared to its non-stripped counterpart (NFO). This might be due to oxidation during the stripping process. On the other hand, this technique was more effective for stripping OMEGA-GOLD oil compared to ARASCO, but it was not effective enough in stripping DHASCO from all of its pigments. It is clear that the chlorophylls were more easily removed from the oils examined by silicic acid and charcoal than carotenoids. This might be related to differences in the chemical structures of chlorophylls and carotenoids that enables chlorophylls to be adsorbed by the stationary phase to a greater degree than carotenoids.

The precise mechanisms of interaction as well as adsorption of minor components of vegetable oils on the multi-layer adsorbents employed in this study are not fully understood. Nonetheless, silicic acid has been used to fractionate the minor components of soybean, corn and canola oils as well as those contributing to colour development in tall oil during oxidation (Endo *et al.*, 1991; Min, 1973). During stripping of vegetable oils, activated charcoal was especially effective in adsorbing tocopherols, as only 15 % of tocopherols were adsorbed in the absence of charcoal (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 autoxidized soybean oil (Boki *et al.*, 1991). Boki *et al.*(1991) suggested, based on the 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. Furthermore, chlorophyll molecules are believed to be adsorbed as protonated species on acid-activated clay (Mokaya *et al.*, 1993).

4.1.2 Fatty acid composition of non-stripped and stripped flax,hemp, ARASCO, DHASCO and OMEGA-GOLD oil

The fatty acid composition of non-stripped and stripped flax and hemp oils are given in Table 4.3. The results in this table indicate that non-stripped and stripped hemp oil contained higher amounts ($p<0.05$) of PUFA than non-stripped and stripped flax oils. The main PUFA in non-stripped and stripped hemp oils was an omega-6 fatty acid; linoleic acid (18:2n-6), which was present at more than 50% , but was available at only 15% in non-stripped and stripped flax oils. Non-stripped hemp oil also contained a reasonable amount of GLA (18:3n-6), up to 3%. Non-stripped flax oil had up to 54 % ALA (18:3n-3), while hemp oil had only 23% of ALA. Thus, flax oil serves as a richer source of this essential fatty acid than hemp oil. The results reported in this work are

Table 4.3 Fatty acid composition (area percent) of non-stripped and stripped flax and hemp oils.

Fatty acid	NFO	SFO	NHO	SHO
C16:0	4.63 ± 0.02^{ab}	4.72 ± 0.03^{ab}	5.42 ± 0.03^{c}	6.31 ± 0.12^{d}
C18:0	4.16 ± 0.05^{cd}	4.40 ± 0.09^{cd}	2.58 ± 0.14^{a}	3.36 ± 0.09^{b}
C18:1n-9	20.3 ± 0.11^{cd}	21.1 ± 0.17^{cd}	9.19 ± 0.88^{a}	10.9 ± 0.24^{b}
C18:2n-6	15.3 ± 0.03^{ab}	15.2 ± 0.03^{ab}	52.1 ± 0.41^{c}	53.8 ± 0.24^{d}
C18:3n-6	-	-	3.37 ± 0.03^{a}	2.71 ± 0.07^{b}
C18:3n-3	54.13 ± 0.18^{d}	53.1 ± 0.29^{c}	23.3 ± 0.45^{b}	18.2 ± 0.42^{a}
Others	1.55	1.5	4.04	4.72
Total PUFA	69.4 ± 0.17^{ab}	68.3 ± 0.18^{ab}	78.8 ± 0.93^{d}	74.7 ± 0.72^{c}

Values are means of three determinations ± standard deviations. Values with different superscripts in each row are different ($p< 0.05$) from one another. Abbreviations are: NFO, non-stripped flax oil; NHO, non-stripped hemp oil; SFO, stripped flax oil; SHO, stripped hemp oil.

similar to those reported by Moes *et al.* (1999), who found that hempseed samples contained about 54 to 57.7% LA, 1.2 to 3.8% GLA, and 15.1 to 17.9% ALA. On the other hand, Table 4.4 illustrates the fatty acid composition of N-ARASCO, N-DHASCO, and N-OMEGA-GOLD oil and their counterparts S-ARASCO, S-DHASCO and S-OMEGA-GOLD oil. The results in this table indicate that non-stripped and stripped ARASCO contained significantly higher amounts ($p< 0.05$) of total PUFA than non-stripped and stripped DHASCO and OMEGA-GOLD oil. The main PUFA in non-stripped and stripped ARASCO was arachidonic acid (C20:4n-6), which was present at more than 40%, but only present at 2% in non-stripped and stripped OMEGA-GOLD oil, and was absent in non-stripped and stripped DHASCO. The results found in this work are similar to those reported by Hamam and Shahidi (2004b), who found that arachidonic acid (AA) single-cell oil derived from the same microfungus species contained about 39% AA. Non-stripped DHASCO contained up to 40% docosahexaenoic acid (C22:6n-3), while OMEGA-GOLD oil contained 34% DHA, and ARASCO lacked this fatty acid. Hamam and Shahidi (2004a) have reported that DHASCO contained 37.1% DHA which is relatively less than what obtained in this study. These authors also reported that OMEGA-GOLD oil contained about 36.8% DHA (Hamam and Shahidi, 2005) which is higher than the amount obtained in this study. The differences between fatty acid profiles presented here and those reported in the literature might represent variation in the source and batch of the oil, method of preparation, and fermentation conditions employed (Hamam and Shahidi, 2004b). Moreover, OMEGA- GOLD oil was also a good source of the omega-6 DPA (up to 13%).

Table 4.4 Fatty acid composition (area percent) of non-stripped and stripped ARASCO, DHASCO and OMEGA- GOLD oil.

Fatty acid	N-ARASCO	S-ARASCO	N-DHASCO	S-DHASCO	N-OMEGA-GOLD	S-OMEGA-GOLD
C10:0	–	–	1.37 ± 0.18^{ab}	1.24 ± 0.04^{ab}	–	–
C12:0	–	–	6.79 ± 0.76^{ab}	6.44 ± 0.17^{ab}	–	–
C14:0	–	–	17.7 ± 0.91^{cd}	17.4 ± 0.25^{cd}	8.73 ± 0.39^{ab}	9.08 ± 0.01^{ab}
C16:0	8.29 ± 0.06^{ab}	8.51 ± 0.03^{ab}	12.1 ± 0.42^{cd}	12.2 ± 0.05^{cd}	23.4 ± 1.04^{e}	32.0 ± 0.49^{f}
C16:1	–	–	2.19 ± 0.07^{db}	2.19 ± 0.03^{c}	1.61 ± 0.07^{a}	2.06 ± 0.02^{bd}
C18:0	8.98 ± 0.03^{a}	9.85 ± 0.06^{b}	–	–	–	–
C18:1n-9	18.0 ± 0.05^{c}	20.1 ± 0.08^{d}	15.9 ± 0.85^{ab}	16.4 ± 0.31^{ab}	–	–
C18:2n-6	7.21 ± 0.03^{d}	7.04 ± 0.03^{c}	0.65 ± 0.00^{ab}	0.65 ± 0.00^{ab}	–	–
C18:3n-6	2.87 ± 0.02^{b}	2.47 ± 0.02^{a}	–	–	–	–
C20:3n-6	2.52 ± 0.02^{ab}	2.52 ± 0^{ab}	–	–	–	–
C20:4n-6	42.8 ± 0.16^{d}	40.2±0.15c	–	–	2.45 ± 0.38^{ab}	2.49 ± 0.04^{ab}
C20:5n-3	1.94 ± 0.03^{b}	2.34 ± 0.02^{c}	–	–	3.21 ± 0.13^{d}	1.28 ± 0.01^{a}
C22:5n-6	1.76 ± 0.05^{ab}	2.11 ± 0.03^{ab}	–	–	13.3 ± 0.64^{c}	17.2 ± 0.26^{d}
C22:6n-3	–	–	41.0 ± 0.45^{cd}	40.9 ± 0.12^{cd}	34.5 ± 1.42^{b}	25.9 ± 0.31^{a}
Others	5.63^{d}	4.93^{c}	2.31^{a}	2.58^{b}	12.84^{f}	7.56^{e}
Total PUFA	59.1 ± 0.010^{f}	56.6 ± 0.15^{e}	41.7 ± 0.66^{ab}	41.6 ± 0.33^{ab}	53.4 ± 1.80^{d}	46.4 ± 0.20^{c}

Values are mean values of triplicate determination ±standard deviation. Abbreviations are: N-ARASCO, non-stripped ARASCO; N-DHASCO, non-stripped DHASCO; N-OMEGA-GOLD, non-stripped OMEGA-GOLD oil; S-ARASCO, stripped ARASCO; S-DHASCO, stripped DHASCO; S-OMEGA-GOLD, stripped OMEGA- GOLD oil.

Thus, ARASCO serves as a rich source of arachidonic acid (C20:4n-6), while, DHASCO and OMEGA-GOLD oil are concentrated sources of DHA (C22:6n-3). DPA(C22:5n-6) present in OMEGA-GOLD oil may retro-convert to arachidonic acid.

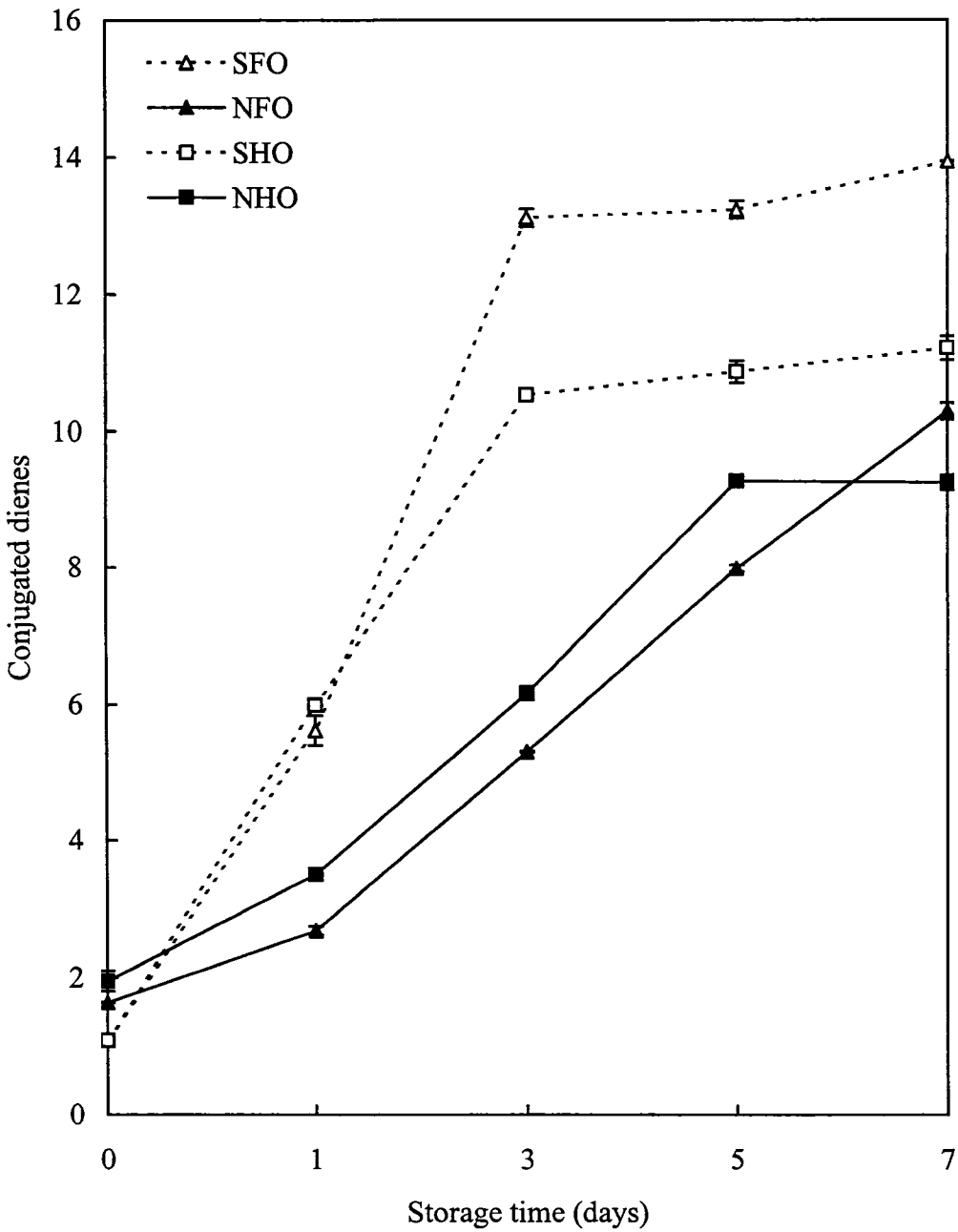
4.2 Oxidative stability of non-stripped and stripped flax, hemp, ARASCO, DHASCO and OMEGA-GOLD oil stored under Schaal oven conditions at 60°C

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, lipid samples are placed in a forced air oven and the temperature is maintained between 60 and 70°C (Frankel, 1993a) or 60-65°C (Malcolmson *et al.*, 1994). It has been observed that 1 day of storage under Schaal oven conditions at 65°C is equivalent to 1 month of 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

Conjugated dienes test is a simple, and rapid physical procedure to evaluate primary products of lipid oxidation. Determination of CD does not require any chemical reactions and needs only a small amount of sample in the milligram range. Moreover, CD can be used to estimate the initial rate of lipid oxidation (Gray and Monahan, 1992). Based on CD values obtained during the oxidation in the dark (Figure 4.1), NFO and NHO were more ($p<0.05$) stable than their corresponding SFO and SHO. However, non-

Figure 4.1 Conjugated dienes values of non-stripped and stripped flax and hemp oils stored under Schaal oven conditions at 60°C.


SFO
NFO
SHO
NHO
Conjugated dienes
Storage time (days)
0
2
4
6
8
10
12
14
16
0
1
3
5
7

stripped flax and non-stripped hemp oils were less stable than expected. Theoretically, non-stripped oils under Schaal oven conditions are expected to be stable due to the presence of different minor components that play a significant role in oil stability (Mounts *et al.,* 1994), but the fatty acid composition of the oils might also influence their stability, especially ALA and GLA which are well known to be readily oxidized during storage and heating of the oil (Dutton *et al.,* 1951). The higher oxidative stability of non-stripped flax and hemp oils compared to their stripped counterparts can be partially attributed to the presence of nutritionally important antioxidants in both non-stripped oils such as tocopherols, carotenoids and phospholipids. Any significant reduction in the levels of these components, particularly tocopherols, could dramatically alter the stability of the stripped 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). However, these minor components might not be effective enough to protect the examined oils from oxidation. Moreover, the fatty acid composition of both oils might contribute highly to their susceptibility to oxidation.

Free radical chain reactions occur during autoxidation of oils and these proceed via three steps, namely initiation, propagation and termination. Tocopherols can interrupt the first two reactions and protect oils 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 this can be marked by a dramatic increase in PV (Rossel, 1994). However, testing single-cell oils; ARASCO, DHASCO, and OMEGA-GOLD oil under Schaal conditions, and based on CD values obtained during the oxidation in the dark (Figure 4.2), non-stripped DHASCO (N-DHASCO) was more ($p< 0.05$) stable than its corresponding stripped counterpart (S-DHASCO). Meanwhile, non-stripped ARASCO (N-ARASCO) and non-stripped OMEGA-GOLD oil (N-OMEGA-GOLD oil), and their counterparts were unstable under these conditions. The stability of N-DHASCO under Schaal oven conditions might be due to the higher amount of tocopherols present in N-DHASCO compared to the amounts detected in N-ARASCO and N-OMEGA-GOLD oil (Table 4.2). However, S-ARASCO, S-DHASCO and S-OMEGA-GOLD oil were devoid of these minor components during stripping, which makes them unstable under Schaal oven conditions.

4.2.2 Secondary oxidation products

Secondary oxidation products of non-stripped and stripped flax, hemp and algal oils were determined by examining TBARS and headspace volatiles, mainly hexanal and propanal. Determination of TBARS is based on colour intensity of the reaction between TBA and secondary oxidation products of polyunsaturated fatty acids (PUFA), including malonaldehyde (MA). The TBARS values, expressed as μmol malonaldahyde equivalents per g of NFO and NHO, were lower than those of their corresponding stripped samples (Figure 4.3), in part due to the presence of minor components such as tocopherols in the

Figure 4.2 Conjugated dienes of non-stripped and stripped ARASCO, DHASCO and OMEGA-GOLD oil stored under Schaal oven conditions at 60°C.

