THE ROLE OF ENDOGENOUS LIPID COMPONENTS ON VEGETABLE OIL STABILITY

By

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In partial fulfilment of the requirements
for Degree of

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Foods and Nutritional Sciences Interdepartmental Program
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RUI C. ZAMBIAZI

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University

of Manitoba in partial fulfillment of the requirements of the degree

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Thirty three vegetable oils were evaluated in this study to determine the effects of effect of the major and minor endogenous components on the storage stability of vegetable oils.

Initially the oils were analysed for fatty acid composition, triacylglycerol species, lipid classes, tocopherols, tocotrienols, sterols, chlorophylls, carotenoids, phenolics and metals. The stability of the oils was monitored by measuring oxygen consumption and chemical indices during storage with and without light at 35^0C and 65^0C, respectively. Changes in the amounts of fatty acids, tocopherols, tocotrienols, chlorophylls, carotenoids, sterols and phenolic acids were determined.

The stability of the oils was dependent on a complex interaction among their endogenous components. During storage without light, the more saturated oils such as coconut and palm kernel were the most stable while the most highly unsaturated oil, conventional flax, was the least stable during storage without light. During storage with light, oil rich in carotenoids, palm golden oil, was the most stable

among the oils analysed, while virgin olive oil containing the highest amount of chlorophyll was the least stable.

Fatty acid composition of oils had a significant effect on oil stability, but the minor components also greatly affected the stability. Chlorophylls acted as pro-oxidants in olive, soybean, canola and cottonseed oils when stored with light. Carotenoids accelerated the oxidation of palm golden oil when stored without light. Metals acted as pro-oxidants when present at trace amounts in palm and coconut oils. Tocopherols and tocotrienols had an antioxidant effect in oils, and their effectiveness was concentration dependent. Phospholipids and phenolic compounds improved the oxidative stability of oils, while a high content of sterols was accompanied by a faster oxidation.

Application of Artificial Neural Networks was effective to predict the stability of oils using composition data. Utilization of fatty acids, tocopherols and tocotrienols composition was sufficient to accurately predict the stability of oils stored without light. The amounts of fatty acids, tocopherols, tocotrienols, chlorophylls and metals was necessary to predict the stability of oils stored in the presence of light.

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1. INTRODUCTION

Oxidative deterioration of fats and oils is one of the most fundamental reactions in lipid chemistry. Once initiated, the oxidation of lipid components results in the formation of primary compounds, which further decompose in a variety of products. These products resulting from lipid oxidation are quite complex and variable. They can produce undesirable flavours and odours, reduce nutritional quality and impart toxicity in fats and oils (Frankel, 1980; Chan *et al.*, 1982; Bradley & Min, 1992).

Lipid oxidation involves mainly the reaction between unsaturated fatty acids and oxygen. Therefore, oil stability is strongly dependent on the degree of unsaturation and the proportion of unsaturated and saturated fatty acids. Higher degrees of unsaturation in fatty acid chains markedly decrease the oil oxidative stability (Frankel & Huang, 1994). Fatty acids, in the form of triacylglycerols, are the major components in vegetable oils, while the presence of free fatty acids, chlorophylls, carotenoids, phospholipids, sterols, phenolic compounds, tocopherols, tocotrienols and trace metals are found at minor levels. These minor components have been reported to affect oxidative stability by different mechanisms (Frankel, 1991; Perrin, 1992; Čmolík *et al.*, 1995). The

influence of many of these minor components on oil oxidation is controversial or remains unclear. In addition, studies involving the effects of minor components on oil stability have been carried out by adding single components in different concentrations, or combinations of a few of them to purified oils. However, vegetable oil composition is very complex, and components present can be neutral, act as antioxidants and/or act as pro-oxidants. The isolation of many components from their natural system causes the destruction of the steric structure and changes their activity (Smouse, 1995). Therefore, the stability characteristic of an oil depends on a complex interaction between its natural components, even those present in only trace amounts. Therefore there is a need to determine and/or clarify the interactions between most endogenous oil components and their cumulative effect on oil stability. The main purpose of this study was to evaluate the effect of the endogenous components on oxidative deterioration in vegetable oils. The specific objectives were:

- To characterize the effect of major and minor endogenous vegetable oil components on the stability of oil during controlled accelerated storage with and without the presence of light;
- To evaluate which components have the greatest effect on oil stability;
- To establish a possible interaction among components which stimulate or depress oil deterioration;

- To use data of oil composition and experimental values of oxygen consumption measurements to predict oil stability by Artificial Neural Networks.

2. LITERATURE REVIEW

2.1. Constituents of oils and fats of plant origin

Oils and fats are the generic denomination for lipids in liquid and solid state at ambient temperature, respectively. The term lipids embraces a variety of chemical substances that are soluble in organic solvents, but practically insoluble in water (Kates, 1975). The major lipid constituents of oils and fats of plant origin are the glycerol esters of fatty acids triesterified as triacylglycerols, which make up to 99% of the total lipid composition (Shahidi & Shukla, 1996). Even though this group of compounds determines the physical and chemical characteristics of an oil or fat, other minor components are present, and to a certain extent, they also contribute to the quality and stability of the plant lipids (Chu & Lin, 1992; Shahidi & Shukla, 1996).

Several other minor components have been identified in oils and fats of plant origin, including tocopherols and tocotrienols, sterols, cholesterol, phospholipids, free fatty acids, fatty alcohols, phenols, chlorophylls and derivatives, carotenoids, waxes, hydrocarbons, diglycerides, monoglycerides and trace metals (Goh *et al.*, 1985; Perrin, 1992; Čmolik *et al.*, 1995). The amount of these components present in an oil is determined by the plant species and by the oil processing

conditions. Their proportion is usually higher in crude oils compared with oils that are refined and deodorized, because most of these components are decomposed or volatilized during the processing steps, resulting in only trace amounts in the refined, bleached and deodorized oils (Jawad *et al.*, 1984; Jung *et al.*, 1989; Manorama & Rukmini, 1991; Čmolik *et al.*, 1995).

2.2. Oil and fat degradation reactions

Oils and fats are subjected to chemical reactions during their processing, storage and utilization. These chemical changes may impart undesirable characteristics to the final products. The degradation process can be initiated either by hydrolysis or oxidation, but indubitably, at ambient temperatures the reaction with oxygen is the most important (Nawar, 1985; Robards *et al.*, 1988).

2.2.1. Lipid hydrolysis

Lipid hydrolysis involves the breakdown of ester bonds of lipid molecules. The lipid breakdown may result from the activity of enzymes (lipolysis), or from the reaction of glycerides with water (hydrolysis), resulting in the formation of diglycerides, monoglycerides, and free fatty acids (Stevenson *et al.*, 1984). Further degradation result in compounds that impart off-flavors and odors to the oil (Robards *et al.*, 1988).

In oilseeds and fruits containing oils, lipolytic reactions may have undergone substantial development by the time they are harvested, transported, and stored prior to their processing. The seeds or fruits that are broken or damaged release their naturally occurring enzymes, mainly phenolases, lipases, and lipoxygenases. These enzymes, under high moisture content and relatively high temperatures, favour a rapid increase in free fatty acid content (Pritchard, 1983; Basiron & Abdullah, 1995). During oil processing, these enzymes are inactivated by thermal treatment applied to the seed or oil. Therefore, lipolytic reactions have no significance after oil extraction, but degradation products produced prior to processing may influence the finished oil quality (Patterson, 1989).

Hydrolytic reactions are more likely to occur in the presence of water and low molecular weight fatty acids at high temperatures (180-200^{0}C). Under these conditions, water reacts also with non-triglyceride components, resulting in precipitates (Clark & Serbia, 1991). The hydrolytic reactions are generally more important during bulk seed storage and oil processing, mainly during the alkali refining step, in which oil and water are in contact at high temperatures. In refined oil and finished products, where water levels are below 0.1%, hydrolytic reactions are not important (Roden & Ullyot, 1984). On the other hand, hydrolytic reactions can be of great importance during food frying. During frying conditions,

water is introduced to the oil medium from the food, and a high temperatures (180°C to 190°C) is maintained for relatively long periods of time (White, 1991). Hydrolysis along with other chemical reactions, including oxidation and thermal decomposition, occur during the frying process (Stevenson *et al.*, 1984).

2.2.2. Lipid oxidation

Lipid oxidation is a complex process involving numerous reactions that give rise to a variety of chemical and physical changes (Labuza, 1971). These reactions often occur simultaneously and competitively (Frankel, 1980; Bradley & Min, 1992). They involve mainly the oxidation of polyunsaturated fatty acids present in different levels in most oils and fats of plant origin. Therefore, they are considered one of the most fundamental reactions in lipid chemistry (Frankel, 1991). In particular, the oxidation of polyunsaturated fatty acids has received much attention due to its involvement in both food spoilage and nutritional losses, resulting in reduced shelf life and a lower overall quality of food products (Sherwin, 1978; Toro-Vazquez *et al.*, 1993). The lipid oxidation products may also have relevant implications for lipid peroxidation in vivo, resulting in undesirable effects on human health (Addis & Warner, 1992).