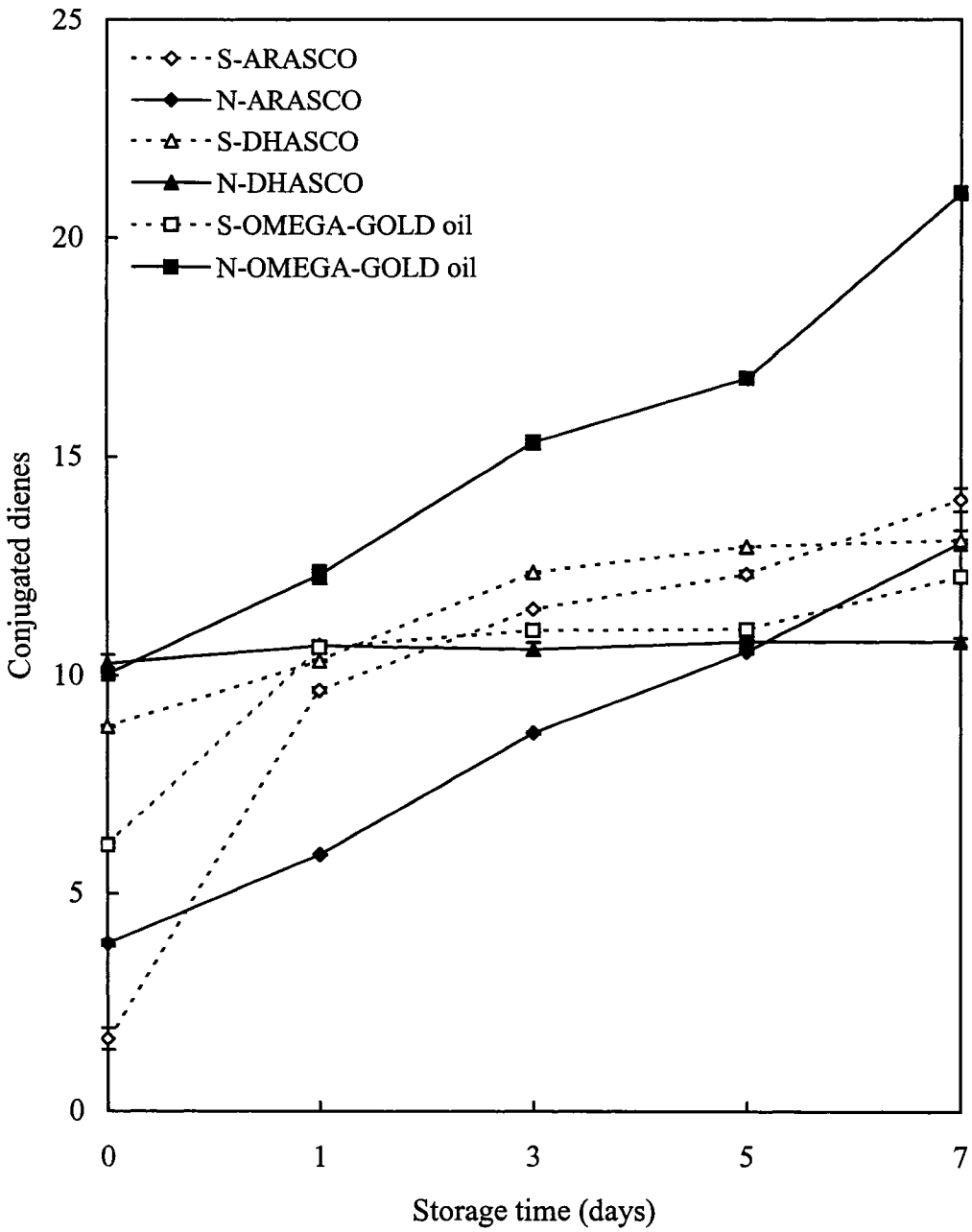
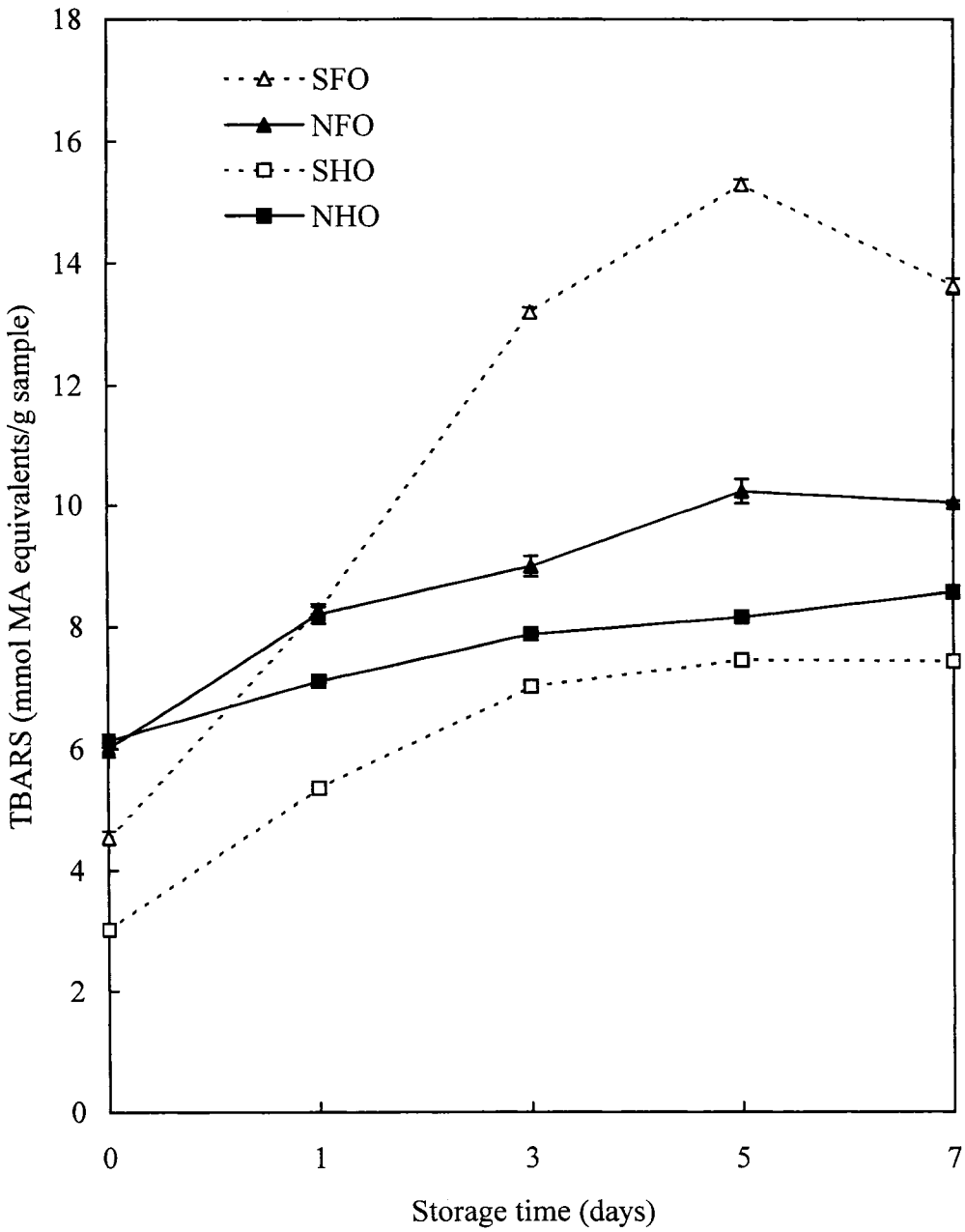
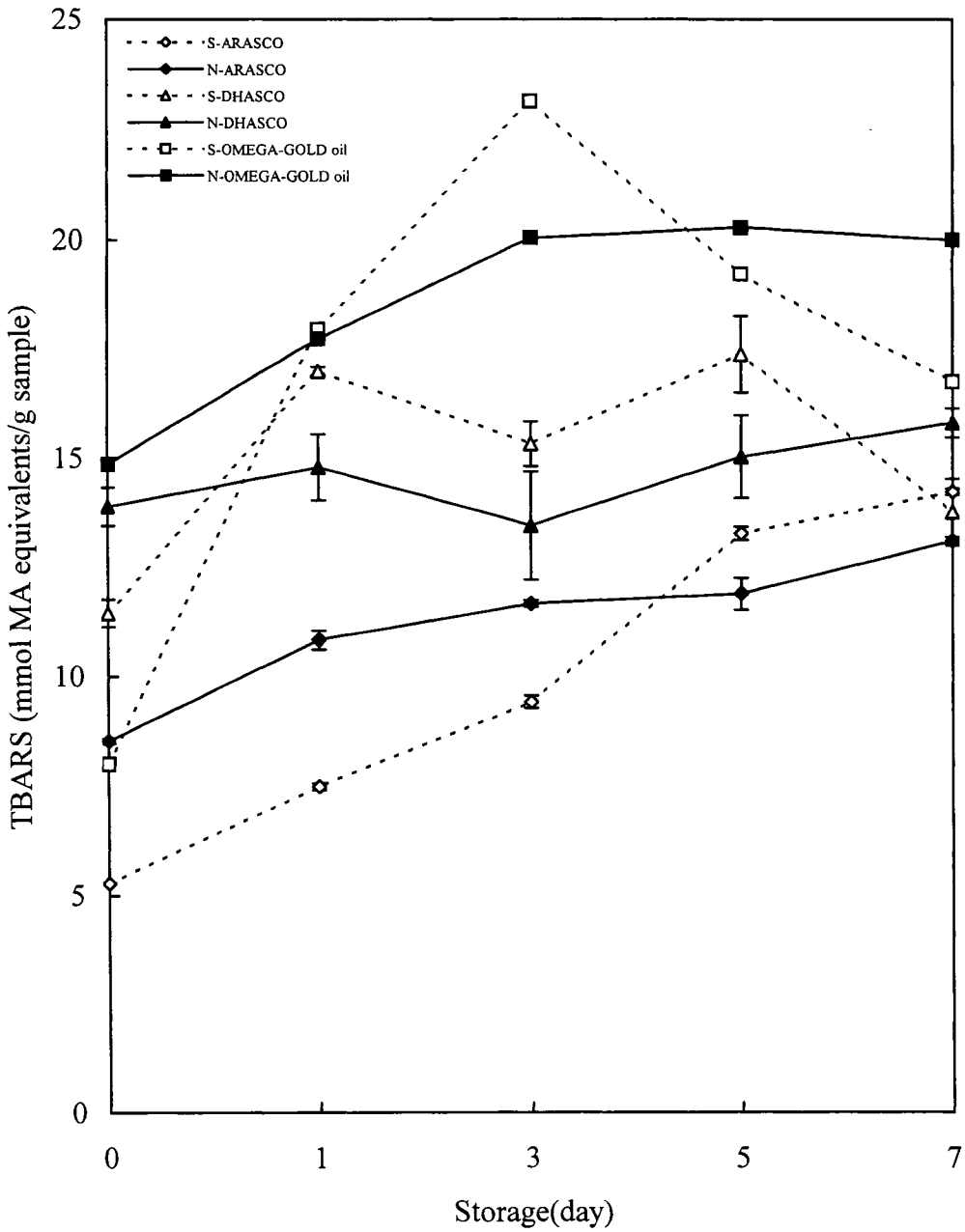

S-ARASCO
N-ARASCO
S-DHASCO
N-DHASCO
S-OMEGA-GOLD oil
N-OMEGA-GOLD oil
Conjugated dienes
Storage time (days)
0
5
10
15
20
25
0
1
3
5
7

Figure 4.3 Thiobarbituric acid reactive substances (TBARS) of non-stripped and stripped flax and hemp oils stored under Schaal oven conditions at 60°C.


SFO
NFO
SHO
NHO
TBARS (mmol MA equivalents/g sample)
Storage time (days)
0
2
4
6
8
10
12
14
16
18
0
1
3
5
7

original oils. The sharp increase in TBARS values was noticed for SFO for the first five days, and then there was a decrease after the fifth day. This might be due to the breaking down of the secondary oxidation products. Meanwhile, TBARS values of SHO were increasing during the first three days, and then there was no difference in the values between the third and the seventh day. Upon further storage, there might be differences in the TBARS values, but this was not tested. The increasing trend in oxidation of stripped flax and hemp oils, as reflected in TBARS values, compared to non-stripped flax and hemp oils, was similar to that obtained for primary oxidation products as reflected in CD values (Figure 4.1). However, NHO was relatively more stable than NFO as shown in Figure 4.3. This might be due to the total amount of tocopherols which was higher ($p<0.05$) in NHO than that found in NFO, as well as fatty acid composition of the oil. Non-stripped flax contained almost double the amount of α-linolenic acid which is highly susceptible to oxidation. On the other hand, when examining N-ARASCO, N-DHASCO, and N-OMEGA-GOLD oil under Schaal conditions, the TBARS values were lower than those of their corresponding stripped counterparts (Figure 4.4). However, the TBARS values for N-ARASCO and N-OMEGA-GOLD oil were higher ($p<0.05$) than those of N-DHASCO which indicates the instability of N-ARASCO and N-OMEGA-GOLD oil. This might be due to the presence of high amounts of minor components, including tocopherols, in DHASCO. These results reported here agree with those of Hamam and Shahidi (2004a). These authors found that TBARS values for DHASCO remained constant during the storage period, indicating good stability of this oil under Schaal oven

Figure 4.4 Thiobarbituric acid reactive substances (TBARS) of non-stripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oil stored under Schaal oven conditions at 60°C.


S-ARASCO
N-ARASCO
S-DHASCO
N-DHASCO
S-OMEGA-GOLD oil
N-OMEGA-GOLD oil
TBARS (mmol MA equivalents/g sample)
25
20
15
10
5
0
0
1
3
5
7
Storage(day)

conditions. However, ARASCO (Hamam and Shahidi (2004a) and OMEGA-GOLD oil (Hamam and Shahidi, 2005) were also unstable under these conditions.

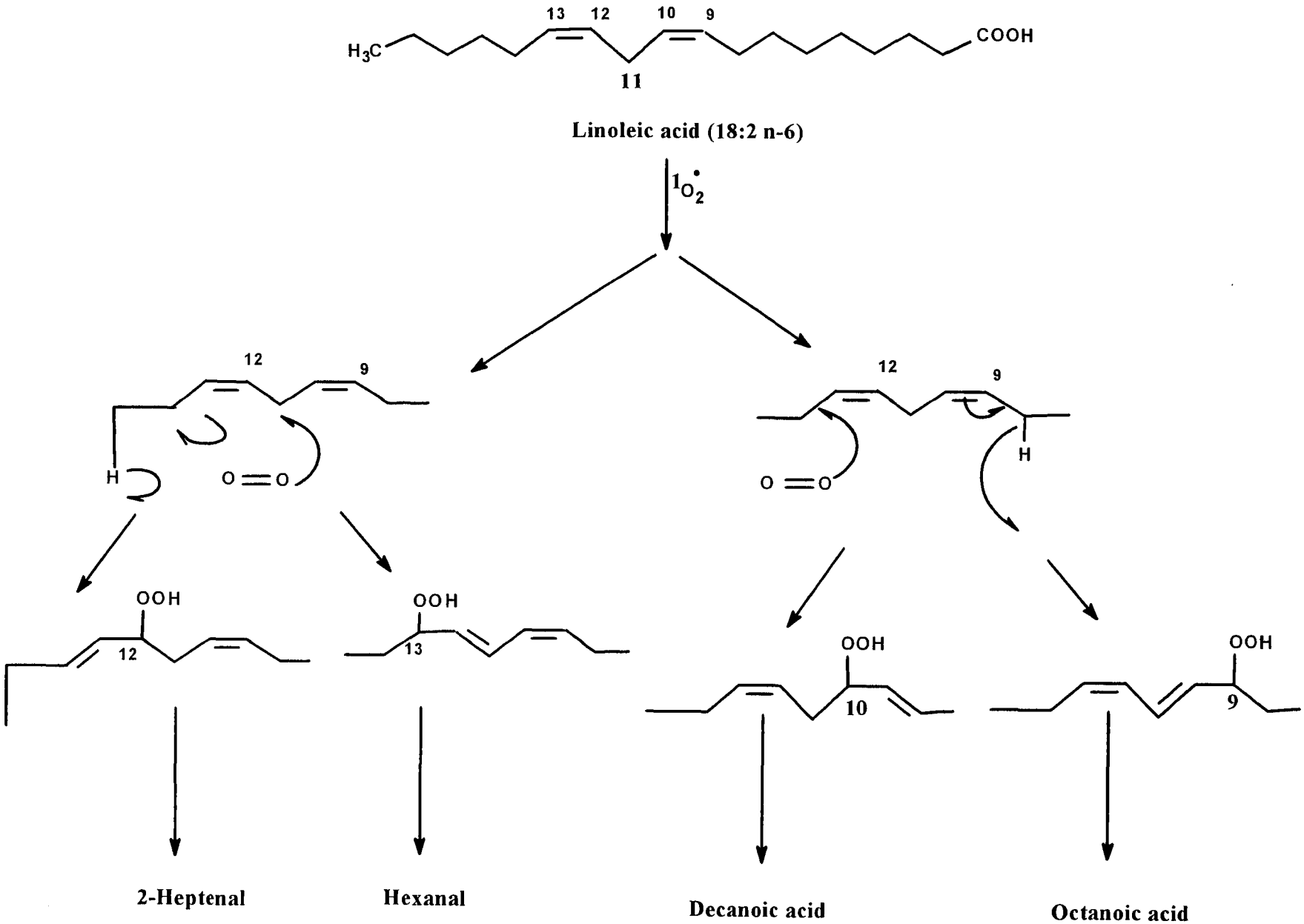
Determination of headspace volatiles, although time consuming and labor intensive, is a very effective method for evaluating the oxidative stability of nutritional and algal oils. The gas chromatographic analysis can provide useful information regarding the origin of flavour and odour volatiles and their precursors and, thus is a suitable method for comparison with the results of sensory analysis (Frankel, 1993a) which is the most useful method for predicting 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 observed during autoxidation of non-stripped and stripped flax oil (Figure A.3a & A.3b) was propanal. Meanwhile, hexanal (Figure A.4a & A.4b) was the major volatile observed during the oxidation of non-stripped and stripped hemp oil.

Hexanal is the major volatile produced from the oxidation of omega-6 polyunsaturated fatty acids (Frankel, 1993a; Shahidi *et al.*, 1997). Hexanal might be formed via autoxidation of GLA and 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 alkoxyl and hydroxyl radicals, which in turn decompose by carbon-carbon cleavage to yield hexanal and pentane from the 13-

Figure 4.5 Formation of hexanal from oxidation of linoleic acid.


13 12 10 9
H3C
COOH
11
Linoleic acid (18:2 n-6)
1O2
12
9
H
O=O
OOH
12
13
10
9
2-Heptenal
Hexanal
Decanoic acid
Octanoic acid

hydroperoxide and methyl octanoate and 2,4-decadienal from 9-hydroperoxides (Frankel, 1984). However, propanal is the major volatile produced from the oxidation of omega-3 PUFA (Frankel, 1993a). On the other hand, the same results were obtained when algal oils were stored under Schaal oven conditions. The major volatile detected during the oxidation of ARASCO (omega-6 algal oil) was hexanal (Figure 4.6). Meanwhile, propanal was the major volatile produced during the oxidation of DHASCO (Figure 4.7) and both hexanal and propanal were detected during oxidation of OMEGA-GOLD oil (Figure 4.8).

Non-stripped flax and hemp oils were more stable ($p<0.05$) than their corresponding stripped counterparts. 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 inhibit 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 deodourized olive oil (Satue *et al.*, 1995). Shahidi *et al.* (1997) reported the loss of endogenous antioxidants in sesame oil, such as sesamin, sesamolin and γ-tocopherol during processing and storage and these paralleled an increase in their hexanal content. Meanwhile, the hexanal content in stripped hemp oil (Figure 4.9) was higher ($p<0.05$) than that of the stripped flax oil (Figure 4.10). This might be explained by the presence of higher amounts of GLA and linoleic acids in hemp oil as compared to that in flax oil. On the other hand, propanal content in stripped flax oil was higher ($p<0.05$) than that of stripped hemp, probably due to the presence of a higher amount of

Figure 4.6 Hexanal, and total volatile of non-stripped and stripped ARASCO stored under Schaal oven conditions at 60°C.

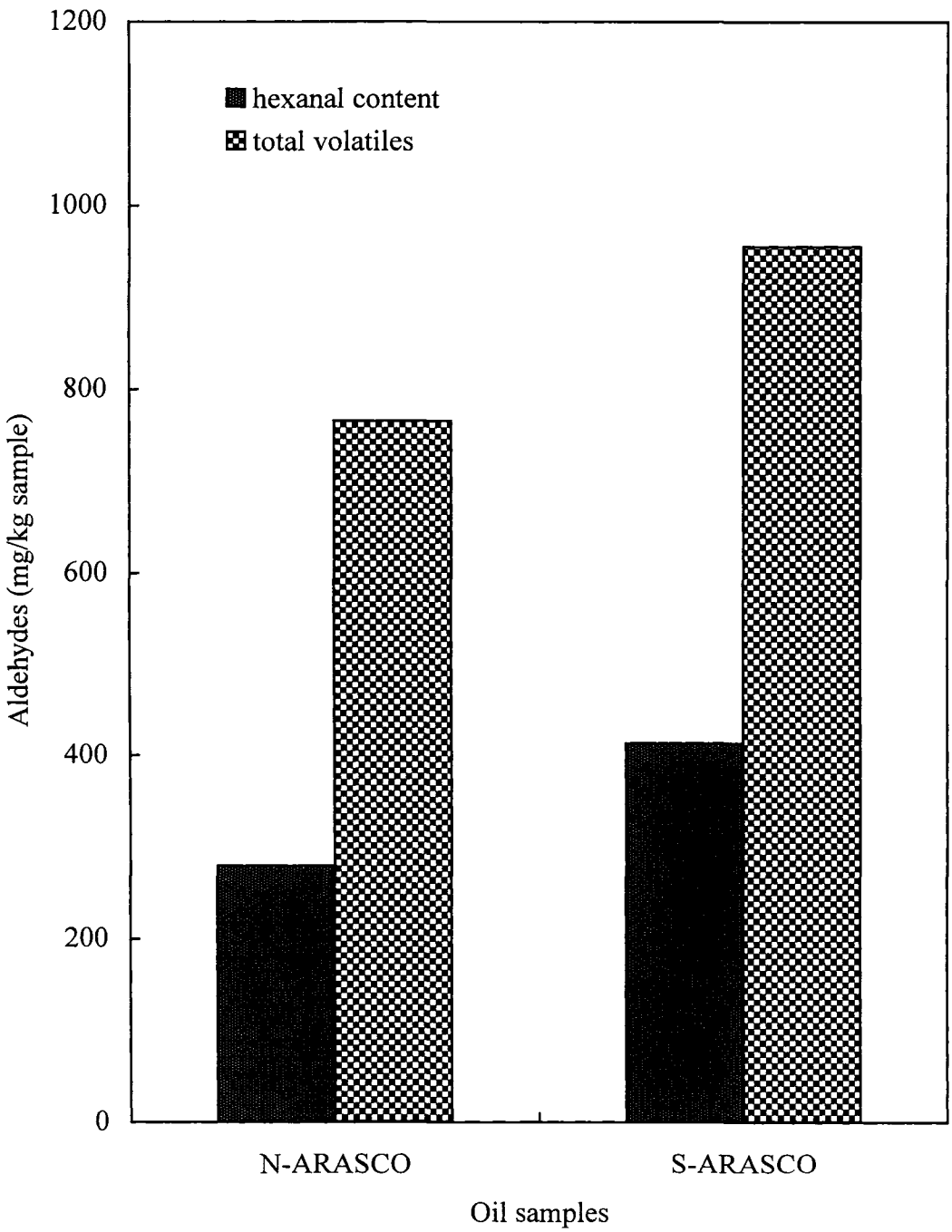

1200
1000
800
600
400
200
0
hexanal content
total volatiles
Aldehydes (mg/kg sample)
N-ARASCO
S-ARASCO
Oil samples

Figure 4.7 Propanal and total volatile of non-stripped and stripped DHASCO stored under Schaal oven conditions at 60°C.

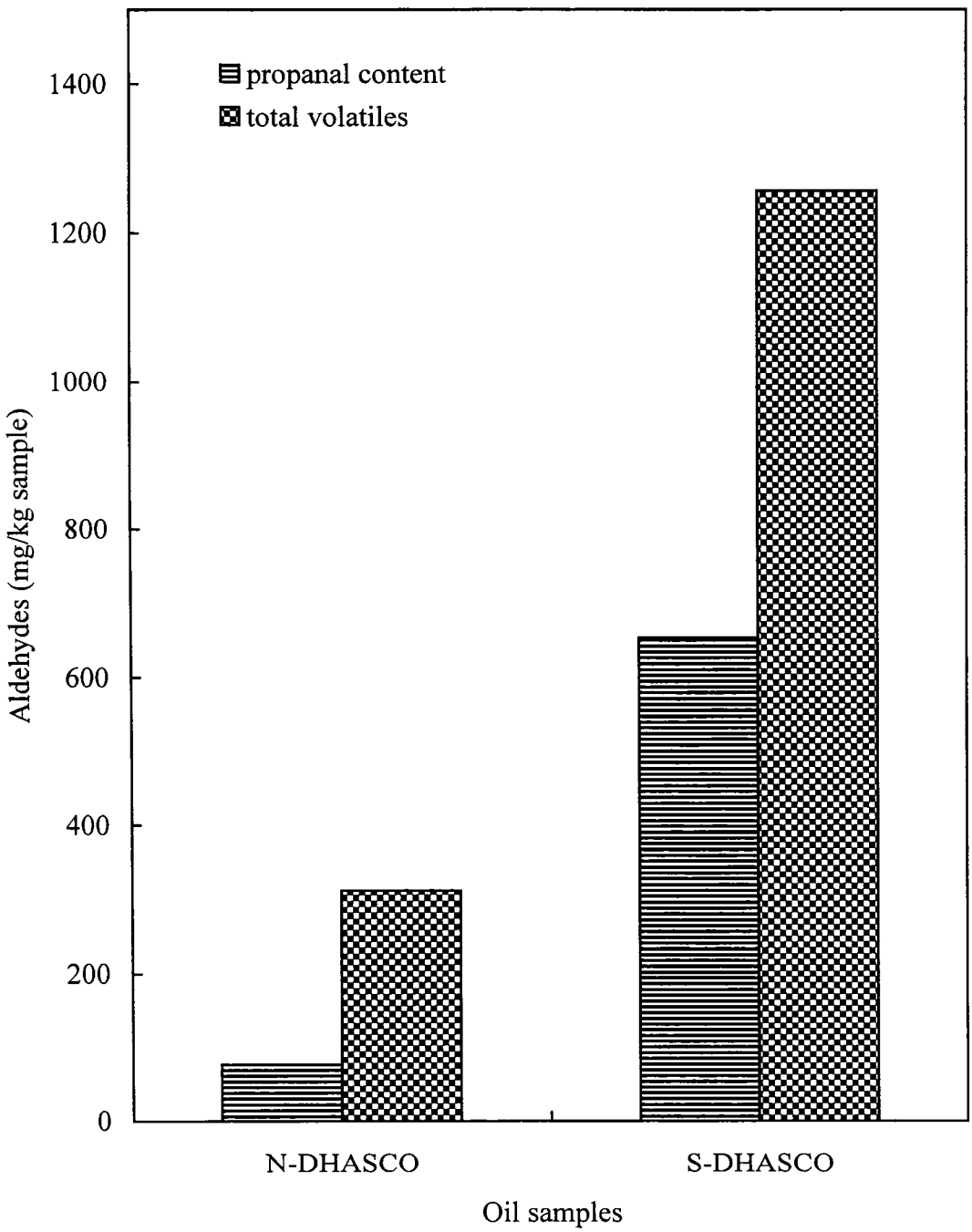

propanal content
total volatiles
1400
1200
1000
800
600
400
200
0
Aldehydes (mg/kg sample)
N-DHASCO
S-DHASCO
Oil samples

Figure 4.8 Propanal, hexanal, and total volatile of non-stripped and stripped OMEGA-GOLD oil stored under Schaal oven conditions at 60°C.

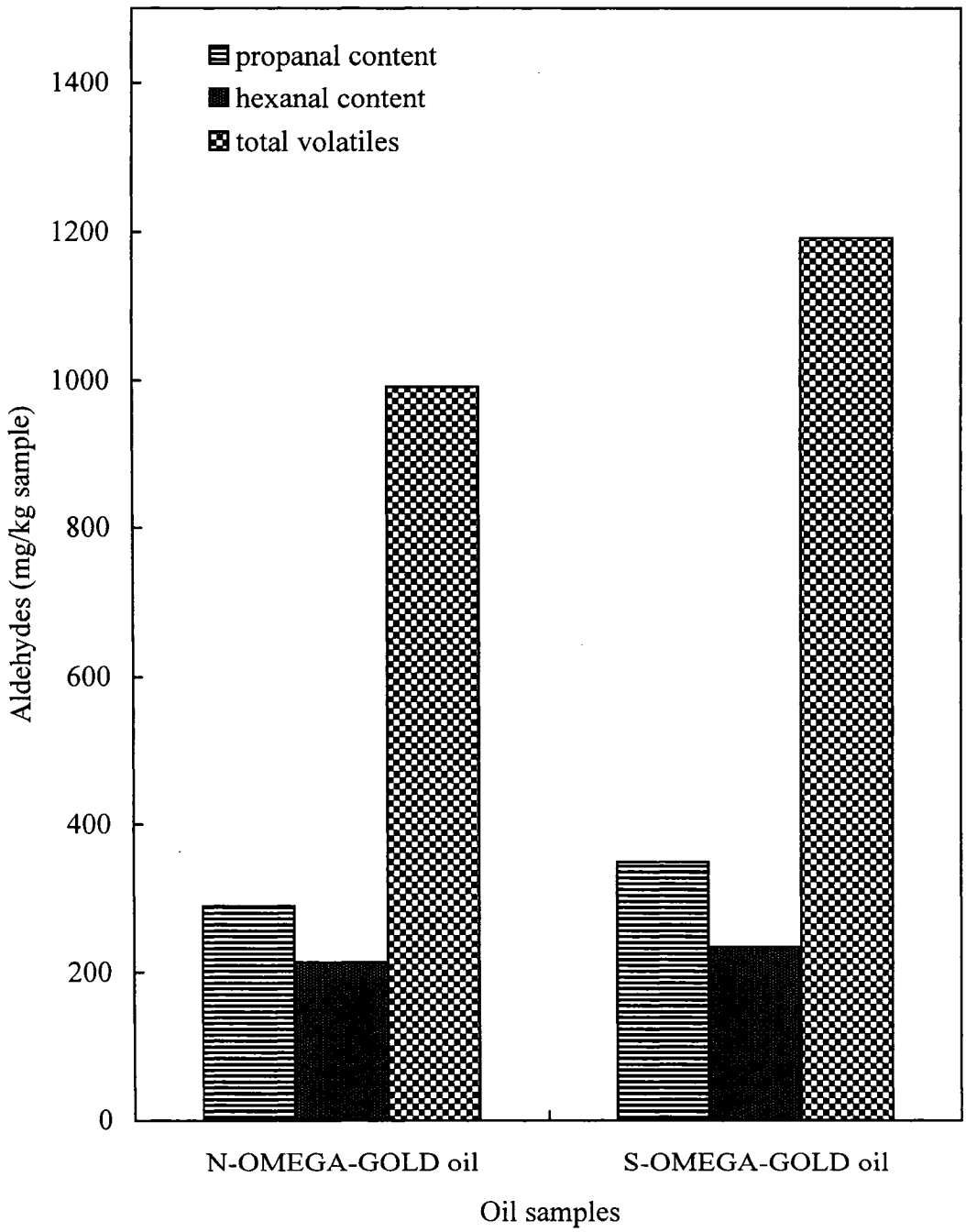

propanal content
hexanal content
total volatiles
1400
1200
1000
800
600
400
200
0
Aldehydes (mg/kg sample)
N-OMEGA-GOLD oil
S-OMEGA-GOLD oil
Oil samples

Figure 4.9 Propanal, hexanal, and total volatile of non-stripped and stripped hemp oil stored under Schaal oven conditions at 60°C.

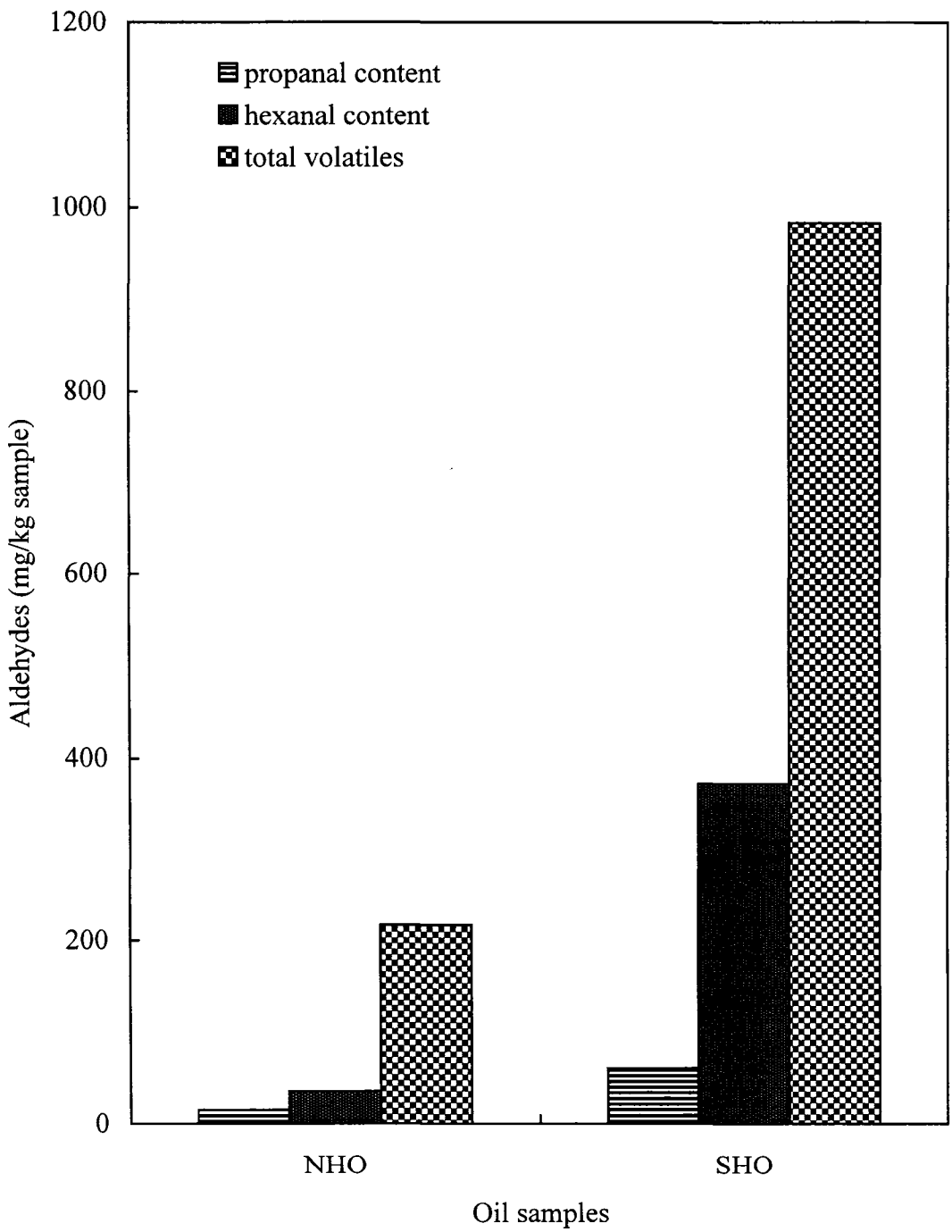
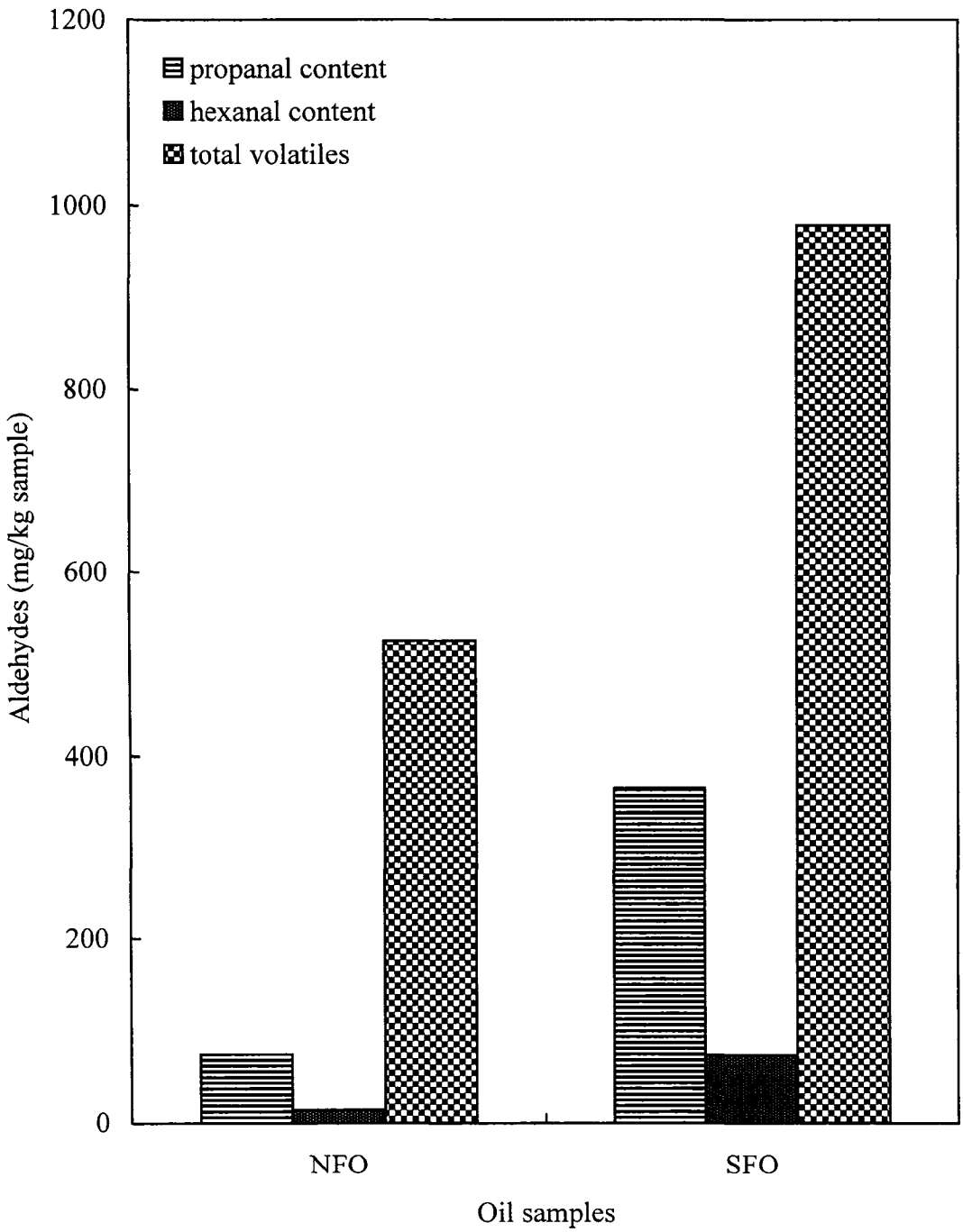

1200
1000
800
600
400
200
0
propanal content
hexanal content
total volatiles
Aldehydes (mg/kg sample)
NHO
SHO
Oil samples

Figure 4.10 Propanal, hexanal, and total volatile of non-stripped and stripped flax oil stored under Schaal oven conditions at 60°C.


1200
1000
800
600
400
200
0
propanal content
hexanal content
total volatiles
Aldehydes (mg/kg sample)
NFO
SFO
Oil samples

ALA in flax oil. However, non-stripped DHASCO was more stable ($p<0.05$) than its corresponding stripped DHASCO. The propanal content in stripped DHASCO was higher ($p<0.05$) than that of non-stripped DHASCO (Figure 4.7). Meanwhile, non-stripped and stripped ARASCO and OMEGA-GOLD oil were not stable under Schaal oven condition. Hexanal content in both stripped and non-stripped ARASCO was high (Figure 4.6), while, propanal and hexanal contents were also high in both stripped and non-stripped OMEGA-GOLD oil (Figure 4.8).

4.3 Oxidative stability of non-stripped and stripped flax, hemp, ARASCO, DHASCO and OMEGA-GOLD oil under fluorescent light at 27°C

4.3.1 Primary oxidation products

The conjugated dienes (CD) values obtained during the photooxidation of non-stripped and stripped flax and hemp oils are presented in Figure 4.11. Photooxidation of NHO, in the initial stages, progressed rapidly compared to NFO. Thereafter, the oxidation, as demonstrated by CD values, progressed gradually for NFO. Meanwhile, the CD of SFO and SHO were lower ($p<0.05$) than those of their corresponding non-stripped counterparts. Therefore, NFO and NHO are more ($p<0.05$) photooxidizable than their corresponding SFO and SHO. This may be explained when considering the presence of pigments, mainly chlorophylls, in non-stripped hemp oil and carotenoids in non-stripped flax oil. The visible spectra of pigments in non-stripped and stripped olive oil (Khan and Shahidi, 2002), non-stripped and stripped flax and hemp oils are presented in Figure 4.12. It is well documented that olive oil contains naturally-occurring photosensitizers such as

Figure 4.11 Conjugated dienes of non-stripped and stripped flax and hemp oils stored under fluorescent light at 27°C.