Even though unsaturated fatty acids are the main compounds involved in lipid oxidation, saturated fatty acids

may also contribute to the oxidation process. Saturated fatty acids and their esters are far more stable than the analogous unsaturated fatty acids. However, with the aid of metal catalysts, or when heated in the presence of air at temperatures higher than 180°C, they may undergo oxidation, forming complex decomposition products (Labuza, 1971). It is generally accepted that the thermal oxidation of saturated fatty acids does not occur selectively at a single location along the chain; therefore, the oxygen attack can occur on all the carbon atoms of the fatty acid chain, initially involving the formation of monohydroperoxides. Further stepwise oxidation causes the formation of several other compounds. As the temperature increases, hydroperoxide decomposition and secondary oxidation occur at faster rates, leading to a wide range of reactions (Brodnitz, 1968). At 100°C the rate of oxidation of saturated fat is about 100 times slower than for unsaturated linoleate, and it is essentially unmeasurable during storage below 50°C to 60°C (Labuza, 1971).

Unsaturated fatty acids are much more reactive than saturated fatty acids, because the presence of a double bond between carbon atoms weakens the neighbouring carbon-hydrogen bonds of the lipid molecule, which then becomes susceptible to oxidation (Brodnitz, 1968). Lipid oxidation reactions can occur either by abstraction of a hydrogen atom from the position adjacent to a double bond, or by a reaction with ground state molecular oxygen generated by a sensitizer

(Gunstone, 1984; Frankel, 1991).

2.2.2.1. Autoxidation

In the presence of initiators, unsaturated lipids react with oxygen to yield carbon-centered alkyl and peroxyl radicals, which propagate in the presence of oxygen by a free radical chain mechanism to form hydroperoxides as the primary products. This process is commonly described in terms of initiation (a), propagation (b), and termination (c) steps (Fig. 2.2.2.1) (Frankel, 1980; Chan *et al.*, 1982; Gunstone, 1984; Cosgrove *et al.*, 1987; Shahidi *et al.*, 1992).

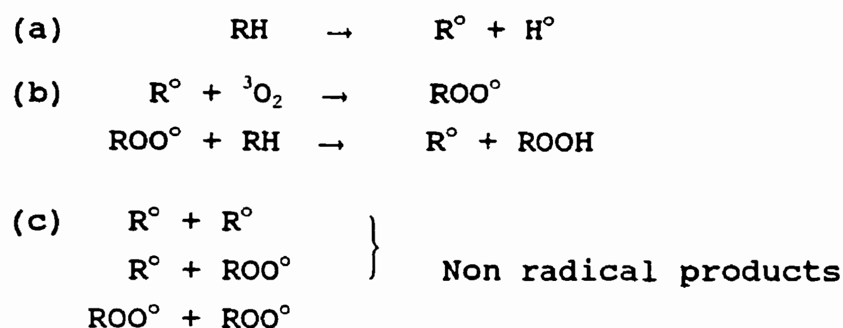


Fig. 2.2.2.1. Scheme of the mechanism of unsaturated lipid autoxidation (Shahidi *et al.*, 1992).

Initially the organic substrate (RH) is subjected to a rupture in the carbon-hydrogen position. Since this reaction is thermodynamically difficult, the production of the first radicals requires the presence of a catalyst to initiate the reaction. This first step is still unclear, but the

production of free radicals may take place by direct thermal dissociation, by hydroperoxide decomposition, by metal catalysis, or by exposure to light with or without the intervention of photo-sensitizers (Frankel, 1980). As a result a hydrogen atom from the organic molecule is lost (H°), generating a carbon-centered radical (R°). The hydrogen lost is an alpha-methylene hydrogen atom, easily detachable due to the influence of the neighbouring double bond(s). Once formed, the carbon-centered radical promptly reacts with molecular oxygen ($^3O_2$), to give an unstable chain-carrying peroxyl radical (ROO°) (Cosgrove *et al.*, 1987). The peroxyl radical reacts with another organic substrate, producing a hydro peroxide (ROOH), and a further carbon-centered radical (Frankel, 1991). The free radicals (R°) resultant from the organic substrate oxidation act as strong initiators or promoters for further oxidation due to their extra electron which has an unpaired spin in the chemical bond (Thomas, 1995). Thus, the oxidative degradation of fats and oils becomes a self-perpetuating process or a chain reaction, often described as being an autocatalytic, or an autoxidative process (Sherwin, 1978).

In the final or terminating step of the autoxidation process, free radicals can interact with their counterparts, or with hydrogen donated from antioxidants, resulting in non-radical products. The combination of two radicals is a process with a low energy of activation; however, this

occurrence is limited both by the concentration of radicals as well as by steric factors when radicals are required to interact at a specific active center on the molecules (Shahidi *et al.*, 1992).

2.2.2.2. Photo-oxidation

The mechanism of photo-oxidation is different from that of the free radical autoxidation which usually occurs in foods (Gunstone, 1984). Photo-sensitized oxidation occurs in the presence of components naturally present in the lipid system and light. These components are known as photo-sensitizers or chromophores, due to their capability to capture and concentrate light energy. The capture and concentration of light is dependent upon the arrangement of electrons around the atomic nuclei of their structure (Bradley & Min, 1992).

The excitation of photo-sensitizers causes the absorption of energy by oxygen, resulting in the formation of singlet oxygen. Light catalysed reactions are complex in nature, and the most pronounced effects are observed with light of short wavelengths, which have the highest quantum energy (Radtke *et al.*, 1970). Through absorption of near ultra violet (UV) or visible light, an electron from the sensitizer (sens) is boosted to a higher energy level; consequently, the sensitizer becomes an electronically excited unstable singlet (^{1}sens). Once excited the sensitizer undergoes an internal conversion by an energy intersystem crossing from one excited state into

another of the same spin state, resulting in two excited states: the singlet (^{1}sens) and the triplet (^{3}sens) states. The longer lifetime of the triplet state makes the initiation of the photo-sensitized oxidation more possible (Bekbölet, 1990).

Once the triplet-excited sensitizer (^{3}sens) is formed, two pathways have been proposed for photo-oxidation (Fig. 2.2.2.2).

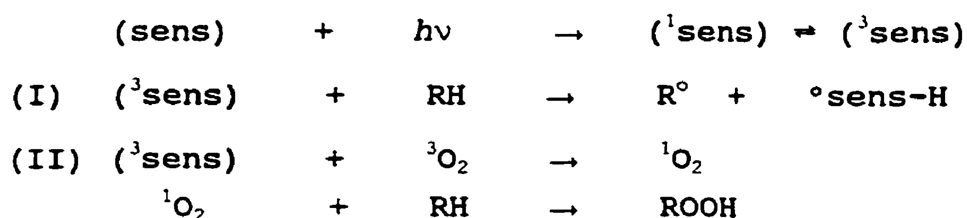


Fig. 2.2.2.2. Pathways of lipid photo-oxidation (Bradley & Min, 1992).

In mechanism I, the sensitizer (sens) absorbs light ($h\nu$) and interacts directly with an organic substrate (RH), resulting in a free radical (R°) and a sensitizer intermediate (sens-H). In mechanism II, molecular oxygen ($^3O_2$) rather than the substrate is presumably the species that reacts with the sensitizer upon light absorption. The energy transfer from the excited triplet sensitizer to a ground state oxygen is very fast (Bradley & Min, 1992), and as a result the triplet oxygen shift one of the unpaired ground state electrons to a higher energy level, producing an excited singlet state of

oxygen ($^1O_2$). The resulting singlet oxygen state ($^1O_2$) is more reactive than the ground state oxygen due to its unstable energy state. As a result, the singlet oxygen releases its excess of energy by reacting directly with electron-rich double bounds (St. Angelo, 1996).

The reaction of singlet oxygen with the high electron density organic substrate is one of the two carbons attached by a double bond of fatty acids, by a symmetrical addition known as the *ene* reaction (Gunstone, 1984).

2.2.2.3. Lipid oxidation products

2.2.2.3.1. Products from lipid oxidation

The oxidation of unsaturated fatty acids proceeds by a multi step reaction with hydroperoxides and cyclic peroxides as the primary products (Labuza, 1971; Carlsson *et al.*, 1976). Due to the resonance stabilization of the carbon-centered radical (R°), the reaction pathway is usually accompanied by a shift in the position of double bonds. This rearrangement results in the formation of isomeric hydroperoxides that often contain conjugated diene groups (Frankel, 1980).