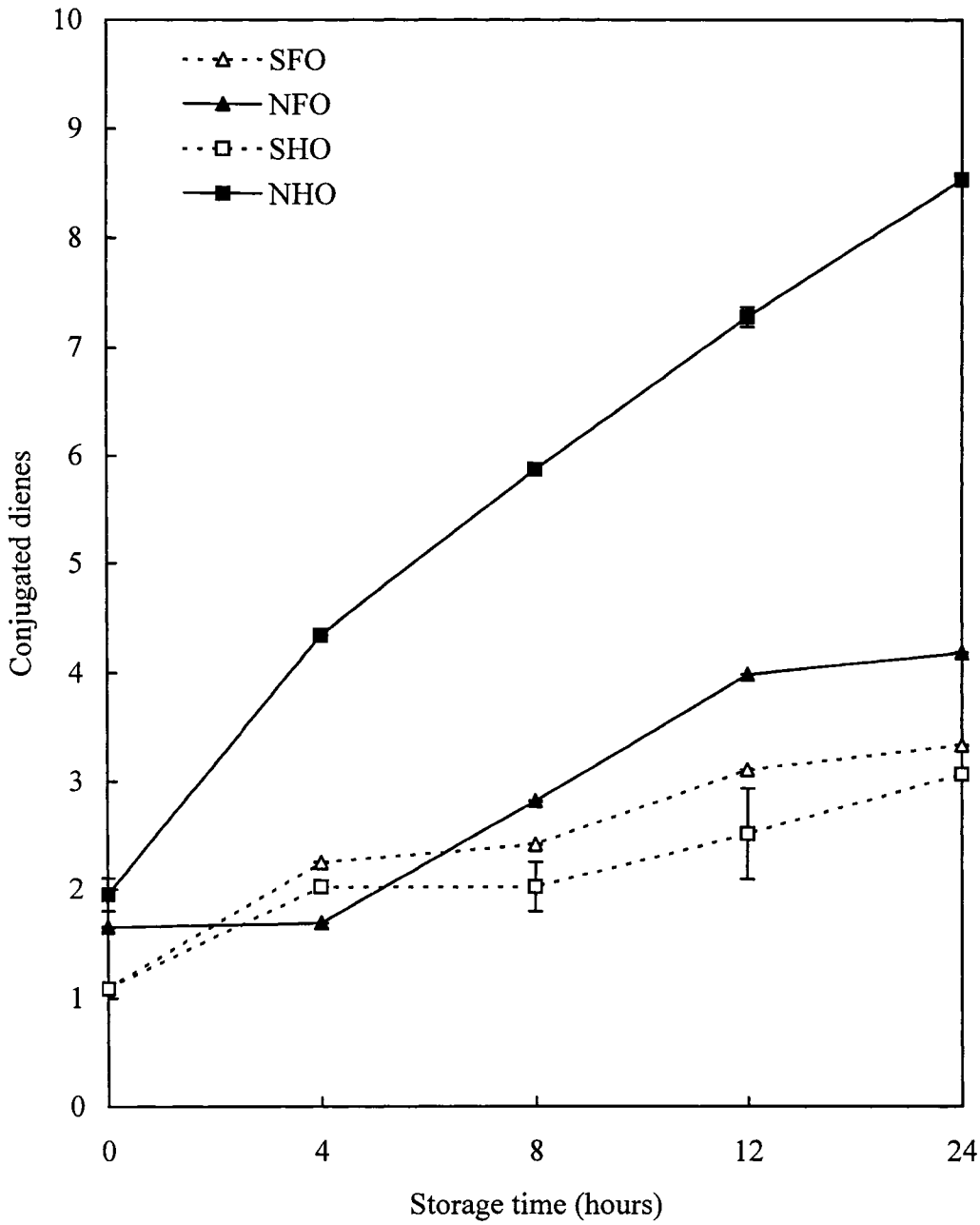
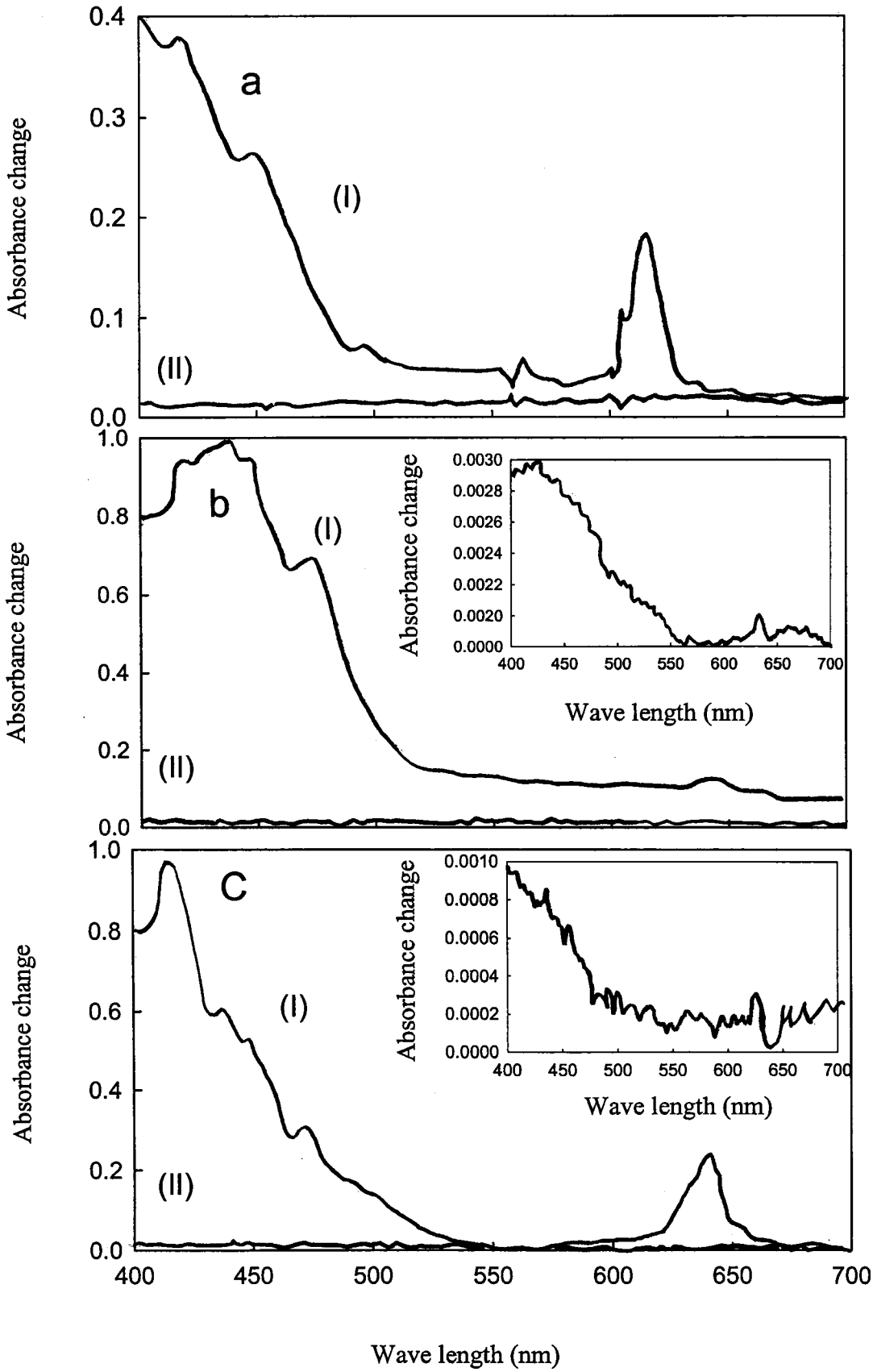

SFO
NFO
SHO
NHO
Conjugated dienes
Storage time (hours)
0
1
2
3
4
5
6
7
8
9
10
0
4
8
12
24

Figure 4.12 Visible spectra of pigments in oil/hexane*[1] of non-stripped (I) and stripped (II)*[2] olive, (a); flax, (b); and hemp, (c) oils.

*[1]Oil/hexane in olive oil (1:1, v/v); flax oil (1:5, v/v); and hemp oil (1:12,v/v).

*[2] (II) spectra is magnified in the right corner of the Figure.


a
(I)
(II)
Absorbance change
0.4
0.3
0.2
0.1
0.0
b
(I)
(II)
Absorbance change
1.0
0.8
0.6
0.4
0.2
0.0
0.0030
0.0028
0.0026
0.0024
0.0022
0.0020
0.0000
400 450 500 550 600 650 700
Absorbance change
Wave length (nm)
C
(I)
(II)
Absorbance change
1.0
0.8
0.6
0.4
0.2
0.0
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
400 450 500 550 600 650 700
Absorbance change
Wave length (nm)
400 450 500 550 600 650 700
Wave length (nm)

chlorophylls and pheophytin as well as quenchers such as carotenoids (Kiritsakis and Dugan, 1985; Rahmani and Csallany, 1991). The characteristic visible absorption peaks occurring below 500 nm corresponds to carotenoids in olive oil, while absorptions at 605, 647 and 667 nm correspond to different types of chlorophylls. These peaks qualitatively confirm the presence of chlorophylls in non-stripped olive and hemp oils, and lower chlorophylls observed in non-stripped flax oil. It has been reported that edible oils containing natural pigments such as chlorophylls and pheophytin are highly susceptible to light-induced oxidation or photooxidation (Hall and Cuppett, 1993; Lee and Kim, 1997). These pigments or photosensitizers that may absorb light and transform to an excited state, which in turn might convert to a less stable triplet state sensitizers. The sensitizer can transfer its energy to the most stable triplet state oxygen, thus converting it to higher energy level singlet oxygen which would in turn attack the double bonds in unsaturated fatty acids. This will generate free peroxyl 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 borage and evening primrose oils (Khan and Shahidi, 2002). Meanwhile, the CD values of NHO were higher ($p<0.05$) than those of NFO. This may be due to the presence of higher levels of cholorophylls in non-stripped hemp oil compared to those found in non-stripped flax oil.

The pigments that act as initiators of photooxidation in edible oils may be destroyed by light (Usuki *et al.*, 1984; Kiritsakis and Dugan, 1985. The pigments, chlorophylls and

caroteniods, were effectively removed from the stripped hemp oil (Figure 4.12) and therefore, the oxidation was not affected by fluorescent light as in stripped flax oil (Figure 4.11). Traces of carotenoids might be retained in stripped flax oil that might be involved in oxidation and act as prooxidants. This might be due to the instability of carotenoids against light and heat, especially β-carotene (Subagio and Morita, 2001). However, formation of conjugated dienes occurred in stripped ARASCO, DHASCO and OMEGA-GOLD oil when exposed to fluorescent light as demonstrated in Figure 4.13. This might also arise from pigments (mainly carotenoids) that are retained in the stripped oils (Figure 4.14). Similarly, an increase in CD values was observed for non-stripped ARASCO and OMEGA-GOLD oil. However, non-stripped DHASCO was stable under light. Although photooxidation of lipids proceeds differently from autoxidation, CD values are formed in both mechanisms (Frankel, 1984), and thus photooxidation can be followed by determining CD (Kiritsakis and Dugan, 1985).

4.3.2 Secondary oxidation products

Figure 4.15 shows TBARS values of non-stripped and stripped flax and hemp oils stored under fluorescent light at 27°C for 24 hours. The TBARS of non-stripped flax and hemp oils were higher ($p<0.05$) than those of stripped flax and hemp oils. However, TBARS values of non-stripped hemp oil were higher ($p<0.05$) than those of non-stripped flax oil. This might be due to higher contents of photosensitizers in non-stripped hemp oil as compared to that of non-stripped flax oil. Photooxidation of α-linolenic acid in non-

Figure 4.13 Conjugated dienes of non-stripped and stripped ARASCO, DHASCO, and OMEGA-GOLD oil under fluorescent light at 27°C.

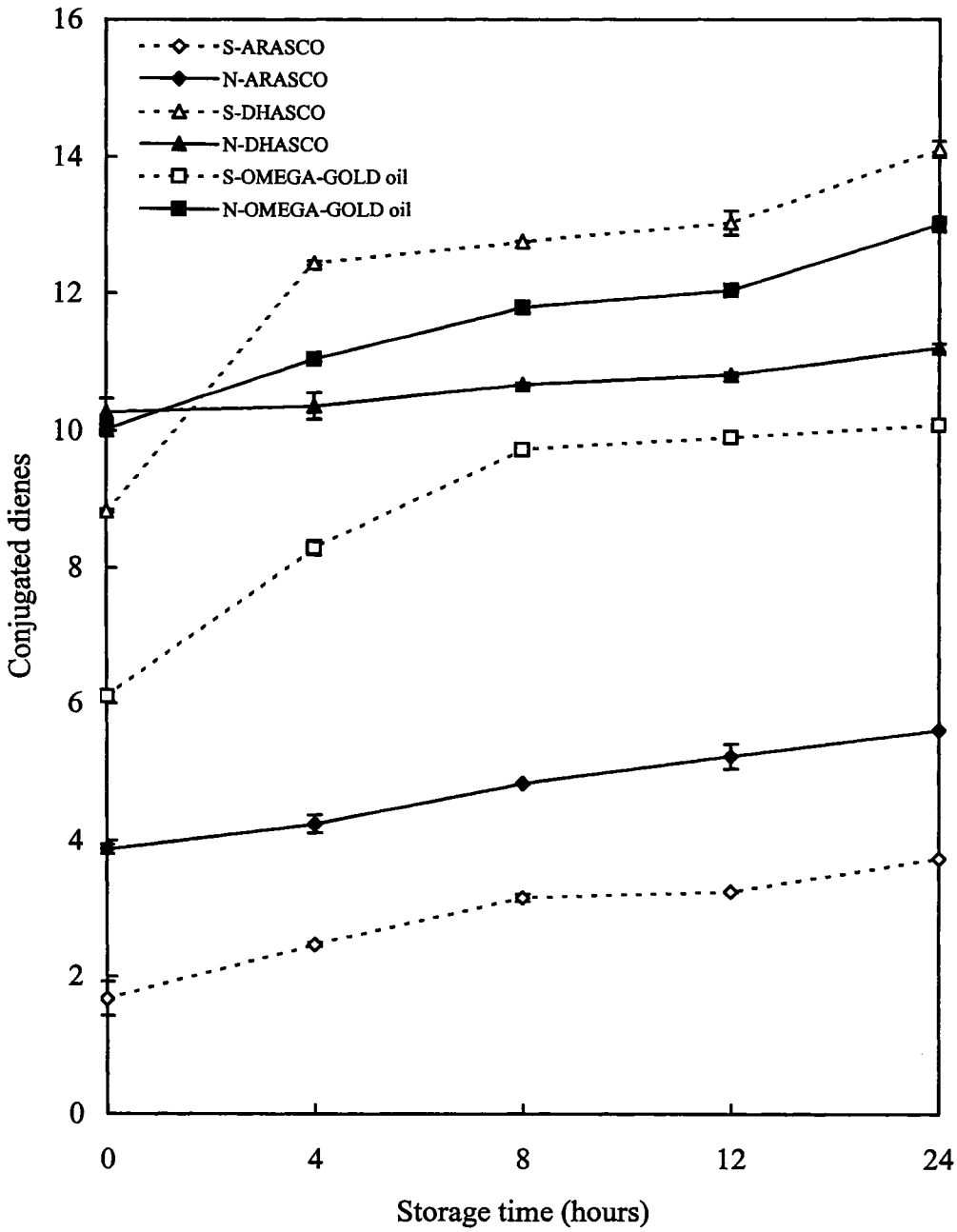

S-ARASCO
N-ARASCO
S-DHASCO
N-DHASCO
S-OMEGA-GOLD oil
N-OMEGA-GOLD oil
Conjugated dienes
Storage time (hours)
0
2
4
6
8
10
12
14
16
0
4
8
12
24

Figure 4.14 Visible spectra of pigments in oil/hexane*[1] of non-stripped (I) and stripped (II)*[2] olive oil, (a); ARASCO, (b); DHASCO, (c); and OMEGA-GOLD oil, (d).

*[1]Oil/hexane in olive oil (1:1, v:v), ARASCO and OMEGA-GOLD oil (1:3, v/v); and DHASCO (1:6,v/v).

*[2] (II) spectra is magnified in the right corner of the Figure.

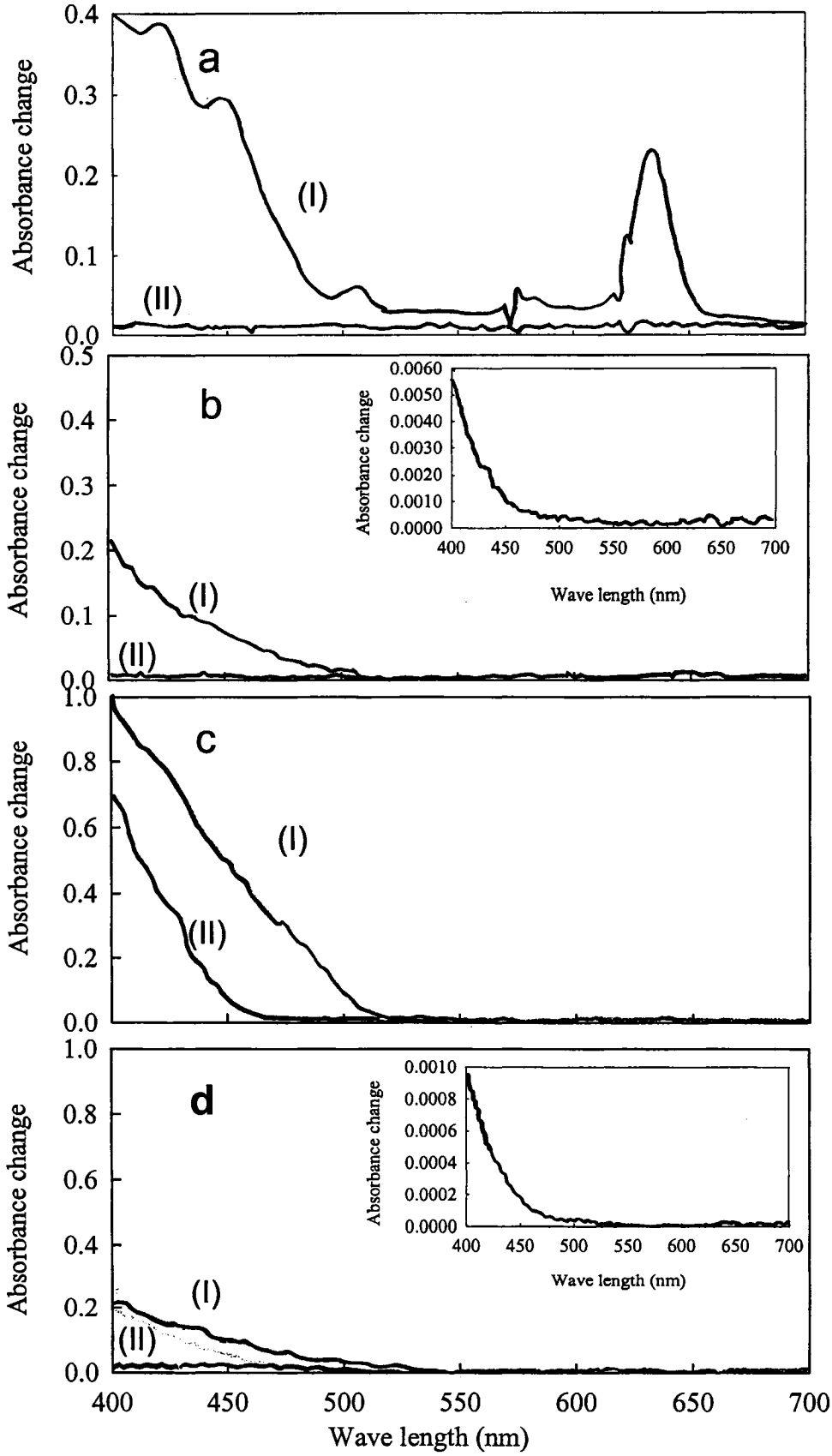
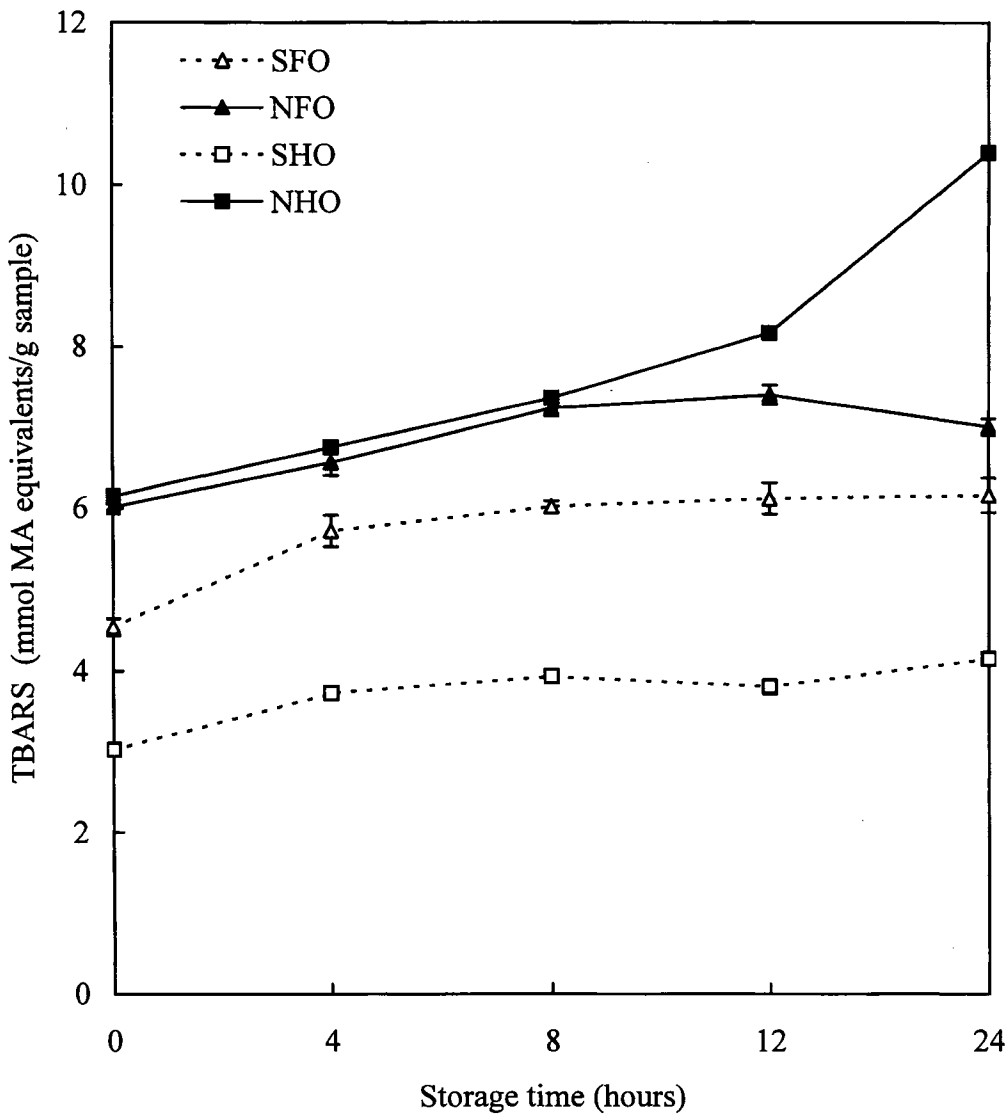
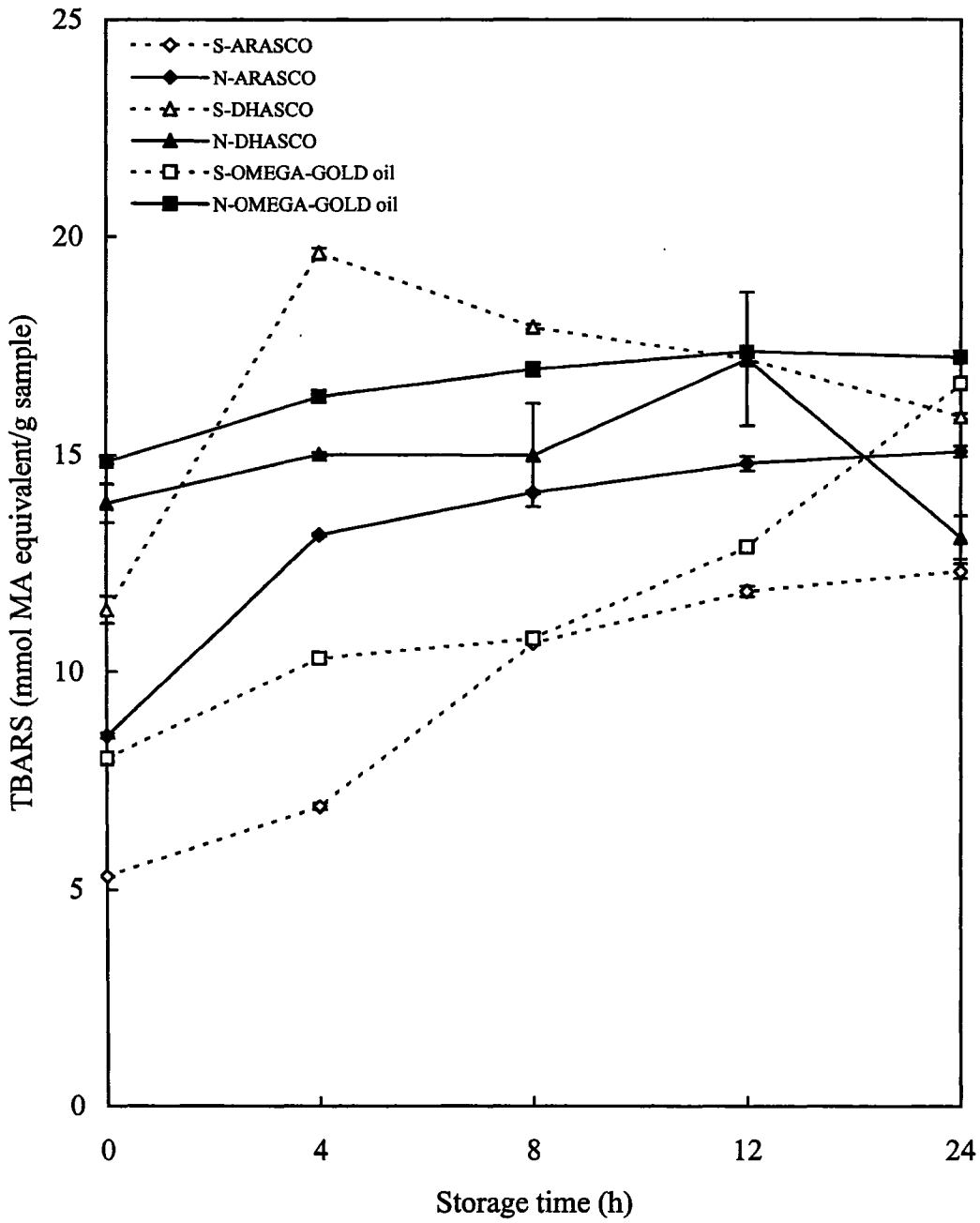

a
b
c
d
(I)
(II)
Absorbance change
Wave length (nm)
0.4
0.3
0.2
0.1
0.0
0.5
1.0
0.8
0.6
400
450
500
550
600
650
700
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0.0000
0.0008
0.0006
0.0004
0.0002

Figure 4.15 Thiobarbituric acid reactive substances (TBARS) of non-stripped and stripped flax and hemp oils under fluorescent light at 27°C.


12
10
8
6
4
2
0
SFO
NFO
SHO
NHO
TBARS (mmol MA equivalents/g sample)
0
4
8
12
24
Storage time (hours)

stripped and stripped flax and hemp oils 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 to the formation of malonaldehyde (Neff *et al.*, 1983). Thiobarbtiuric acid (TBA) reacts with malonaldehyde (MA) and selected compounds to give a pink-coloured complex with a maximum absorbance at 532 nm (Shahidi and Wanasundara, 1998). Based on their TBARS values, non-stripped borage (NBO) and non-stripped evening primrose oil (NEPO) were more ($p<0.05$) susceptible to oxidation than stripped borage oil (SBO) and stripped evening primrose oil (EPO) (Khan and Shahidi, 2002). On the other hand, TBARS values of non-stripped and stripped ARASCO, DHASCO and OMEGA-GOLD oil under fluorescent light are illustrated in Figure 4.16. Data in this figure indicates that, non-stripped DHASCO had the highest stability compared with other oils. Meanwhile, non-stripped ARASCO and non-stripped OMEGA-GOLD oil were unstable under the same conditions. However, stripped ARASCO, stripped DHASCO and stripped OMEGA-GOLD oil were more unstable than their corresponding non-stripped oils under fluorescent light. This might be due to retaining of some of the pigments (mainly carotnoids) in S-ARASCO, S-DHASCO, and S-OMEGA-GOLD oil (Figure 4.14), which might contribute to the instability of these stripped oils. Stripped DHASCO, devoid of most of its antioxidants, but with retention of some caroteniods, may oxidize rapidly as indicated by the formation of high amounts of TBARS.

Figure 4.16 Thiobarbituric acid reactive substances (TBARS) of non-stripped and stripped ARASCO, DHASCO and OMEGA-GOLD oil under fluorescent light at 27°C.


S-ARASCO
N-ARASCO
S-DHASCO
N-DHASCO
S-OMEGA-GOLD oil
N-OMEGA-GOLD oil
TBARS (mmol MA equivalent/g sample)
Storage time (h)
0
5
10
15
20
25
0
4
8
12
24

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 flax oil (Figure A.5) was propanal, and the main volatile obtained from the oxidation of hemp oil under fluorescent light was hexanal (Figure A.6) which has been shown to cause undesirable flavours in lipids (Shahidi *et al.*, 1997). Hexanal may be formed via photooxidation of linoleic acid as well as gamma-linolenic acid. Meanwhile, propanal might be formed via photooxidation of linolenic acid. Based on propanal content (Figure 4.17), stripped flax oil had a higher ($p<0.05$) content of propanal as it is more susceptible to oxidation than NFO. Meanwhile, hexanal content of non-stripped hemp was more ($p<0.05$) than that of its corresponding counterpart SH (Figure 4.18). On the other hand, non-stripped DHASCO was more stable than its corresponding stripped counterpart based on propanal contents (Figure 4.19). This might be due to the pigments retained in S-DHASCO. However, non-stripped and stripped ARASCO and OMEGA-GOLD oil were not stable under fluorescent light, as indicated in the total volatile content found in S-ARASCO (Figure 4.20) and S-OMEGA-GOLD oil (Figure 4.21).

Figure 4.17 Propanal, hexanal and total volatile contents in non-stripped and stripped flax oil under fluorescent light at 27°C.

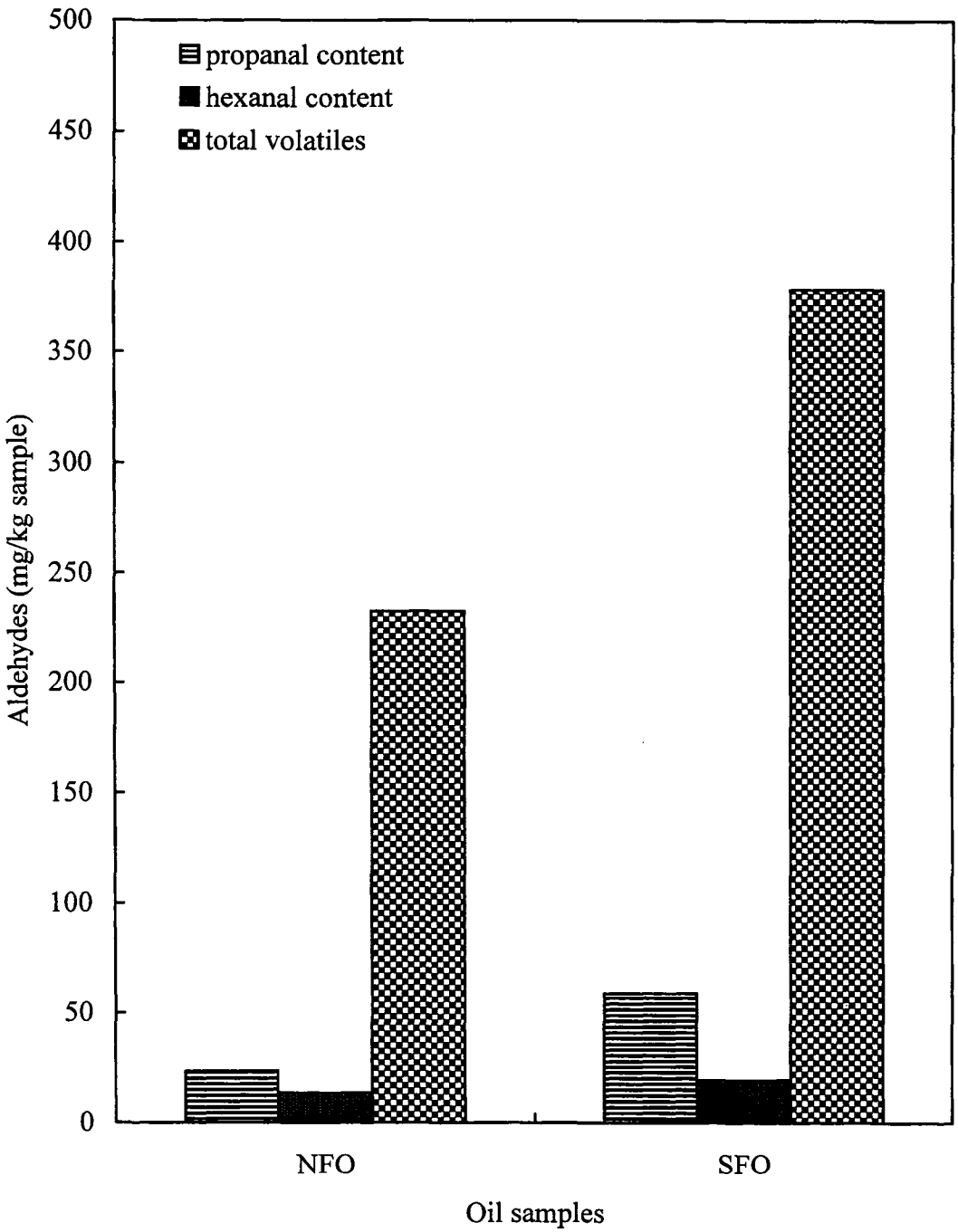

500
450
400
350
300
250
200
150
100
50
0
propanal content
hexanal content
total volatiles
Aldehydes (mg/kg sample)
NFO
SFO
Oil samples

Figure 4.18 Propanal, hexanal and total volatile contents in non-stripped and stripped hemp oil under fluorescent light at 27°C.

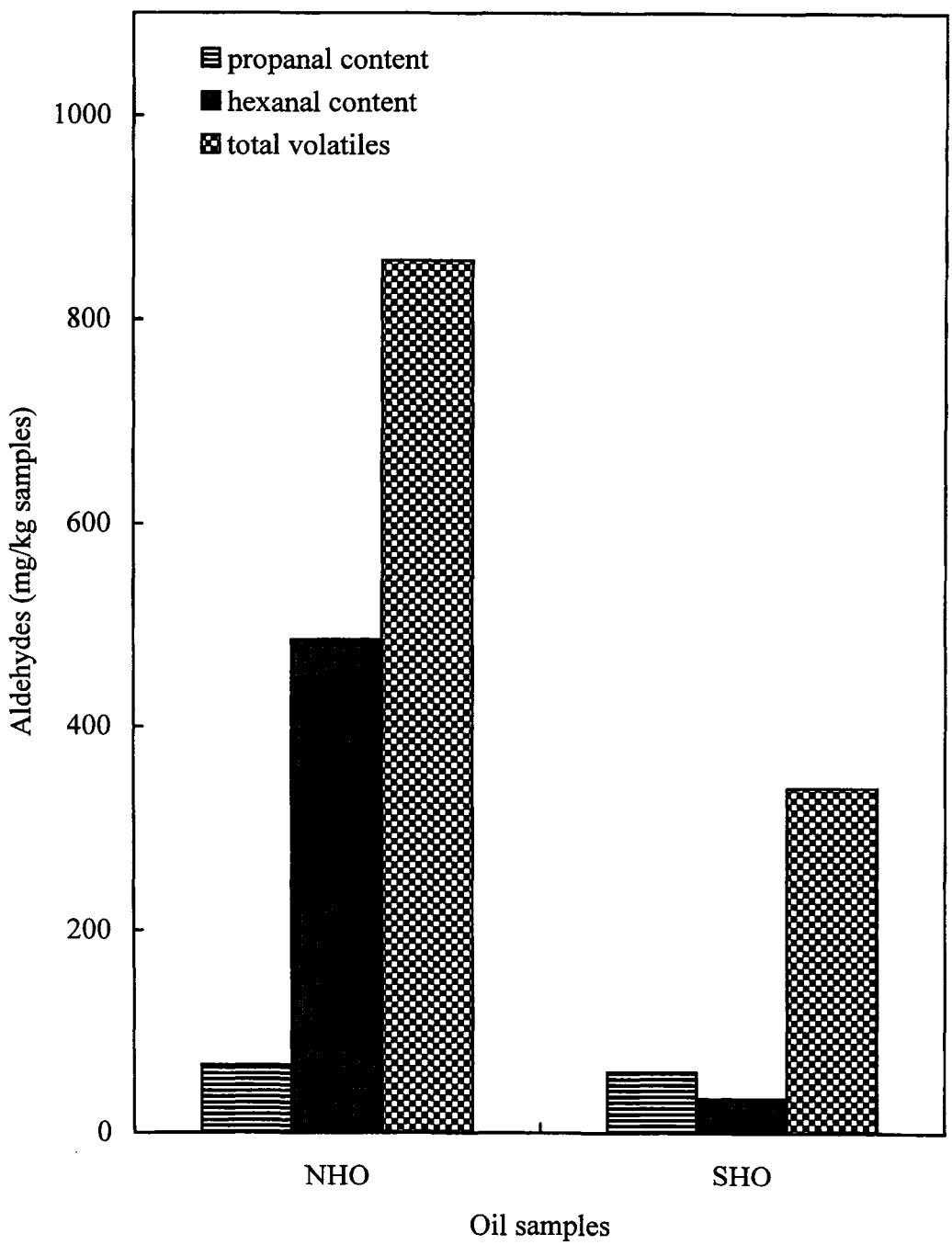

propanal content
hexanal content
total volatiles
1000
800
600
400
200
0
Aldehydes (mg/kg samples)
NHO
SHO
Oil samples

Figure 4.19 Propanal and total volatile contents in non-stripped and stripped DHASCO under fluorescent light at 27°C.