Autoxidation and photo-oxidation proceed in different pathways, resulting in the formation of different primary hydroperoxides (Labuza, 1971; Gunstone, 1984).

Methyl oleate autoxidation involves hydrogen abstraction of carbon-8 (C_8) and carbon-11 (C_{11}), producing two allylic radicals. The oxygen attack on the end carbon positions of

the delocalized radicals produces four isomeric hydro peroxides (Frankel, 1985). However, the photo-oxidation of methyl oleate follows a direct attack by oxygen on its double bond, initially generating only two hydroperoxides (Gunstone, 1984). Methyl linoleate autoxidation (Fig. 2.2.2.3.1.1) leads to the formation of a mixture of two conjugated hydroperoxides, while photo-oxidation generates four isomeric, two conjugated and two unconjugated, hydroperoxides (Frankel, 1980; Wong, 1989). Oxidation of methyl linolenate results in four hydroperoxides by autoxidation, while six hydroperoxides are generated by photo-oxidation (Frankel, 1985).

Lipid hydroperoxides formed by either autoxidation or photo-oxidation reactions are highly unstable; therefore, they are readily decomposed by several mechanisms yielding radical and nonradical products. The first monohydroperoxides formed may produce hydroperoxides, dihydroperoxides, bicycloendo-peroxides, and hydroperoxide epidioxides (Frankel, 1980, 1985, 1991). Further reactions of these hydroperoxides and hydroperoxide derivatives may cause the formation of several different compounds, such as carbonyls, hydrocarbons, ketones, oxyacids, epoxides, oxo-esters, and alcohols (Chan *et al.*, 1982; Frankel, 1980; Frankel, 1991), which are also susceptible to subsequent chemical reactions, generating different compounds (Fig. 2.2.2.3.1.2) (Nawar, 1985).

The actual amount and type of final compounds formed during oxidation depends on the type and amounts of initial

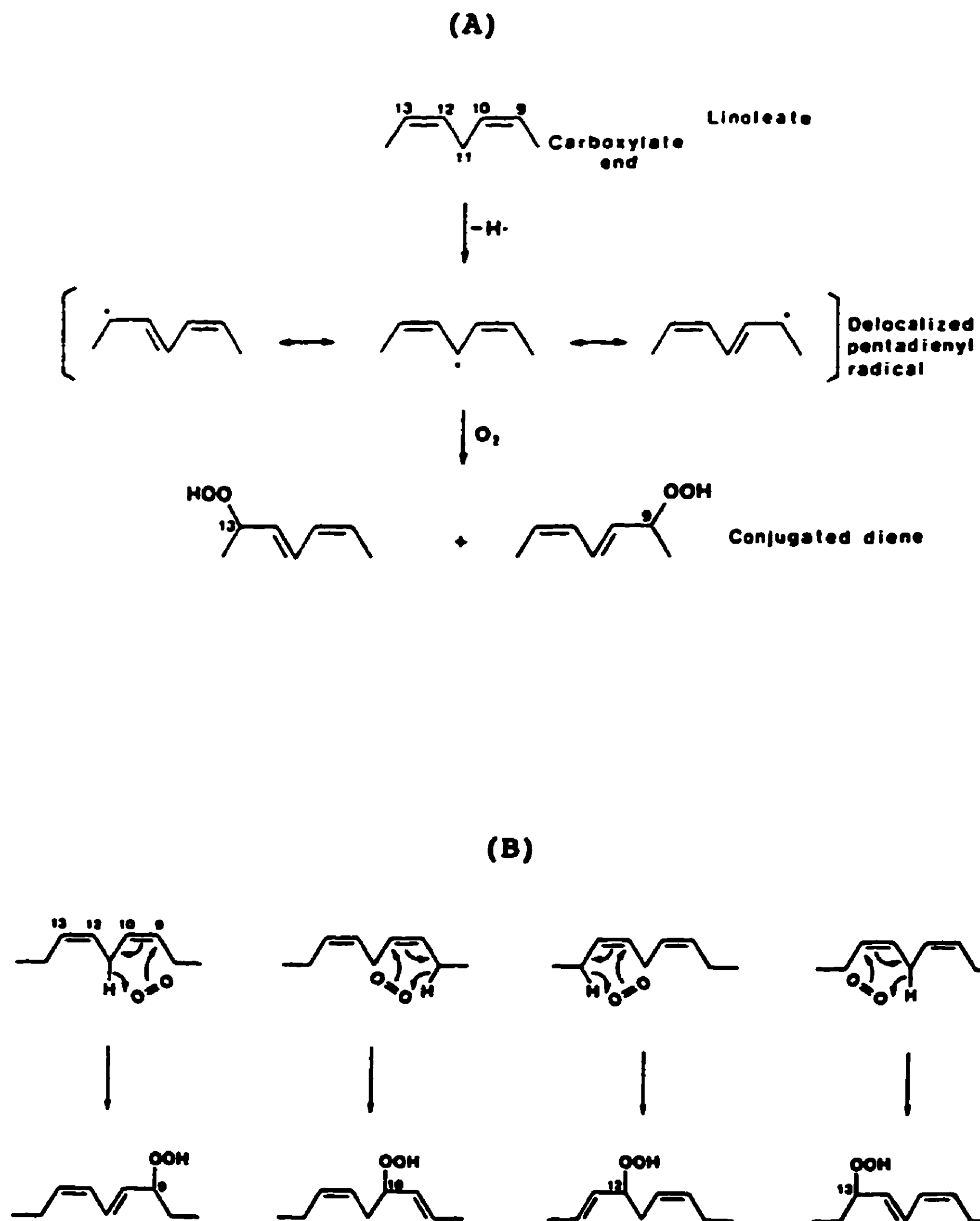


Fig. 2.2.2.3.1.1. **Mechanism of methyl linoleate oxidation.** A)autoxidation; B)photo-oxidation (Wong, 1989).

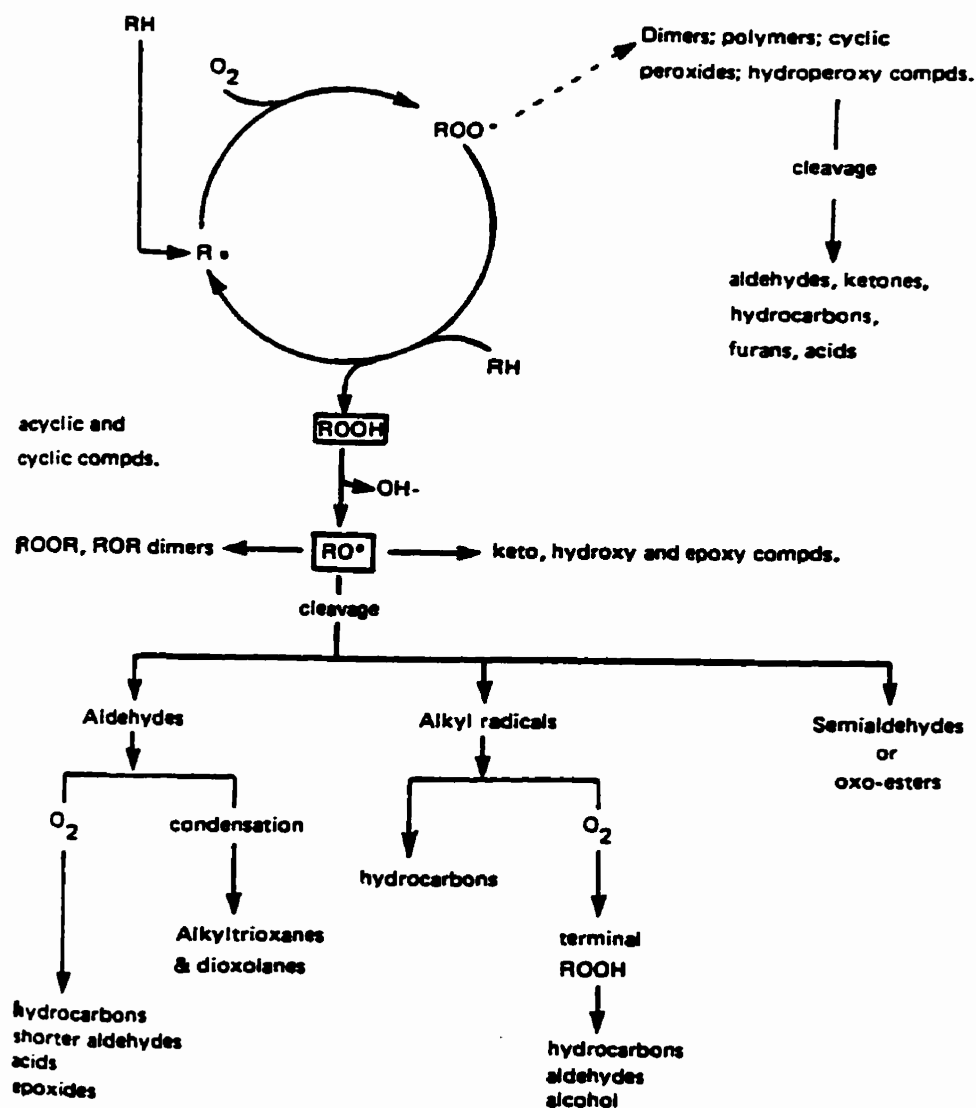


Fig. 2.2.2.3.1.2. Overview of lipid oxidation and their products (Nawar, 1985).

hydroperoxides, trace metals, and the conditions under which oxidation takes place (Labuza, 1971; Brimberg, 1993). Different volatile decomposition products are formed according to the thermal stabilities of the lipid oxidation precursors (Frankel, 1980). The concentration of decomposition products may become high under certain conditions, mainly at high temperatures, leading to the formation of cyclic monomeric, dimeric, trimeric and higher polymers (Clark & Serbia, 1991).