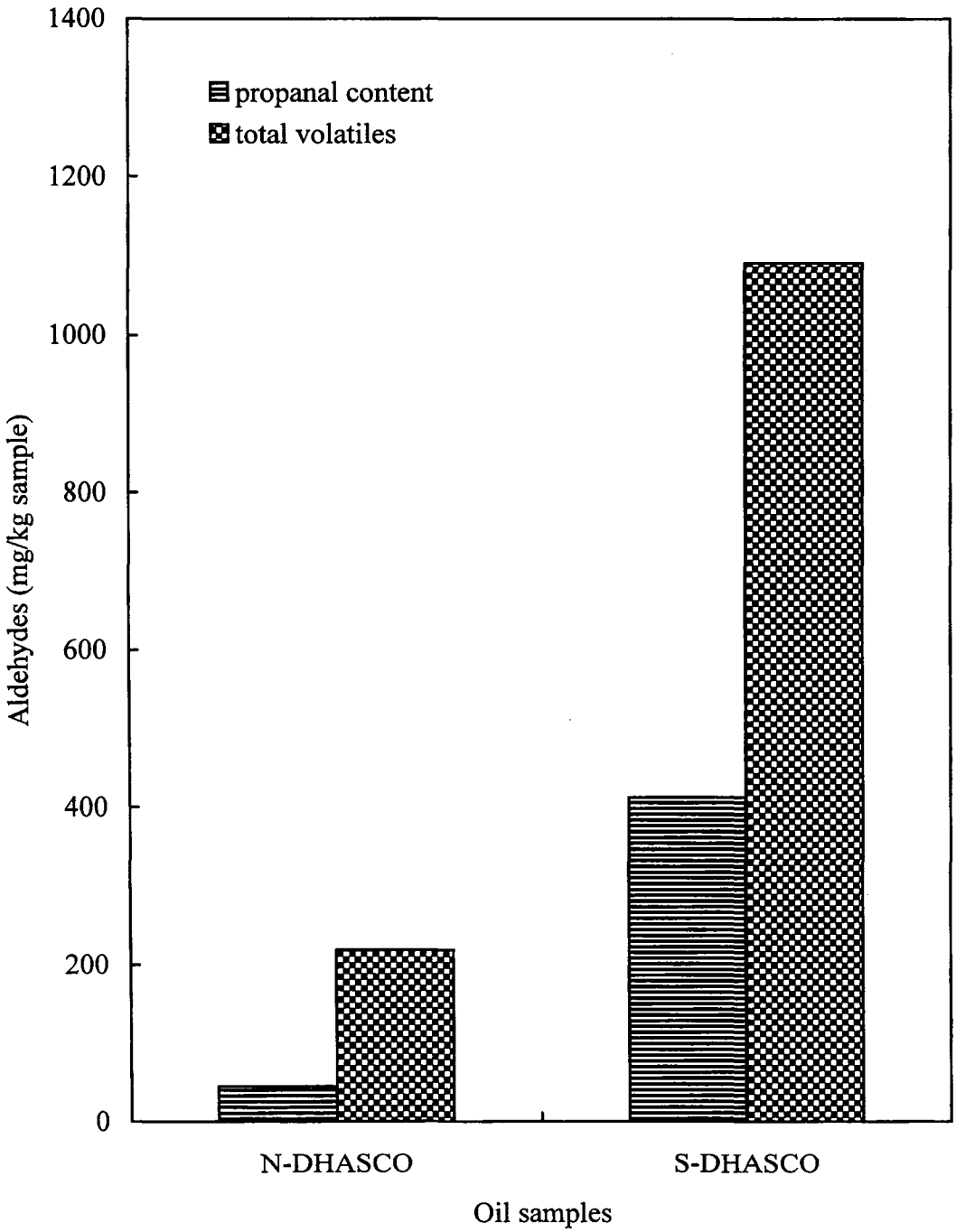

1400
1200
1000
800
600
400
200
0
propanal content
total volatiles
Aldehydes (mg/kg sample)
N-DHASCO
S-DHASCO
Oil samples

Figure 4.20 Hexanal and total volatile contents in non-stripped and stripped ARASCO under fluorescent light at 27°C.

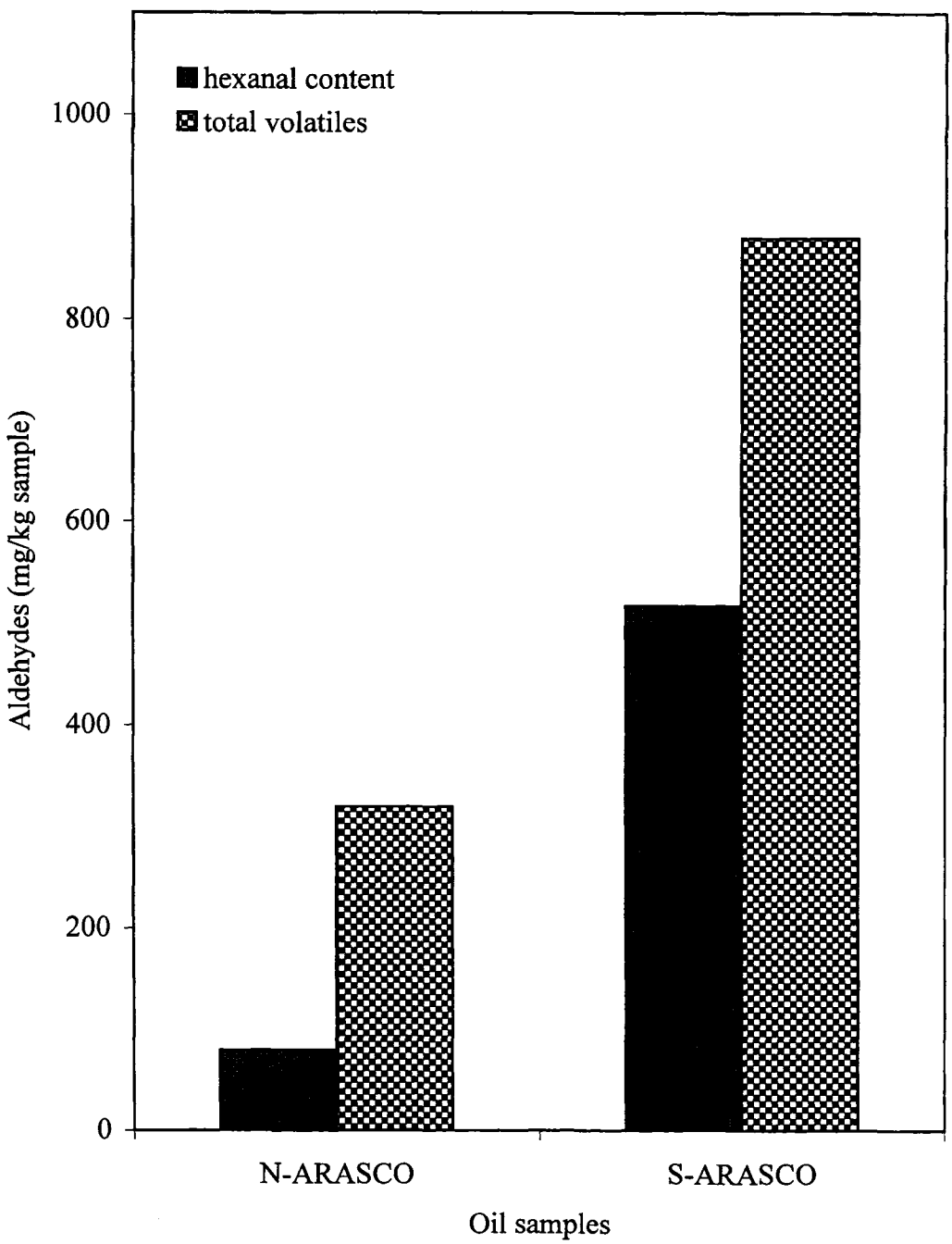
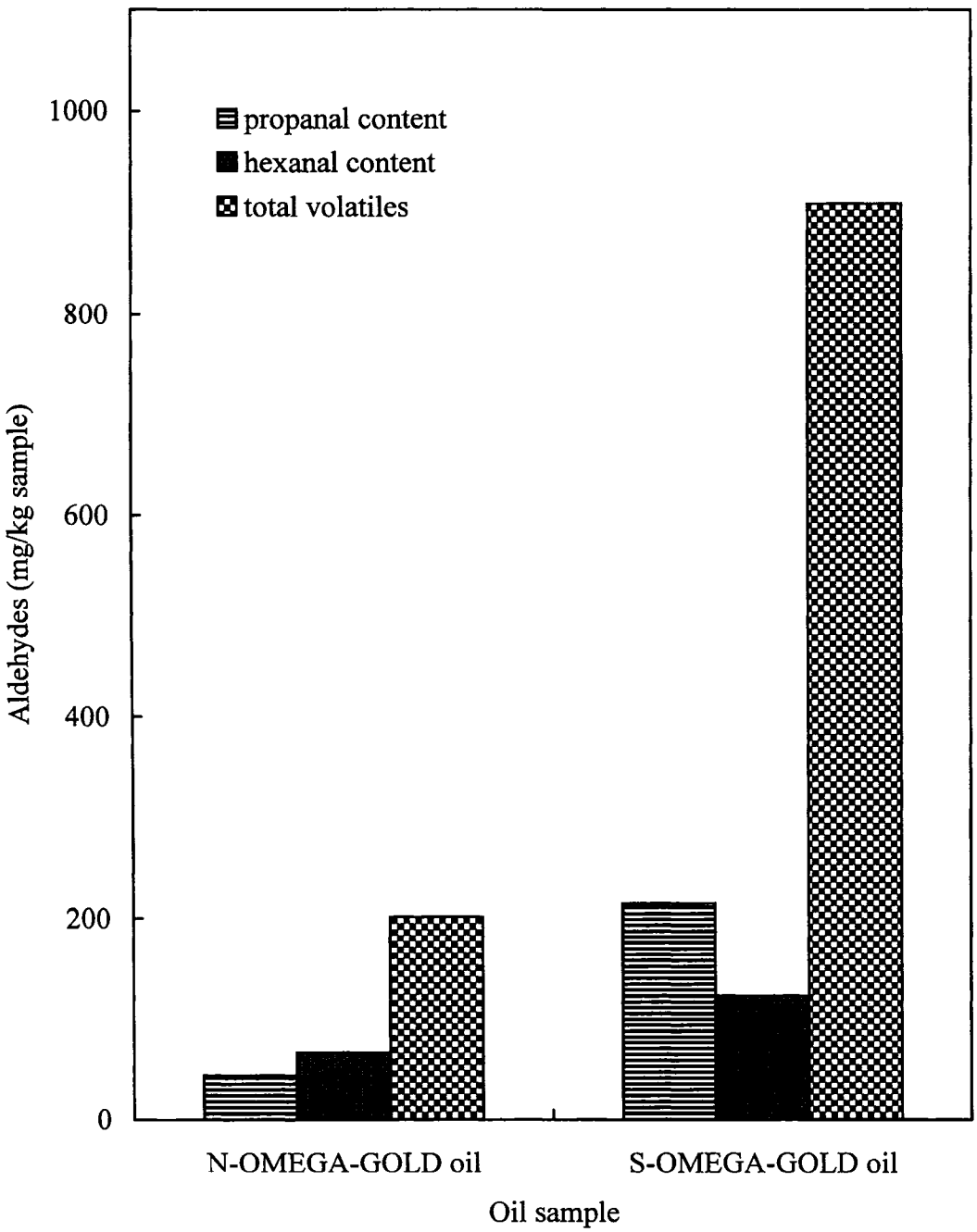

hexanal content
total volatiles
1000
800
600
400
200
0
Aldehydes (mg/kg sample)
N-ARASCO
S-ARASCO
Oil samples

Figure 4.21 Propanal, hexanal and total volatile contents in non-stripped and stripped OMEGA-GOLD oil under fluorescent light at 27°C.


1000
800
600
400
200
0
propanal content
hexanal content
total volatiles
Aldehydes (mg/kg sample)
N-OMEGA-GOLD oil
S-OMEGA-GOLD oil
Oil sample

4.4 Radical scavenging activity of flax, hemp, ARASCO, DHASCO, and OMEGA-GOLD oil

Examination of free radical scavenging properties of selected oils might identify the potential utilization of these oils in promoting human health. Generally known antioxidants, including radical scavengers, might protect important biomolecules from radical attacks and consequently reduce the risk of aging-associated health problems, such as cancer and heart disease (Aruoma, 1998).

4.4.1 DPPH radical scavenging activity of flax and hemp oil methanolic extracts

The model of scavenging stable free radicals is a widely used method for evaluation of the antioxidant properties of products in a relatively short time (Schwarz *et al.*, 2000). DPPH radical was used to evaluate free radical scavenging properties of flax and hemp oil extracts, mainly their minor components (Figure 4.22). Hemp oil extract, after 20 min of reaction with DPPH radical, exhibited a greater ($p<0.05$) DPPH radical scavenging activity than that observed for flax oil extract. This was indicated by the least amount of DPPH radical remaining after 20 min. The more the DPPH remaining after reaction of free radical with antioxidant extracts, the lower the antioxidant capacity. Similar kinetics were detected in antioxidant-DPPH radical reaction for the two oil extracts examined (Figure 4.23). Control represents the reaction mixture containing no extract. Similar studies were carried out using algal oils (Figure 4.22). Results shown in this figure demonstrate that DHASCO extract has the best ability to scavenge DPPH radical as the least amount of free radical remaining rather than ARASCO and OMEGA-GOLD oil

Figure 4.22 Comparison of radical DPPH scavenging activity of flax, hemp, ARASCO, DHASCO, and OMEGA-GOLD oil extracts after 20 min.

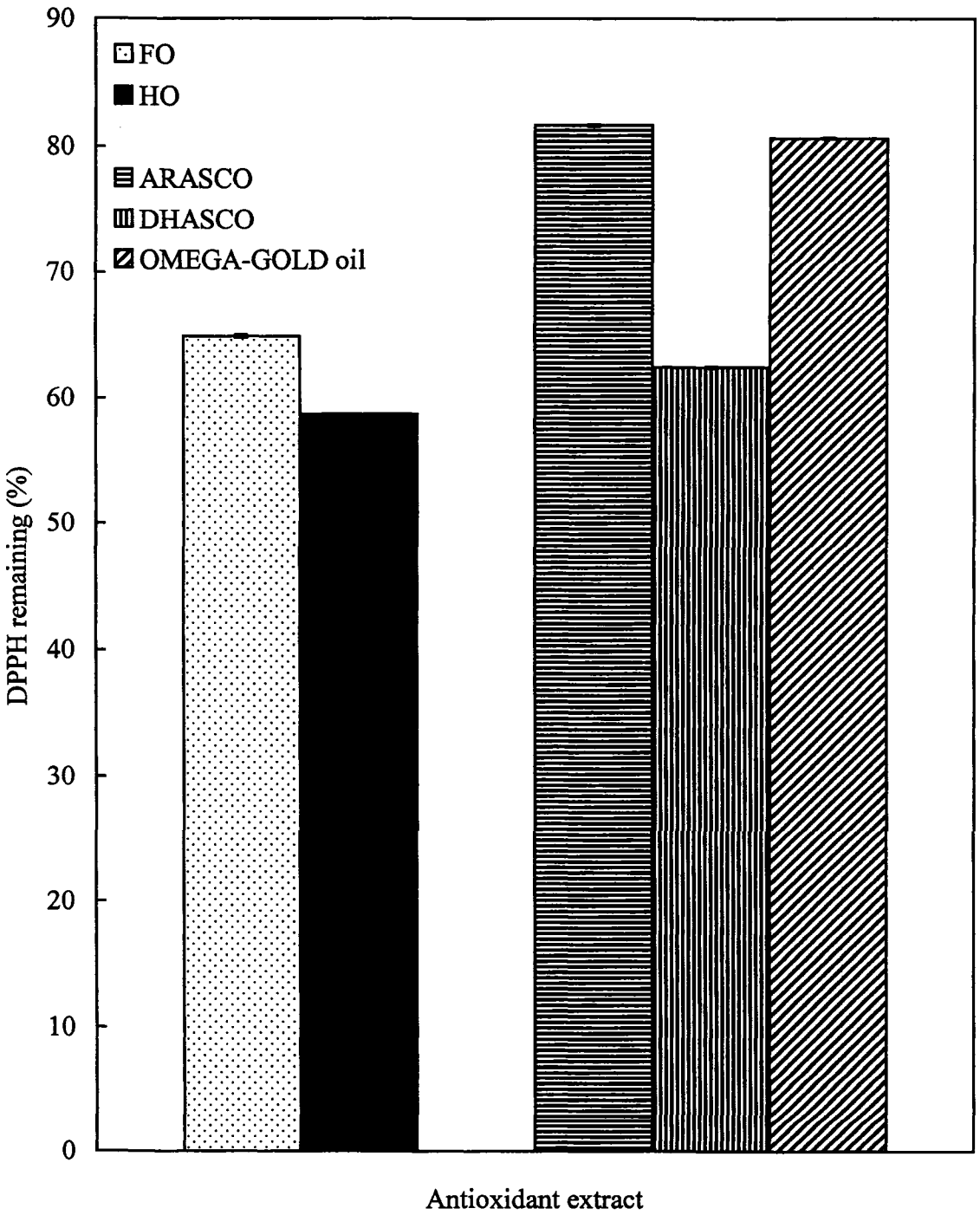
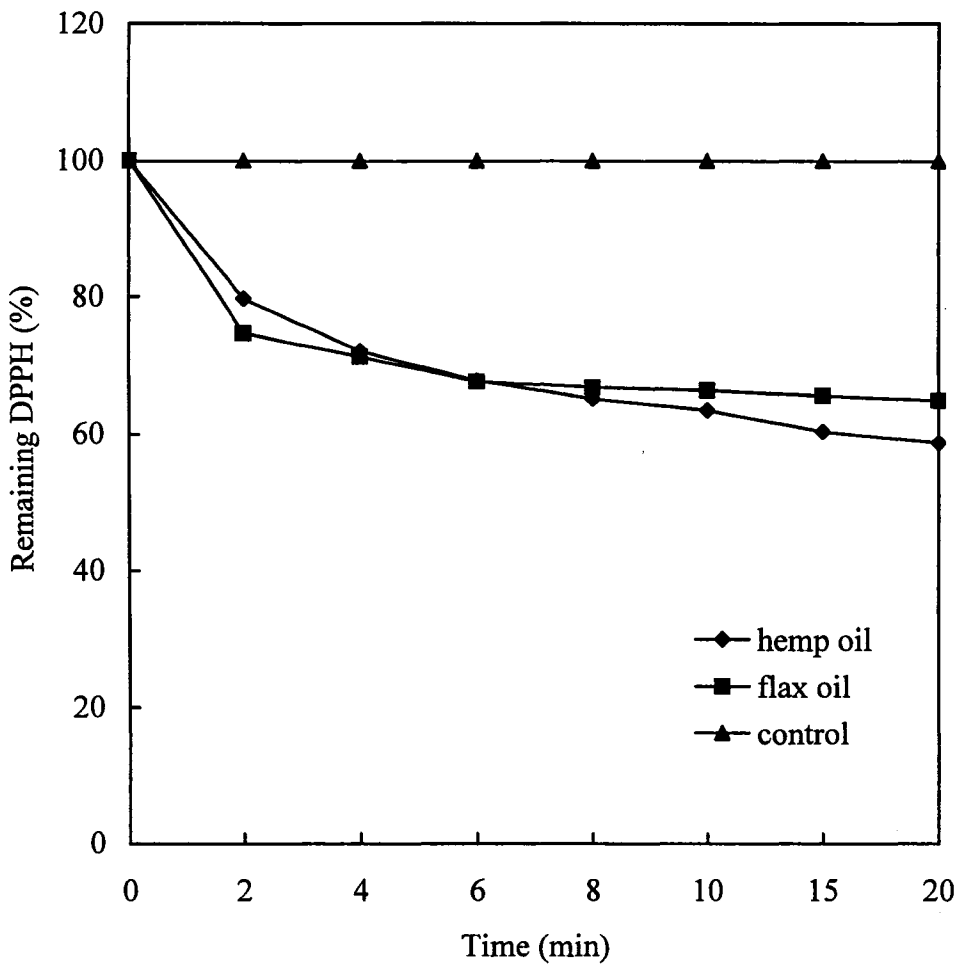

90
80
70
60
50
40
30
20
10
0
FO
HO
ARASCO
DHASCO
OMEGA-GOLD oil
DPPH remaining (%)
Antioxidant extract

Figure 4.23 Reaction of flax and hemp oil extracts with DPPH radical.