2.2.2.3.2. Effects of oxidation on food quality and human health

Lipid oxidation products affect the quality attributes of oils, fats and products containing lipids. Quality attributes such as the flavor and odor from the formation or modification of volatile compounds (Chan *et al.*, 1982), the taste formed by hydroxy-acids, the colour from Maillard-type reactions, and the texture created by cross-linking reactions of proteins have been reported (Eriksson, 1982). Another implication that has been related to lipid oxidation products includes the reduction in the nutritional value of the fat by decreasing essential fatty acids, as well as the destruction of fat-soluble vitamins (Shahidi *et al.*, 1992).

Many attempts have been made to relate the effect of oxidation products to diseases, but the health aspects of lipid oxidation products still remain controversial (Addis & Warner, 1992). Lipid peroxidation may have substantial

effects related to atherosclerotic plaque formation, cell membrane cytotoxicity and coronary heart disease. Furthermore, other research supports the idea that oxidized lipids are more deleterious to arterial health than are native lipids (Perkins,1976; Frankel, 1985; Addis & Warner, 1992).

There is some indication that cell injury may result from the presence of fatty acid peroxides and hydroperoxides. Lipid peroxides and some of their breakdown products may interact with proteins, membranes and enzymes as well as with vital cell functions (Jacob, 1994). Linoleic hydroperoxides were found to induce endothelial damage, to accelerate uptake of low density lipoprotein-LDL (Eunjoo *et al.*, 1994), to accelerate atherosclerosis, and also to stimulate thrombosis (Addis & Warner, 1992).

Free-radicals have been associated with the oxidation of low density lipoprotein (LDL), which may accelerate the development of an atherosclerotic plaque on the walls of blood vessels (Amer. Soc. Clin. Nutr., 1991). They also may damage cell membranes and other vital cell components, such as genetic material in the cell nucleus (Sies, 1991). The free radical damage to DNA is also believed to play a role in the initiation of carcinogenesis (Jacob, 1994).

There is evidence of cytotoxicity and membrane effects of oxysterols (Addis & Warner, 1992). The oxidation products of phytosterols are thought to be more atherogenic than their natural sterol counterparts (Smith, 1980). Oxidized

cholesterol has been related to increased atherosclerosis, with cholestanetriol and 25-hydroxy-cholesterol the most effective inducers (Taylor *et al.*, 1979).

2.2.2.4. Antioxidants

Antioxidants are defined by the United States Food and Drugs Administration (FDA) as substances used to preserve food by retarding deterioration, rancidity or discolouration due to oxidation (Dziezak, 1986). They are compounds that in small quantities prevent or greatly retard the oxidation of oils and fats (Pokorný, 1991). Antioxidants are naturally present in oils of plant origin (Labuza, 1971).

Antioxidants can act as a free radical scavengers, metal chelators, peroxide destroyers, oxygen scavengers, and as singlet oxygen quenchers (Labuza, 1971; Dziezak, 1986; Pokorný, 1991).

Antioxidants acting as free radical scavengers work by breaking the free radical chain reaction through removal of the alkylperoxy radical (ROO°) (Fig. 2.2.2.4.1) (Nawar, 1985).

$$ROO^{\circ} + AH \rightarrow ROOH + A^{\circ}$$

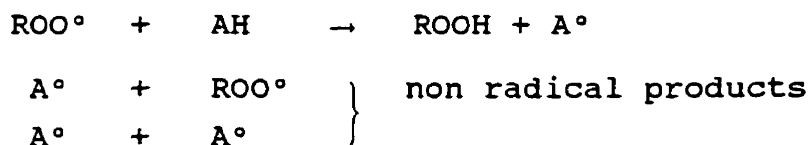


Fig. 2.2.2.4.1. Mechanism of free radical terminators (Nawar, 1985).

Free radical terminators interrupt the free radical chain of oxidative reaction by contributing hydrogen (H°) from the phenolic hydroxyl groups (AH). The resulting aroxyl radicals (A°) are relatively stable; therefore, they do not initiate or propagate further oxidation (Dziezak, 1986; Clark *et al.*, 1990). The resulting radicals may regenerate an initial phenol molecule or react with another radical to produce non-radical products (Clark *et al.*, 1990). Their action is based on the phenolic structure or the phenolic configuration within their molecular structure (Sherwin, 1976). This class of antioxidants must be able to have an effective delocalization of the unpaired electrons produced during reaction with the free radicals. The substitution of hydroxyl or other groups at the *ortho* and *para* positions are much more effective than at the *meta* position on the phenol molecule, because of the greater number of resonance forms possible. Another important factor is the size of a substituting group. Groups with several carbon atoms help to protect the antioxidant radical and give more stability towards further reaction, but this also makes it more difficult to react with the peroxy radical (Labuza, 1971). The various natural antioxidants that function by radical interception are considered substrate specific; therefore, no single free radical interceptor can effectively neutralize the effect of all the free radicals that are generated from fats with different fatty acid compositions (Thomas, 1995). Tocopherols and phenolic

compounds are generally included in this class of antioxidants (St. Angelo, 1996).

Metal chelators act as antioxidants by complexing metal ions, making them less reactive (Frankel, 1991). These agents indirectly affect the initiation step of oxidation by controlling the source of the production of free radicals before the propagation step (Labuza, 1971). Metal chelators include citric, phosphoric, ascorbic, phytic, tartaric, oxalic, succinic and malic acids (St. Angelo, 1996).

Another group of antioxidants react with hydroperoxides to give stable products which do not form radicals. They are not usually present naturally in food systems; some examples of these compounds include phosphites and sulfur compounds (Frankel, 1980).

Oxygen scavengers protect oil by scavenging oxygen. The oxygen is removed and usually chemically converted to water at the expense of the antioxidant. Vitamin *C* is an oxygen scavenger (St. Angelo, 1996).

Other group of antioxidants have the ability to quench singlet oxygen molecules, either by a physical or chemical process (St. Angelo, 1996). Physical quenching leads to the degeneration of singlet oxygen to ground state oxygen by an energy or charge transfer; whereas, chemical quenching involves an irreversible reaction of singlet oxygen with a quencher (Fig. 2.2.2.4.2) (Bradley & Min, 1992). α-Tocopherol, carotenoids, and Vitamin *C* have been found to act as singlet

oxygen quenchers (St. Angelo, 1996).

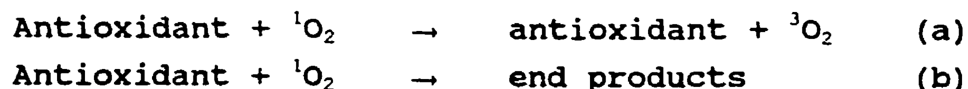

Antioxidant + $^1O_2$ → antioxidant + $^3O_2$ (a)
Antioxidant + $^1O_2$ → end products (b)

Fig. 2.2.2.4.2. Quenching action of antioxidants. a) physical process; b) chemical process (St. Angelo, 1996).

The effectiveness of antioxidants is related to the temperature of the reaction. Ragnarsson *et al.* (1977) showed that the action of primary antioxidants depends on their participation in a series of reactions involving radicals, and the rates of these reactions are affected by temperature. They also show that Q_{10} (the rate increase for a 10^0C temperature increase) varies due to a change in mechanism within the systems. Marinova & Yanishlieva (1992) also observed that the effectiveness of an antioxidant was related to temperature, due to changes in the mechanism of its participation in the different reactions of the inhibited autoxidation process. Therefore, single high temperature studies are not adequate to predict antioxidant effectiveness at lower temperatures more characteristic of storage conditions.

2.3. Oil and fat components involved in lipid oxidation

2.3.1. Fatty acid composition

The predominant fatty acids present in fats and oils are saturated and unsaturated compounds with straight aliphatic chains. An even number of carbon atoms, from 16 to 18, with a single carboxyl group, is the most common. A number of other minor fatty acids are present in edible oils including a small amount of branched chain, cyclic, and odd number straight chain acids (Christie, 1992). An important feature common to most plant origin oils and fats is the high percentage of unsaturated fatty acids in the triglycerides.

In general, the higher the degree of unsaturation of a vegetable oil's fatty acids, the more susceptible they are to oxidative deterioration. When the oxidative stability for several edible oils was compared it was found that the stability decreased from refined olive, through canola, corn, soybean, sunflower, and safflower oils, respectively (Gordon & Mursi, 1994). These results agree with their degree of fatty acid composition, refined olive oil being higher in oleic acid and more stable, and safflower oil being higher in polyunsaturated fatty acids with lower stability. Similar results were found with olive oil compared to sunflower oil (Kaya *et al.*, 1993), and with soybean oil compared to low erucic acid rapeseed. Low erucic acid rapeseed is higher in linolenic acid, and sunflower oil is higher in linoleic acid when compared to soybean oil (Warner *et al.*, 1989).

The stability of vegetable oils stored under light follows different oxidation patterns when compared with dark

storage. Warner *et al.* (1989) found sunflower oil more stable than soybean and low erucic acid rapeseed oils, both being higher in linolenic and lower in linoleic acid compared to sunflower oil. Malcolmson *et al.* (1994) reported that sunflower oil stored under light conditions was more stable than canola, cottonseed and soybean oils. Neuman *et al.* (1991) also reported that soybean was more unstable than sunflower and peanut oils. The stability of corn oil, as measured by peroxide values, was found to be more than twice as good in daylight and 1.6 times better in the dark, than sunflower oil at room temperature, even though the flavour stability for both oils was very close (Oštrić-Matijaševic *et al.*, 1982).

Hydrogenation is widely used practice to change either the physical characteristics or to increase the oxidative stability of an oil by introducing hydrogen at the double bonds of unsaturated fatty acids (Gunstone & Noris, 1983). Jackson *et al.* (1986) showed that partial hydrogenation increased the stability of soybean oil, compared with non-hydrogenated soybean oil, when measured by the Active Oxygen Method.

Greater stability has also been achieved by blending highly unsaturated vegetable oils with highly saturated oils. Frankel & Huang (1994) blended different proportions of high oleic sunflower oil with soybean and canola oils, to reduce linolenic acid content from 9% to 2%, and with corn oil, to

reduce linoleic acid content from 60% to 20%. The blended oils had greater oxidative stability compared to soybean and corn oils alone. The canola/high oleic sunflower oil blend was equivalent to or better in oxidative stability than the hydrogenated canola oil, both with 1% linolenic acid.

More recently, improvement in the oxidative stability of vegetable oils has been achieved by the modification of fatty acid compositions in the oilseeds through breeding programs. Purdy (1985) found that high oleic safflower and sunflower oils obtained from seed modified by chemical metagenesis showed improved oxidative stability, compared with oils from conventional lines. These new lines contained 80% to 89% oleic acid, with an accompanying reduction in linoleic acid content to 7%. A further reduction in linoleic acid content to 1% in high oleic sunflower oils resulted in further improvements in stability.

White & Miller (1988) compared the stability of low linolenic (3.7%), high stearic (24%) and conventional varieties of soybean oils (4.2% stearic, 7.2% linolenic acids). The high stearic and low linolenic acid varieties were similar in oxidative stability, but both were more stable than the conventional variety of soybean oil. Similar results were obtained in studies using genetic modified soybean oils, with 1.3% to 4.8% of linolenic acid, compared with regular varieties (Mounts *et al.*, 1988; Liu & White, 1992).

This same trend has been observed with other genetically

modified oils. Przybylski *et al.* (1993) showed that low linolenic canola oil with 3% linolenic acid, showed markedly greater stability during accelerated storage at 60^{0}C when compared with conventional canola oil (11.5% linolenic acid). High oleic peanut oil, 76% oleic and 4.7% linoleic acids, has also been shown to have greater stability than conventional peanut oil with 56% oleic and 24% linoleic acids (O'Keefe *et al.*, 1993).

2.3.2. Glycerides

In nature, lipid classes do not exist as single pure compounds, but rather as complex mixtures of related components in which the composition of the aliphatic residues varies from molecule to molecule. Fatty acids are attached to glycerol molecule forming triacylglycerols (Gunstone & Norris, 1983). Monoacyl and diacylglycerols are also present in vegetable oils in low proportion (Roden & Ullyot, 1984).

2.3.2.1. Triacylglycerols

Each position on the glycerol molecule may be esterified by a different fatty acid following plant patterns. The most common forms are the triacylglycerols (TAG) in which two or three kinds of fatty acid moieties are present in the molecule (Shibamoto, 1994). Triacylglycerols are very complex with n^3 possible molecular species, where n is the number of fatty acids present in the oil. Both the physical and chemical

characteristics of fats are greatly influenced by the kinds and proportions of the fatty acids and how those are positioned on the glycerol moiety (Miyashita *et al.*, 1990; Neff *et al.*, 1994). It is well known that the biosynthesis of triacylglycerols in vegetable oils involves a preferential esterification of unsaturated fatty acids in the *sn*-2 position, and saturated fatty acids at the *sn*-1,3 positions of glycerol (Sacchi *et al.*, 1992).

Several studies have showed that the oxidation rate depends on the degree of triacylglycerol unsaturation. The oxidative stability, during storage in the dark, of different vegetable oils was found to decrease when unsaturation increased (Park *et al.*, 1983a; Wada & Koizumi, 1983; Tautorus & McCurdy, 1992; Neff *et al.*, 1993), or when the oils were stored under light (Neff *et al.*, 1992; Neff *et al.*, 1994). Park *et al.* (1983a) found that trilinolein oxidized 6 times faster than triolein. Other studies showed that an increase in the number of double bonds in a fatty acid or ester caused the rate of oxidation to increase by a factor of two for each added methylene-interrupted double bond (Miyashita *et al.*, 1990).

The effect of randomization on oil stability has been studied with model systems and vegetable oils. Early studies by Raghuveer & Hammond (1967) showed that the stability of several fats randomized by interesterification decreased when oxidized at 37^{0}C and 50^{0}C. Other workers reported that

randomization had a drastic effect in lowering the stability of palm oil, but had an insignificant effect on lard (Hofmman *et al.*, 1973).

Park *et al.* (1983a,b) found that randomization of the fatty acids had no significant effect on the autoxidative stability of soybean oil and a synthetic mixture of trilinolein and trilaurin. These authors also observed no differences in the rates of oxidation for triacylglycerols where unsaturated fatty acids were located at different positions in the glycerol molecule. They concluded that selective attacks of oxygen molecules on the unsaturated acyl groups in triacylglycerols does not occur. The carbon chain length of saturated fatty acids was also observed to have no effect on the oxidation rates of unsaturated fatty acids in triacylglycerols (Wada & Koizumi, 1983).

Tautorus & McCurdy (1992), working with linseed oil and trioleoylglycerol, found that randomization of the mixture formed a more stable product than blending in the same proportions. Wada & Koizumi (1983) found similar trends when interesterifying triacylglycerols of tripalmitin, tristearin, triolein and trilinolein. These authors showed that randomized triglycerides were more stable than the original mixture of triacylglycerols.

Wada & Koizumi (1983) found that triacylglycerols with unsaturated fatty acids linked at the *sn*-2 position were more stable than those linked at the *sn*-1,3 position. However,

Miyashita *et al.* (1990) found that synthetic dilinolenoyl-linoleoyl triacylglycerols were more easily oxidized when linolenic acid was in the *sn*-1,2 compared to the *sn*-1,3 position.

Neff *et al.* (1992) studying twenty different genetically modified soybean oils found a high correlation between peroxide values and the presence of linoleic and linolenic acids at the *sn*-2 position. They also reported that oleic acid, when located in the *sn*-2 position, increased oil stability compared to its location in the *sn*-1,3 position of the glycerol. Similar studies evaluating the photo-oxidative stability of soybean oil showed that the position of oleic or linolenic acids on the second carbon (*sn*-2) had little influence on oil stability when compared to the external positions. However, when linoleic acid was positioned on the second glycerol carbon (*sn*-2) the oxidative stability of the oils was reduced (Neff *et al.*, 1993). Further studies with purified canola oil found that for both light and without light storage, stability increased for oils with oleic acid present at the *sn*-2 position. However, linoleic acid positioned on that same carbon reduced the oxidative stability of purified canola oil (Neff *et al.*, 1994).