120
100
80
60
40
20
0
Remaining DPPH (%)
0
2
4
6
8
10
15
20
Time (min)
hemp oil
flax oil
control

extracts. Similar kinetics were detected in antioxidant-DPPH radical for these algal oil extracts (Figure 4.24). Meanwhile, depending on the standard curve prepared to calculate the tocopherol equivalent shown in Figure A.1 ($y= 0.0102x + 0.04252$, $r^2 = 0.9566$), hemp oil had a higher ($p<0.05$) capacity, expressed as α-tocopherol equivalent (mM), than flax oil extract (Figure 4.25). Similar studies were performed on ARASCO, DHASCO and OMEGA-GOLD oil extracts (Figure 4.25). Data indicated that DHASCO extract had the highest ($p<0.05$) antioxidant capacity expressed as α-tocopherol equivalents compared to ARASCO and OMEGA-GOLD oil extracts, which might explain the stability of DHASCO during autoxidation and photooxidation rather than the other two algal oils.

4.5 Determination of total phenolic contents of oil samples

It is well known that phenolic compounds contribute to the overall antioxidant capacity of oils. It is reported that phenolics have a great effect on the stability, sensory, and nutritional characteristics of oil samples and might prevent their deterioration through quenching of radical reactions responsible for lipid oxidation (Cai *et al.*, 2003; Tovar *et al.*, 2001). It has been reported that oil stability is correlated not only with the total amount of phenolics, but also with the type of phenolics present (Tovar *et al.*, 2001).

Total phenolics of flax and hemp oils, obtained by methanol extraction, are given in Figure 4.26 as gallic acid equivalents (GAE) ($y= 0.0113x-0.0232$, $r^2 = 0.9979$) as shown in Figure A.2. The levels of total phenolics determined in this way are not absolute measurements of the amounts of phenolic materials, but are in fact based on chemical

Figure 4.24 Reaction of ARASCO, DHASCO and OMEGA-GOLD oil extracts with DPPH radical.

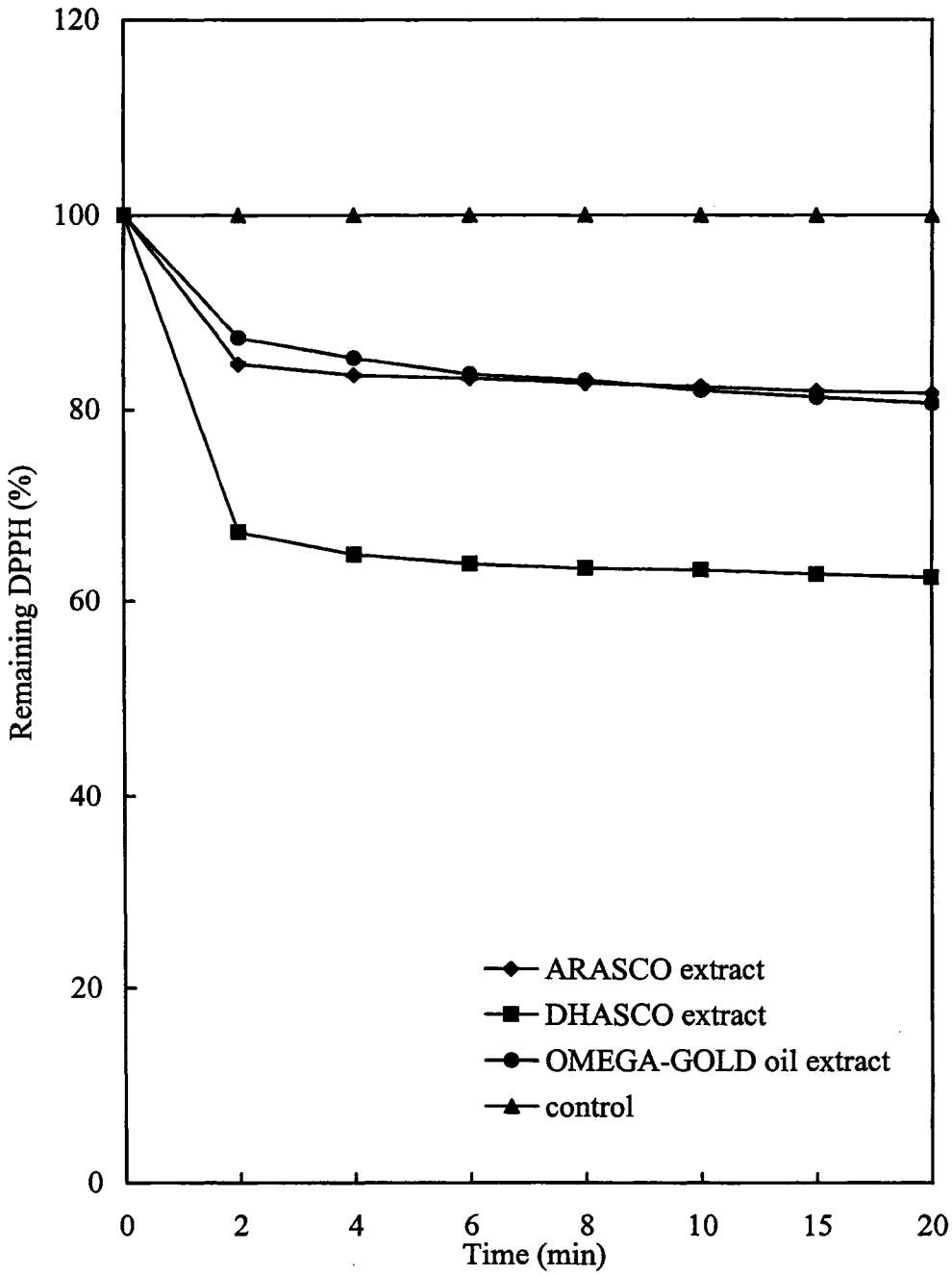

120
100
80
60
40
20
0
Remaining DPPH (%)
0
2
4
6
8
10
15
20
Time (min)
ARASCO extract
DHASCO extract
OMEGA-GOLD oil extract
control

Figure 4.25 Antioxidant capacity of flax, hemp, ARASCO, DHASCO and OMEGA-GOLD oil extracts with DPPH as α-tocopherol equivalents.

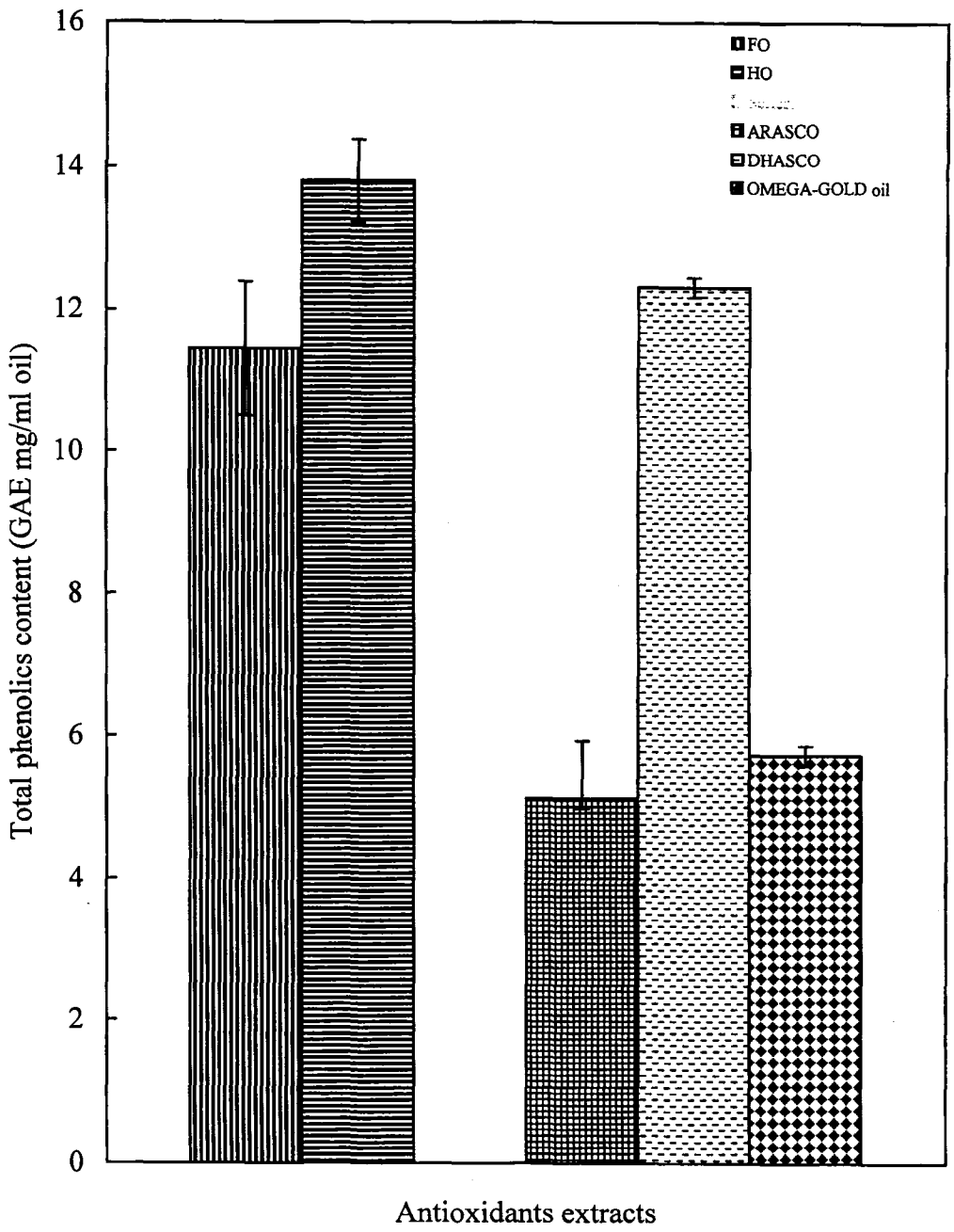
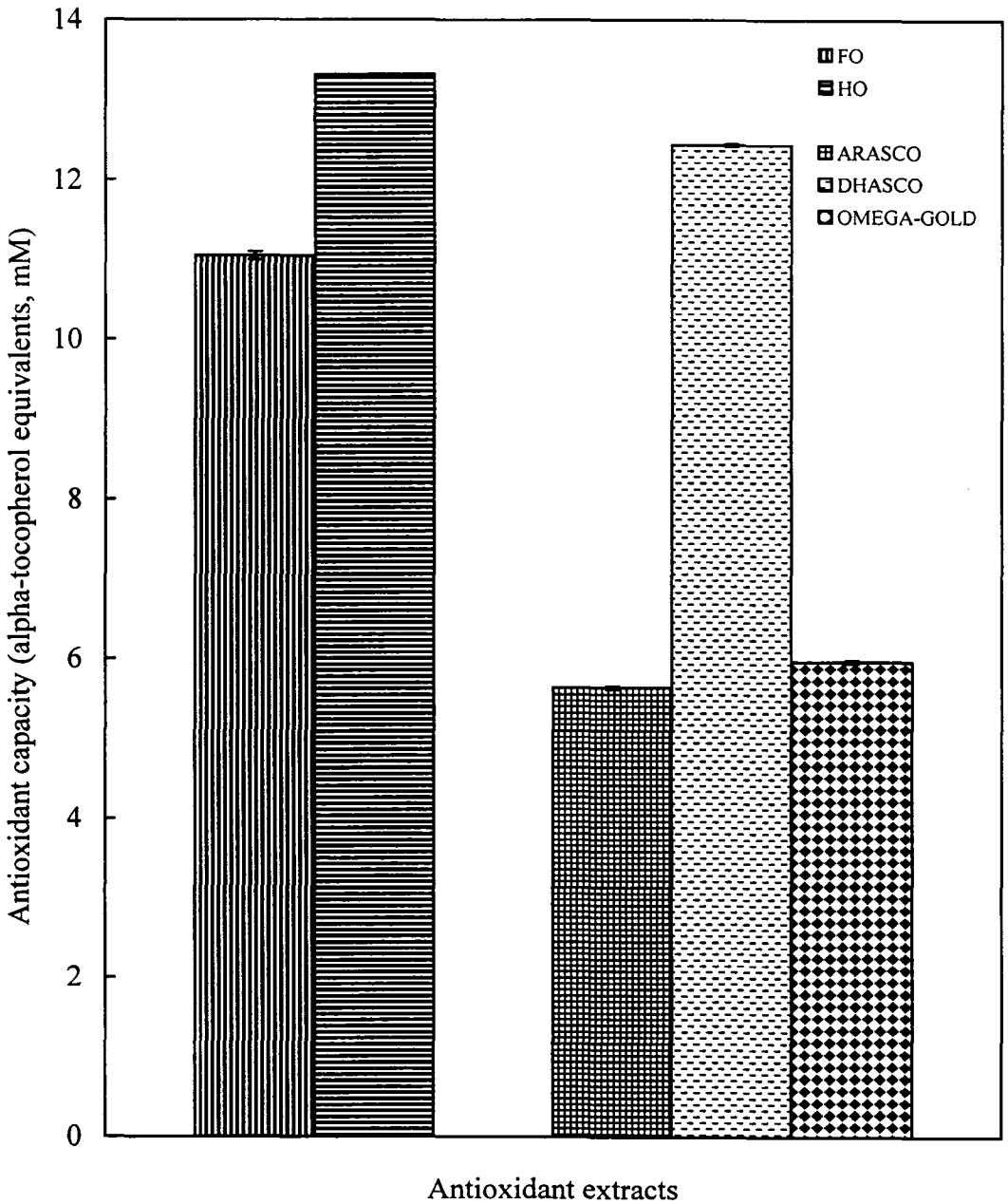

16
14
12
10
8
6
4
2
0
Total phenolics content (GAE mg/ml oil)
FO
HO
ARASCO
DHASCO
OMEGA-GOLD oil
Antioxidants extracts

Figure 4.26 Total phenolic contents of flax, hemp, ARASCO, DHASCO and OMEGA-GOLD oil extracts expressed as mg of gallic acid equivalents per ml of oil.


14
12
10
8
6
4
2
0
Antioxidant capacity (alpha-tocopherol equivalents, mM)
FO
HO
ARASCO
DHASCO
OMEGA-GOLD
Antioxidant extracts

reducing capacity of gallic acid. Data in Figure 4.26 shows that flax and hemp oil extracts had nearly the same ability to reduce Folin-Ciocalteu's reagent. The presence of these phenols might contribute to better stability of non-stripped flax and hemp oils under Schaal oven conditions as compared to those of their stripped counterparts. The total phenolics also expressed as GAE for ARASCO, DHASCO and OMEGA-GOLD oil are extracts as in Figure 4.26. Data in this figure demonstrate that DHASCO extract had the best ability ($p < 0.05$) to reduce the Folin-Ciocalteu's reagent compared to ARASCO, and OMEGA-GOLD oil extracts. These results are supported by the high stability of DHASCO under Schaal oven conditions as shown in section 4.2.

CONCLUSIONS AND RECOMMENDATIONS

A column chromatographic technique was employed as an effective means for stripping the minor components from flax and hemp oils, as well as ARASCO, DHASCO and the OMEGA-GOLD oil. Removal of the minor, non-triacylglycerol, components from oils influenced their oxidative stability in both the dark and under fluorescent light. Moreover, the behaviour of endogenous antioxidants in these oils was dependent on the pigments involved, and the amount of tocopherols present as well as other factors. The radical scavenging activity of these oils can be interpreted as the combined action of these endogenous antioxidants.

The results of this work (1) provide simple and reliable analytical methods to follow the oxidation of selected vegetable oils such as those of stripped and non-stripped flax and hemp oils, as well as algal oils such as those of ARASCO, DHASCO and OMEGA-GOLD oil, and (2) enhance our understanding of the parameters involved in the oxidation of nutritional and medicinal oils examined.

Based on the results represented in this thesis, it is recommended to: (1) develop new and more effective techniques to prepare stripped oils in large scale; (2) examine the effects of adding synthetic antioxidants to hemp, flax, ARASCO and OMEGA-GOLD oil to improve their oxidative stability and improve their safety and extend their shelf life; and (3) examine the link between antiradical properties and biological activities.