2.3.2.2. Monoacyl and diacylglycerols

Only a few studies have discussed the effect of mono-acylglycerols (MAG) and diacylglycerols (DAG) on vegetable oil

stability. These glycerides are present in vegetable oils in low amounts, and their presence is basically due to partial hydrolysis during processing and storage of oils and fats (Roden & Ullyot, 1984). Also during interesterification a small amount of diacylglycerides and monoacylglycerides is formed (Haumann, 1994).

Mistry & Min (1988) analysed the effect of monoacyl and diacylglycerols on the oxidative stability of purified soybean oils stored without light at 55^{0}C. They found that 0.25% and 0.5% of monostearin, distearin, monolinolein and dilinolein caused a greater depletion of oxygen during storage when compared with pure soybean oil. This effect was more pronounced at higher levels of these components, indicating that monoacylglycerols and diacylglycerols acted as pro-oxidants.

2.3.3. Free fatty acids

The free fatty acid content is closely associated with oil quality (Roden & Ullyot, 1984). A high content of free fatty acids can be related to enzymatic or chemical hydrolysis prior to or during oil processing (Patterson, 1989).

Free fatty acids present in the oil may also affect the oil stability. Miyashita & Takagi (1986) found that the oxidative rate of methyl linoleate and soybean oil increased with increasing concentrations of stearic acid. They also showed that the stearic acid contents of samples did not

decrease as autoxidation proceeded. Based on these results the authors suggested that oxidation was accelerated by the carboxyl group of stearic acid. The probable mechanism was attributed to the catalytic action of the carboxyl group in the stearic acid on the decomposition of a small amount of hydroperoxides formed in the initial stage of autoxidation.

The pro-oxidant effect of free fatty acids was also supported by Mistry & Min (1987) when they studied purified soybean oil with added free fatty acids. They observed that the faster there was an increase in the amount of volatiles and peroxide value, the faster there was a decrease in oxygen in the head space of the samples containing free fatty acids when compared with purified soybean oil. Octadecane, which has the same number of carbons (C-18) as these fatty acids but no carboxylic group, did not influence the oxidative stability. Based on these results the authors concluded that stearic, oleic, linoleic and linolenic acids acted as pro-oxidants, probably due to the catalytic action of the carboxyl group.

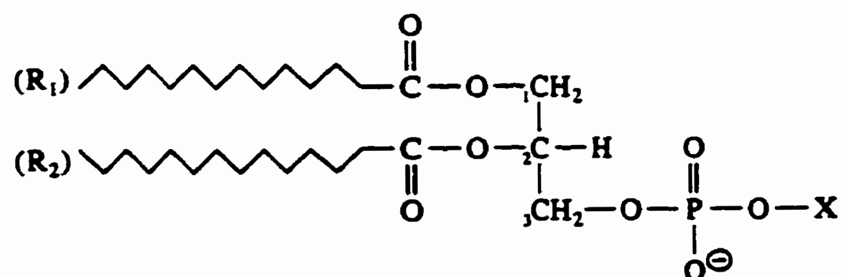
2.3.4. Phospholipids

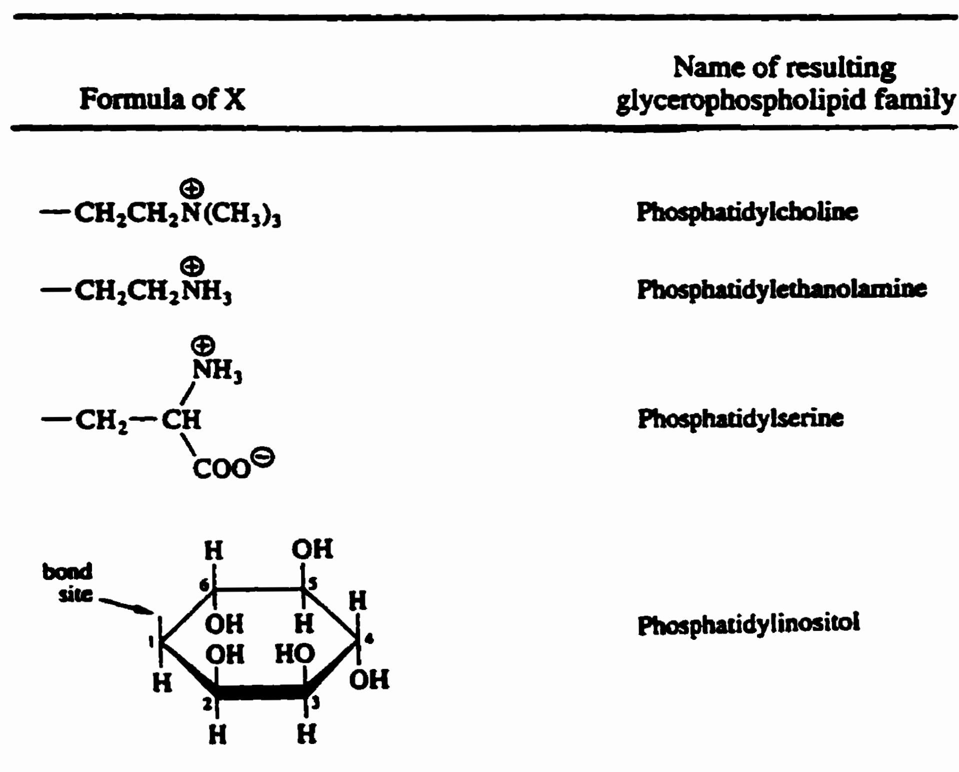
Phospholipids, also denominated phosphatides, occur normally at low levels in freshly refined and deodorized edible oils, because their complete or partial removal by degumming is usually regarded as an essential first step in oil processing. If not removed, some phospholipids form

sludges through hydration, contributing to poor flavours, undesirable colours, and unwanted surface activity at later stages in the refining process or in oil utilization (Hudson & Ghavami, 1984; Patterson, 1989). They are chemically complex, and in vegetable oils they are usually present as phosphatidyl choline (PC-lecithin), phosphatidyl ethanolamine (PE-cephalin), phosphatidyl inositol (PI), phosphatidic acid (PA), and phosphatidyl serine (PS) (Fig. 2.3.4) (Hudson & Ghavami, 1984).

There have been some controversial results in the published literature about the effect of phospholipids on oil oxidation. Husain *et al.* (1986) speculated that this confusion may be due to the structural complexity of phospholipids containing different chemically active groups and various fatty acid compositions.

Several studies have shown an antioxidant effect of phospholipids on vegetable oil stability. Pokorný *et al.* (1990) found that phosphatidyl choline and phosphatidyl ethanolamine, when added at a concentration of 3% in linseed oil and methyl linoleate, resulted in lower peroxide values during accelerated storage. Hildebrand *et al.* (1984) demonstrated that added phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl choline and phosphatidyl inositol at levels of 0.5% each, resulted in better oxidative stability of soybean oil. Phosphatidyl inositol appeared to be the most effective, while PA was ineffective at the





Formula of X	Name of resulting glycerophospholipid family
$-CH_2CH_2\overset{\oplus}{N}(CH_3)_3$	Phosphatidylcholine
$-CH_2CH_2\overset{\oplus}{N}H_3$	Phosphatidylethanolamine
$-CH_2-CH(\overset{\oplus}{N}H_3)(COO^{\ominus})$	Phosphatidylserine
	Phosphatidylinositol

Fig. 2.3.4. Structure of phospholipids commonly present in vegetables oils (Moran et *al.*, 1994).

concentration used. A study by Bhatia *et al.* (1978) showed that phosphatidyl ethanolamine provided better protection against oxidation in ghee during storage at 37^{0}C than did phosphatidic acid and phosphatidyl choline (Bhatia *et al.*, 1978). The authors attributed the greater potential of PE as an antioxidant to its amino group, which has the capability of chelating pro-oxidative metals likely to be present in trace quantities in the fat.