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APPENDIX

Figure A.1 Depletion in absorbance of tocopherol at 517nm at different concentrations (μM)

Correlation coefficient (r) = 0.9566

Equation of the line is Y= aX+b where,

Y= absorbance at 517nm (A_{517nm})

X= Concentration of tocopherols in (μM)

a = 0.0102

b= 0.0425

Therefore, Y= 0.0102X+0.0425

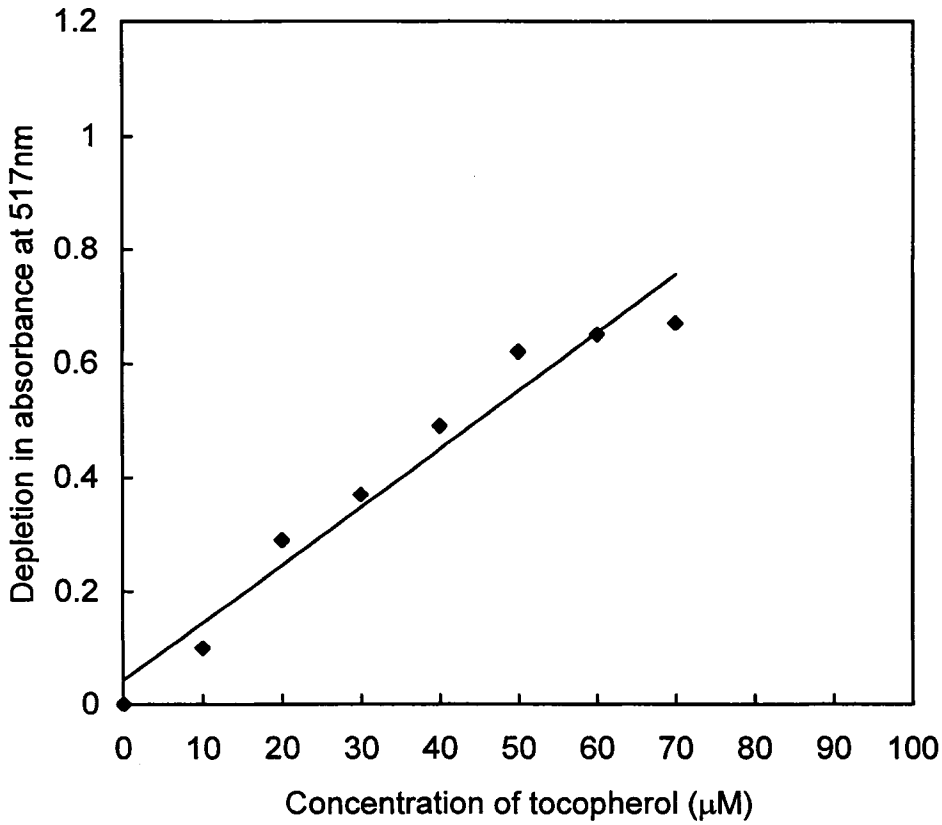

1.2
1
0.8
0.6
0.4
0.2
0
Depletion in absorbance at 517nm
0
10
20
30
40
50
60
70
80
90
100
Concentration of tocopherol (μM)

Figure A.2 The absorbance of gallic acid at 765nm at different concentrations (μg/ml)

Correlation coefficient (r) = 0.9979

Equation of the line is Y= aX-b where,

Y= absorbance at 765nm (A_{765nm})

X= Concentration of gallic acid in (μg/ml)

a = 0.0113

b= 0.0232

Therefore, Y= 0.0113X-0.0232

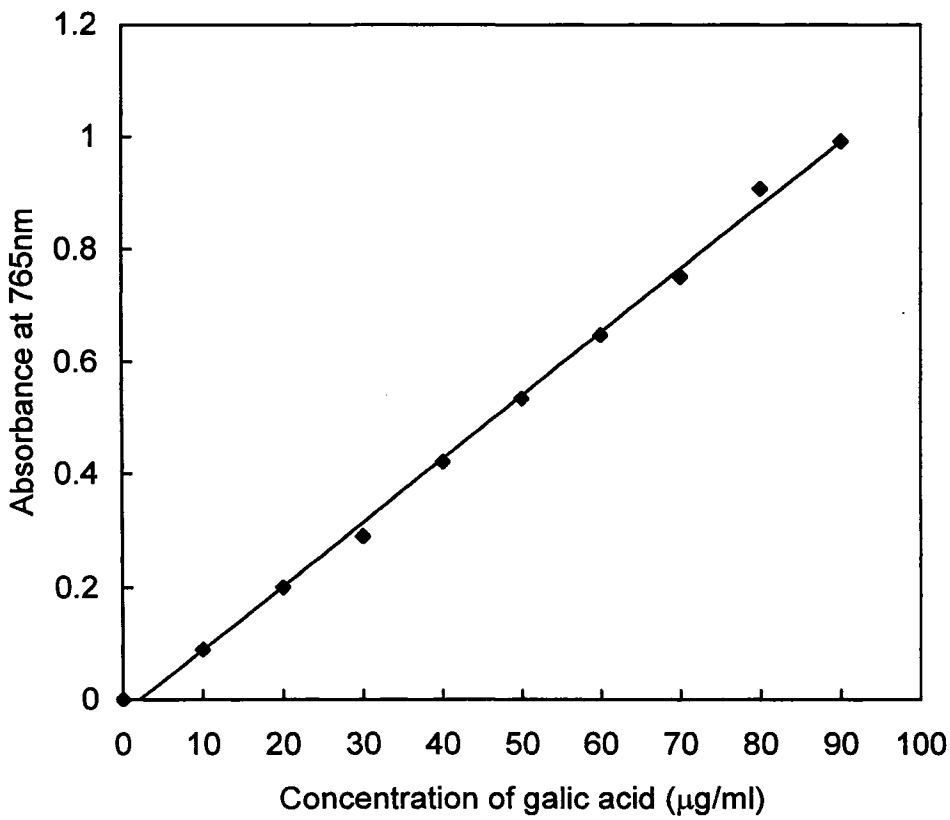

1.2
1
0.8
0.6
0.4
0.2
0
Absorbance at 765nm
0
10
20
30
40
50
60
70
80
90
100
Concentration of galic acid (μg/ml)

Figure A.3 Gas chromatogram of headspace volatiles of non-stripped (a) and stripped (b) flax oil after 7 days of storage under Schaal oven conditions at 60 C°.

Peak: (1) propanal; (2) hexanal; and (3) heptanone(internal standard).

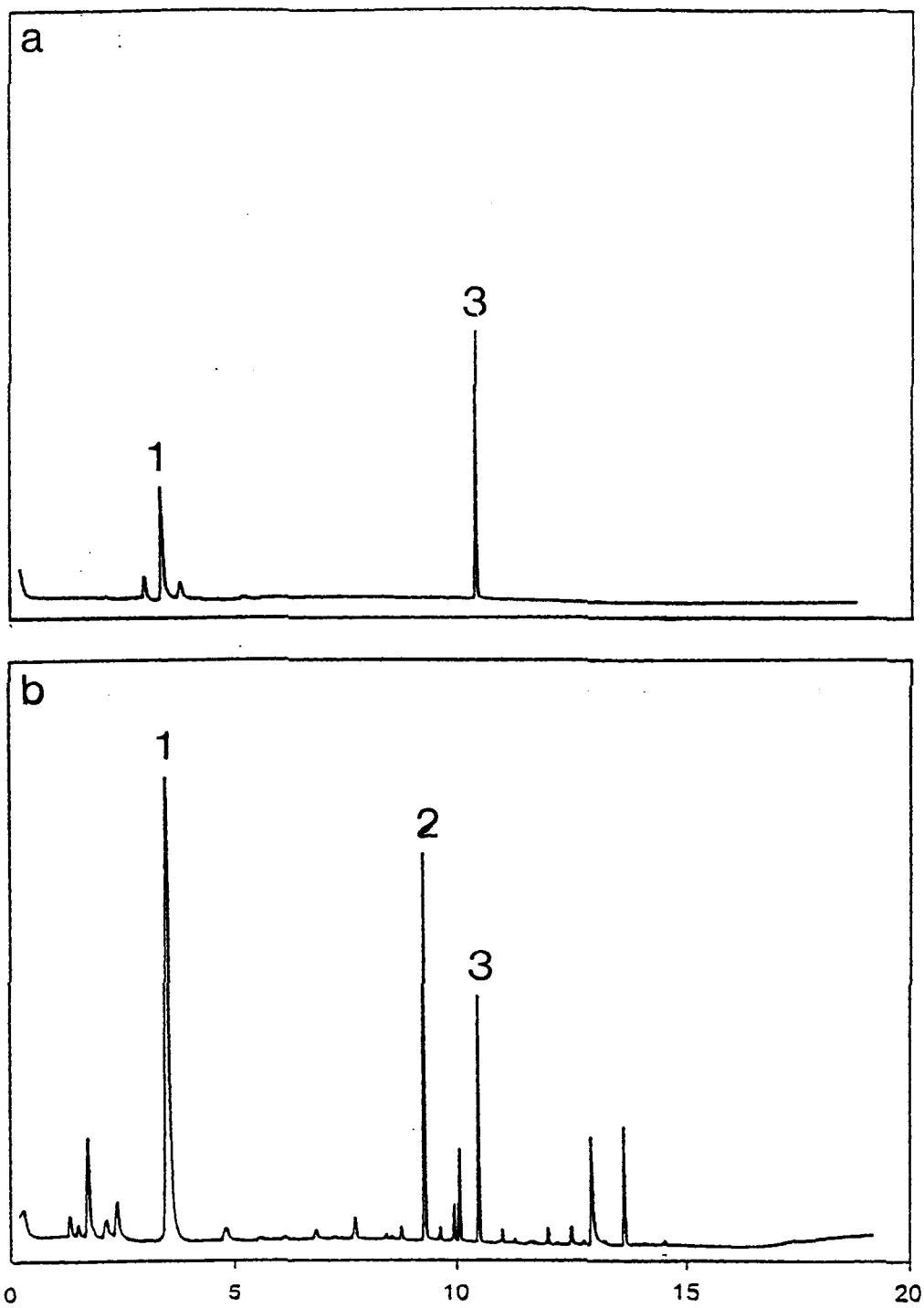

a
3
1
b
1
2
3
0
5
10
15
20
Time (min)

Figure A.4 Gas chromatogrâm of headspace volatiles of non-stripped (a) and stripped (b) hemp oil after 7 days of storage under Schaal oven conditions at 60 C°.

Peak: (1) propanal; (2) hexanal; and (3) heptanone (internal standard).

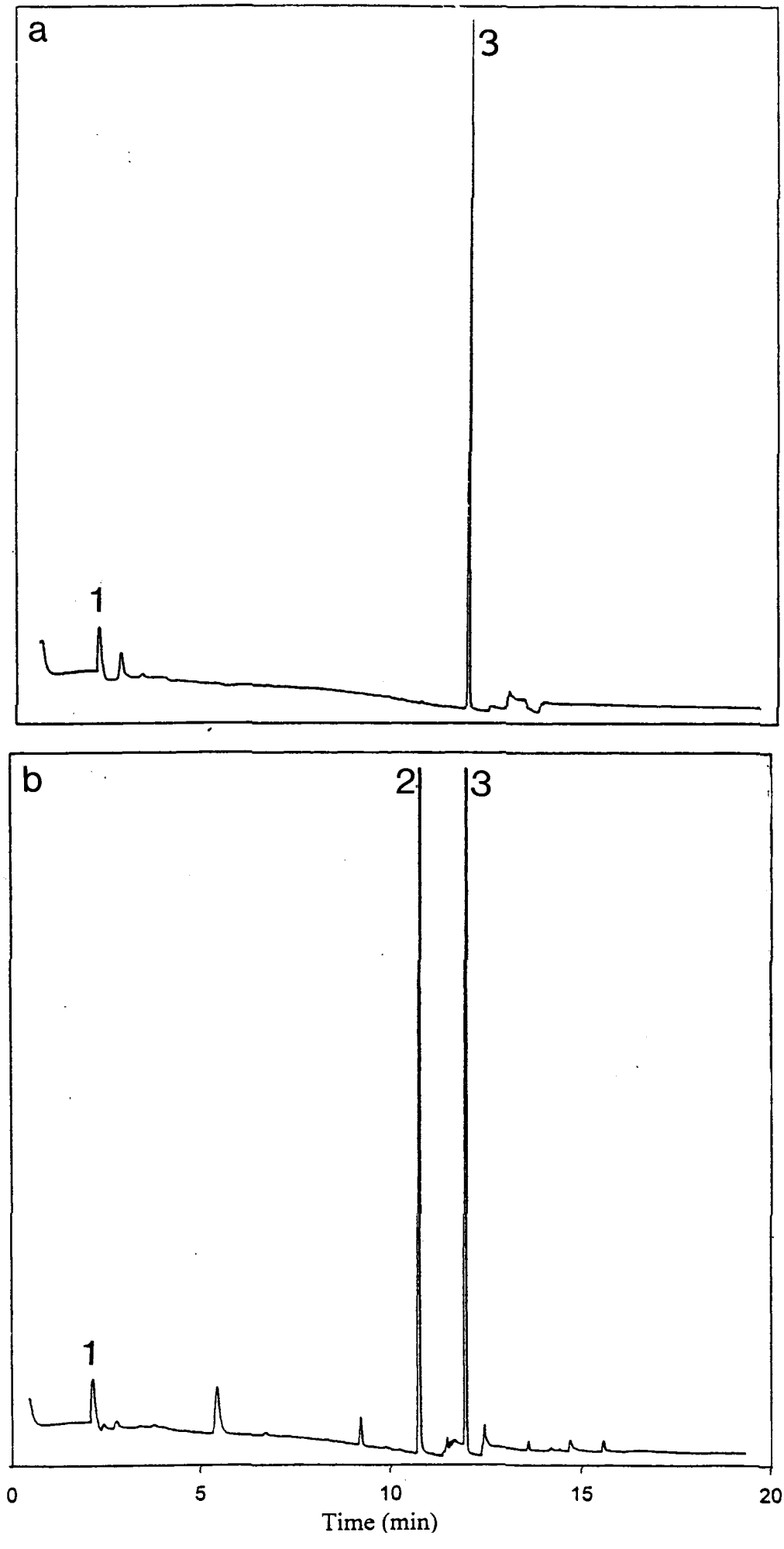

a
3
1
b
2
3
1
0
5
10
15
20
Time (min)

Figure A.5 Gas chromatogram of headspace volatiles of non-stripped (a) and stripped (b) flax oil after 24 hours under fluorescent light at 27C°.

Peak: (1) propanal; (2) hexanal; and (3) heptanone (internal standard).

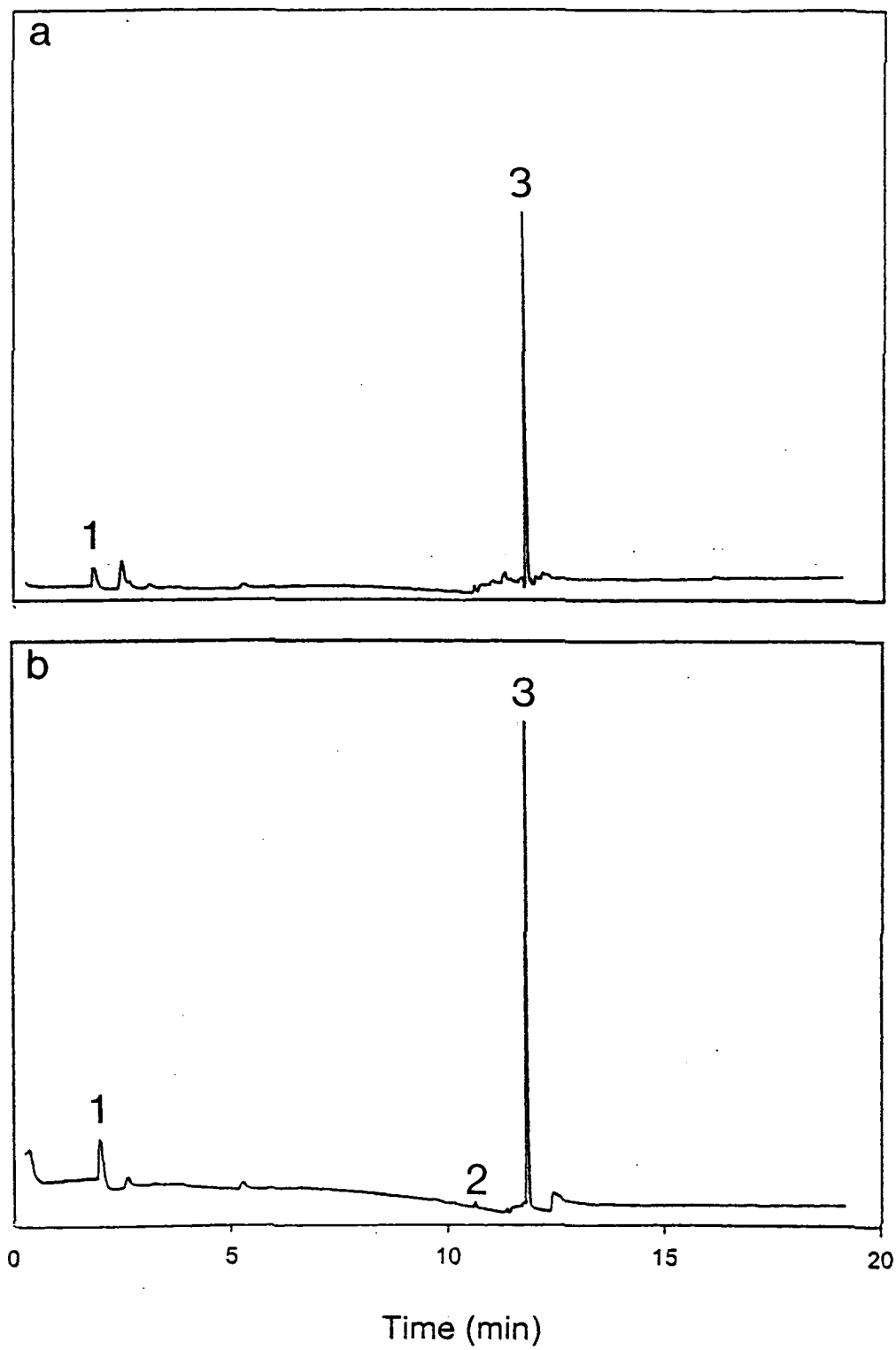
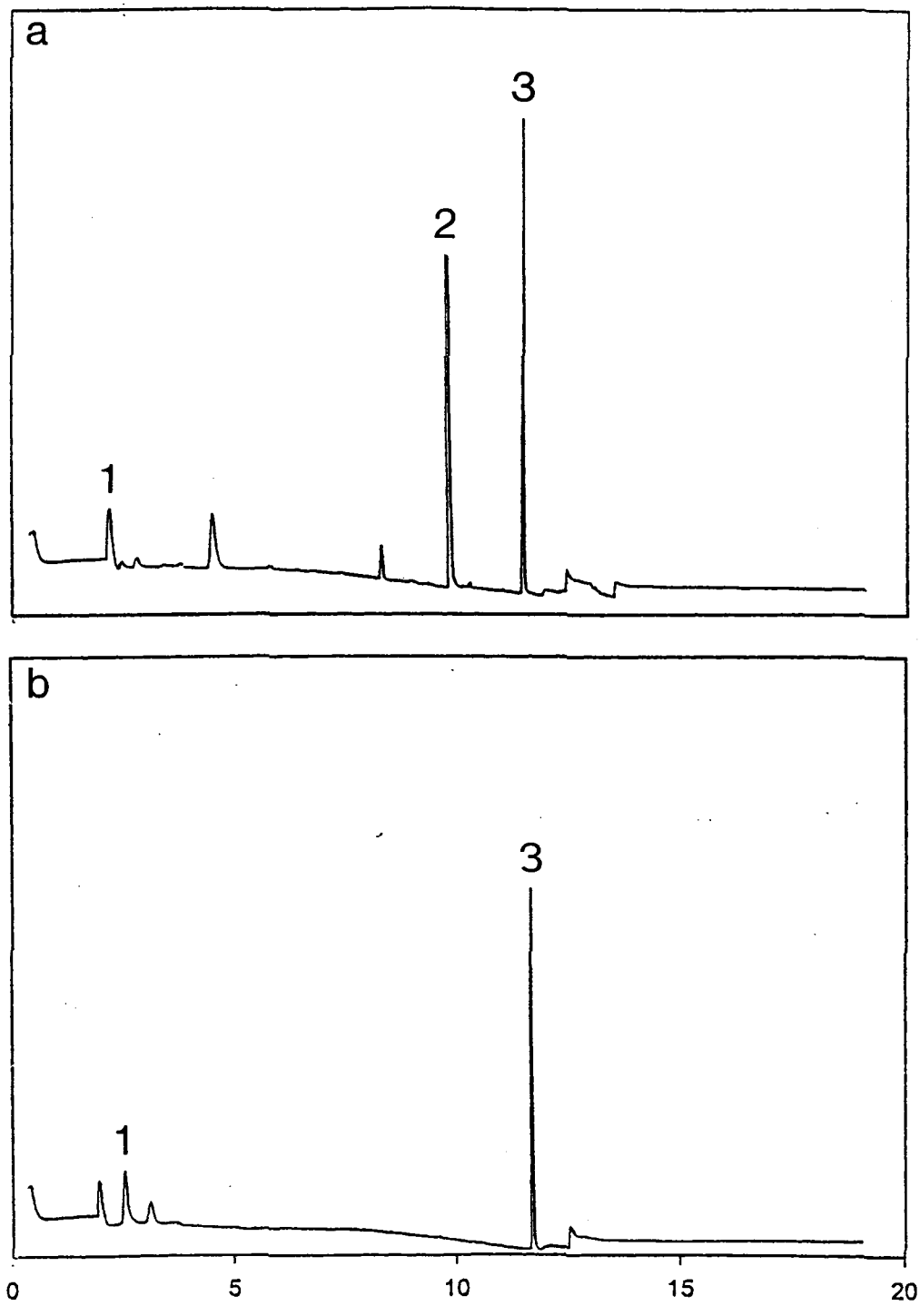

a
3
1
b
3
1
2
0
5
10
15
20
Time (min)

Figure A.6 Gas chromatogram of headspace volatiles of non-stripped (a) and stripped (b) hemp oil after 24 hours under fluorescent light at 27C°.

Peak: (1) propanal; (2) hexanal; and (3) heptanone (internal standard).


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