Phospholipids are also often considered antioxidants when used as synergists in combination with phenolics. Hudson & Ghavami (1984) showed that phospholipids had little antioxidant effect *per se*, but were effective synergists when used with tocopherols. The addition of 0.5% of dipalmitoyl phosphatidyl ethanolamine and dipalmitoyl phosphatidyl choline acted as antioxidants in refined soybean oil containing tocopherols, while the same concentration of these components in soybean oil without was found to be ineffective. The phospholipids were most likely involved in a chemical reaction that stabilized the tocopherol free-radical hybrids, which resulted from the donation of hydrogen by tocopherols. Kashima *et al.* (1991) found that phosphatidyl choline and phosphatidyl ethanolamine remarkably suppressed oxidation of refined and perilla oils when tocopherols were present. Hildebrand *et al.* (1984) also observed that the effectiveness of phospholipids increased in soybean oil when tocopherol was added. They proposed that the amine group of phosphatidyl

choline and phosphatidyl ethanolamine, as well as the reducing sugar of phosphatidyl inositol, most likely are responsible for facilitating the hydrogen or electron donation to the tocopherols. Hudson & Mahgoub (1981) observed the same pattern of ineffectiveness of phosphatidyl ethanolamine and phosphatidyl choline in lard, but enhanced stability when α-tocopherol was present. The possible mechanism was attributed to phospholipids acting as powerful synergists by chelating traces of pro-oxidant metals.

There is evidence that phospholipids could be synergistic only at elevated temperatures. Dipalmitoyl phosphatidyl ethanolamine has been shown to be an efficient synergist with tocopherols in lard only at temperatures above 80°C. As the concentration of phospholipid increased up to 1% for a given concentration of tocopherol, the synergistic efficiencies improved. However, the same phospholipids added to rapeseed oil stored below 80°C showed very low synergistic activity (Dziedzic & Hudson, 1984b). Husain et al. (1986) also showed that neither saturated phosphatidyl ethanolamine nor phosphatidyl choline exhibited synergism with tocopherol at temperatures below 50°C.

Other studies found that phospholipids may act as pro-oxidants. Krishna & Prabhakar (1994) showed that extracted hydrogenated gums from crude peanut oil exhibited antioxidant activity when added to methyl linoleate. However, the phospholipids isolated by a silicic acid column showed pro-

oxidant activity in the same medium. The authors concluded that possibly the phospholipids lost antioxidant activity due to changes in polarity during the isolation procedure. Husain *et al.* (1986) observed that egg yolk phospholipids with a high degree of unsaturation showed pro-oxidant activity during the initial stage of the autoxidation of methyl linoleate. The authors proposed that the pro-oxidant effect of egg yolk might be due to the presence of unstable polyunsaturated fatty acids such as arachidonic and docosahexaenoic acids. On the other hand, they also found that egg yolk phospholipids retarded hydroperoxide formation when heating methyl linoleate at 180°C, but this could be related to polar brown colour products formed from heated phospholipids, which have chelating properties.

2.3.5. Tocopherols and tocotrienols

Tocopherols and tocotrienols comprise compounds related to the vitamin *E* group. The term vitamin *E* covers a family of compounds that possesses as a common feature a hydroxychromane ring and a terpenoid side chain located at position 2 of the ring (Diack & Saska, 1994). These compounds present different biological activities that are related to different levels of absorption from the intestine (Moran *et al.*, 1994). Among them, α-tocopherol has the highest vitamin *E* activity (100%), followed by β-(50%), γ-(26%), and δ-tocopherols(10%) (Wong, 1989).

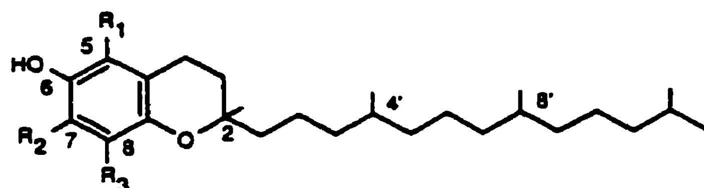
This group is composed of eight different isomers and derivatives belonging to two families, tocopherols and tocotrienols (Fig. 2.3.5) (Bauernfeind, 1977). These compounds are methyl-substituted chromanols with three-isoprene moity side chains. Tocopherols present a saturated side chain with chiral carbons at positions 2', 4' and 8', and tocotrienols present an ethylenic bonds at positions 3', 7' and 11' (Tonolo & Marzo, 1989). Within these two groups, a distinction is made between α-(5,7,8-trimethyltocol), β-(5,8-dimethyltocol), γ-(7,8-dimethyltocol), and δ-(8-methyltocol) tocopherols and tocotrienols, according to the number and position of the methyl substituents on the chromane ring (Tan, 1989).

All natural tocopherols and tocotrienols are derived from plant sources where they are found mainly in the seeds (Slover, 1971). Vegetable oils generally contain mainly α-, γ-, and δ-tocopherols (Slover, 1971; Carpenter, 1979; Tan, 1989). Tocotrienols have been found in palm, coconut, corn and rice bran oils (Clark *et al.*, 1990; Shin & Godber, 1993; Orthoefer, 1994).

2.3.5.1. Tocopherols

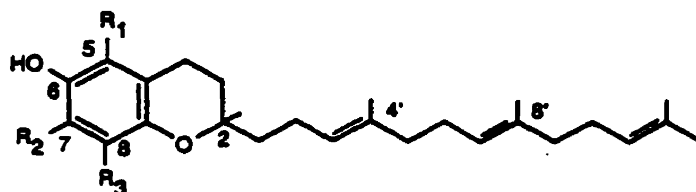
Tocopherols are considered to be the major natural antioxidants present in vegetable oils. Their antioxidant activities are dependent mainly on their concentration and the presence of synergistic compounds (Pokorný, 1991).

(A)



	R_1	R_2	R_3
5,7,8-Trimethyl tocol (α-tocopherol)	CH_3	CH_3	CH_3
7,8-Dimethyl tocol (β-tocopherol)	H	CH_3	CH_3
5,8-Dimethyl tocol (γ-tocopherol)	CH_3	H	CH_3
8-Methyl tocol (δ-tocopherol)	H	H	CH_3

(B)



	R_1	R_2	R_3
5,7,8-Trimethyl tocotrienol (α-tocotrienol)	CH_3	CH_3	CH_3
7,8-Dimethyl tocotrienol (β-tocotrienol)	H	CH_3	CH_3
5,8-Dimethyl tocotrienol (γ-tocotrienol)	CH_3	H	CH_3
8-Methyl tocotrienol (δ-tocotrienol)	H	H	CH_3

Fig. 2.3.5. Structure of (A)tocopherols and (B)tocotrienols present in vegetable oils (Bauernfeind, 1977).

Tocopherols have been reported to act essentially as free radical scavengers, based on the tocopherol-tocopheryl semiquinone redox system (Wong, 1989).

Clark *et al.* (1990), using several vegetable oils and animal fats, found that the maximum stability was achieved when the tocopherol level was about 600ppm. The addition of tocopherols up to 2000ppm resulted in only a slight improvement in stability. These authors also reported that the addition of tocopherols to animal fats was more efficient than it was in vegetable oils. This was explained as due to the fact that most deodorized vegetable oils already contain tocopherols at concentrations close to the optimal level as antioxidants.

Other studies have shown that tocopherols may act as antioxidants or pro-oxidants in vegetables oils, depending on their concentrations and the oxidation stage. Jung & Min (1990), using purified soybean oil in chromatographic columns packed with activated silicic acid, found that the optimum concentration of tocopherols for oxidative stability was between 100ppm and 500ppm. At the upper level, α-tocopherol acted as a pro-oxidant, γ-tocopherol had no effect, whereas δ-tocopherol acted as an antioxidant. The authors found that the optimum concentration of tocopherol for the oxidative stability of soybean oil seems to be related to the oxidative stability of each individual tocopherol. The lower the individual oxidative stability of each tocopherol, the less

the optimum concentration of that tocopherol necessary to achieve maximum stability. Among tocopherols, the highest oxidative stability was found to be for δ-tocopherol and the lowest for α-tocopherol.

Studies by Huang *et al.* (1994) showed that tocopherols may act as pro-oxidants at concentrations lower than 500ppm. α-Tocopherol showed pro-oxidant activity when it was present over 250ppm in corn oil, while γ-tocopherol showed maximum antioxidant activity at concentrations of 250 to 530ppm. Blekas *et al.* (1995) suggested that the pro-oxidant activity of α-tocopherol occurs only during the early stages of oil oxidation. They found that α-tocopherol showed antioxidant activity at concentrations of 100 to 1000ppm when purified and refined olive oils were used, except for the beginning stages of oxidation.

Tocopherols may also act as singlet oxygen quenchers via a charge-transfer quenching mechanism. The singlet oxygen reactivity of tocopherol seems to be related to vitamin *E* activity, since α-tocopherol shows the highest capacity for singlet oxygen quenching, and is totally absorbed by the organism (Wong, 1989; Moran *et al.*, 1994). Yamauchi & Matsushita (1977) found α-tocopherol the most effective singlet oxygen quencher during methyl linoleate oxidation. However, the rate of singlet oxygen quenching by tocopherols was lower than the rate of quenching by β-carotene. Jung *et al.* (1991) found that tocopherols increased the stability of

soybean oil during photo-sensitized oxidation when chlorophyll *b* was present. These effects were more pronounced when the concentrations of tocopherols was increased. The antioxidant activity was higher for α-tocopherol while it was lower for γ- and δ-tocopherols.

The optimum concentration of tocopherols may be affected by the type and quantity of synergists such as phospholipids and other minor compounds present in oils (Prior *et al.*, 1991). Tocopherols have shown a synergistic effect with phospholipids and enhanced oxidative stability of vegetable oils (Hudson & Mahgoub, 1981; Hildebrand *et al.*, 1984; Hudson & Ghavami, 1984; Kashima *et al.*, 1991). Nishina (1991) found that L-ascorbic acid had a strong synergistic effect on tocopherols in fats and oils with low amounts of unsaturated fatty acids. These interactions were less effective when unsaturated fats and oils were used. On the other hand, Yoshida *et al.* (1992) found that free fatty acids showed pro-oxidant activity toward tocopherols in purified vegetable oils when heated in a microwave oven. The shorter the chain length (among C_6 to C_{12}) and the higher the level of the unsaturated fatty acids, the greater was the reduction of tocopherols in the oil.

Temperature may also affect tocopherol efficiency as an antioxidant. Marinova & Yanishlieva (1992) found that the effectiveness of α-tocopherol added to lard increased as the temperature increased from 25°C to 100°C. The authors

suggested that this was due to the change in the mechanism of tocopherol participation in the different reactions of the inhibited autoxidation process, mainly in the reactions associated with the regeneration of the inhibitor molecule.

Other factors may interfere in tocopherol activities. Jacobsberg *et al.*(1978) found that the rate of destruction of tocopherol at 70°C was directly influenced by the nature of the fatty acid chain. α-Tocopherol was destroyed 20 to 40 times and 5 to 10 times faster in linolenate and linoleate mediums, respectively, than in an oleate medium.

Oxidized tocopherols also showed pro-oxidant effects when added to purified soybean oil, at concentrations of 100ppm to 1000ppm. Oxidized α-tocopherol had the greatest pro-oxidant effect when compared to oxidized γ- and δ-tocopherols (Jung & Min, 1992).

2.3.5.2. Tocotrienols

Tocotrienols were reported to have antioxidant activity similar to tocopherols. All tocotrienols have a phenolic group similar to tocopherols, which is essential to their mode of action (Clark *et al.*, 1990).

Studies on the antioxidant activity of tocotrienols in the oxidation of phospholipid liposomes suggested that tocotrienols have the same antioxidative activity as or even higher than corresponding tocopherols (Yamaoka *et al.*, 1991).

Few studies have demonstrated the antioxidant activity of

tocotrienols in oil and fat systems. Tests conducted in tallow showed that α-tocotrienol alone (Yanishlieva *et al.*, 1977) and all tocotrienols together were more efficient as antioxidants than the corresponding tocopherols (Seher & Ivanov, 1976).

Jacobsberg *et al.* (1978) found that tocotrienols showed a slightly faster oxidation rate than the corresponding tocopherols in palm oils. The oxidation rate decreased for α-tocotrienol, α-tocopherol, γ-tocotrienol, δ-tocotrienol, and γ-tocopherol, in that order respectively. The authors concluded that the faster oxidation of tocotrienols indicated better antioxidant activity than corresponding tocopherols.

2.3.6. Phenolic compounds

Phenolic acids and their derivatives are widely distributed in plants, many being essential metabolites. Only a few specific compounds occur in relatively high concentrations in oilseeds (Fig. 2.3.6) (Krygier *et al.*, 1982).

Only small amount of phenolic compounds are present in the polar fraction in commercial vegetable oils (Tsimidou *et al.*, 1992a). An exception is in virgin olive oil, which contain a significant amount of phenolics that are usually removed from other edible oils during refining stages (Tsimidou et al., 1992a,b). These compounds play an important role in the sensory characteristics of virgin olive oils

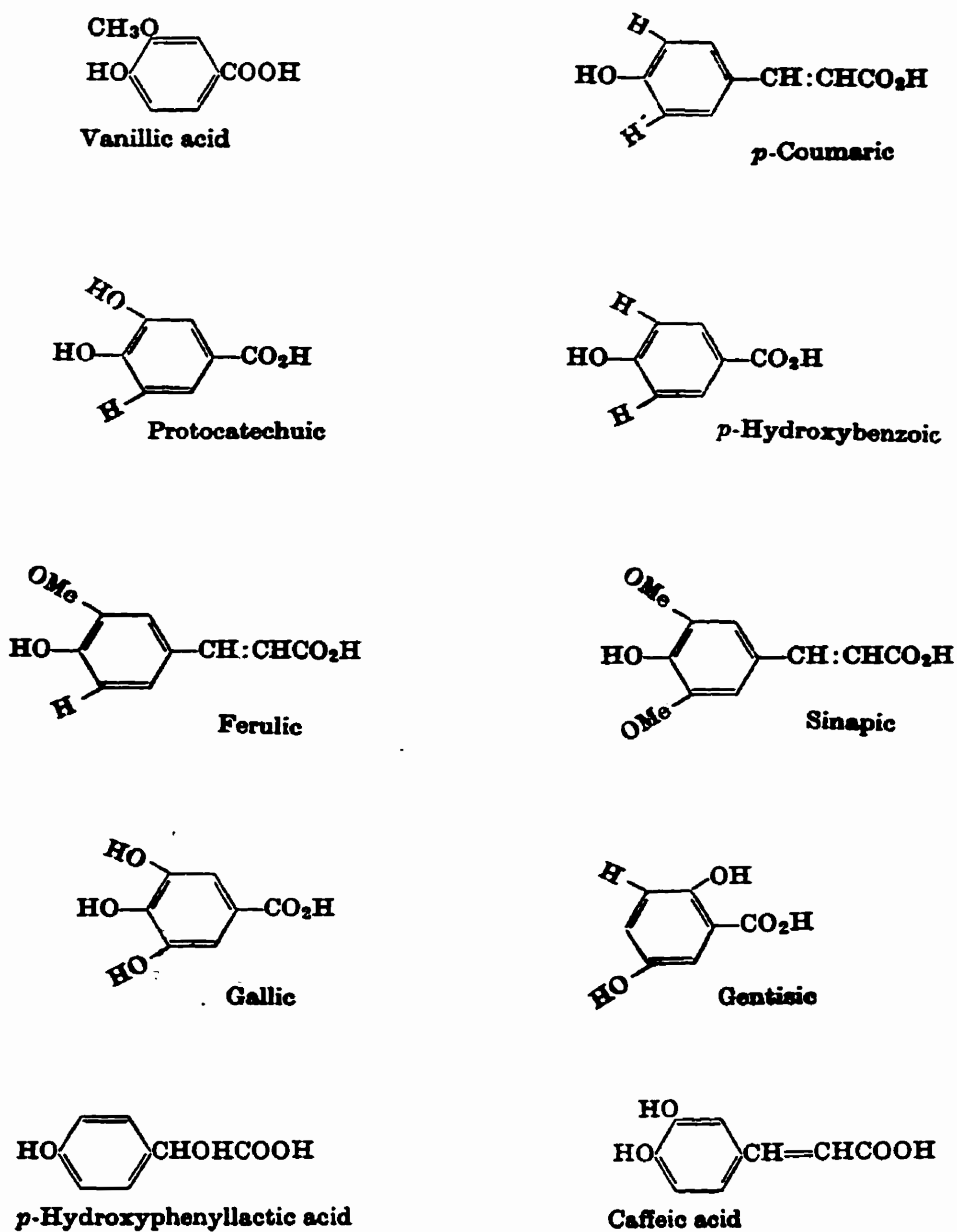


Fig. 2.3.6. Structure of some phenolic compounds found in vegetable oils (Marinova & Yanishlieva, 1994).

(Nergiz & Ünal, 1991; Montedoro *et al.*, 1992).

Phenolic compounds and some of their derivatives are considered very efficient in preventing autoxidation of vegetable oils (Shahidi *et al.*, 1992; Tsimidou *et al.*, 1992b). Natural polyphenols, whether simple compounds such as phenolic acids or their esters, or more complex, though chemically related compounds such as lactones, chalcones, and/or flavonoids, have marked antioxidant activity towards fat oxidation (Dziedzic & Hudson, 1984a; Dimberg *et al.*, 1993).

Early studies evaluating the antioxidant activity of oat extracts suggested that its activity was largely related to phenolics present in the grain (Daniels *et al.*, 1963), mainly caffeic acid (Daniels & Martin, 1961). The relative long shelf life of virgin olive oil compared to other vegetable oils was also attributed to the presence of phenolics (Papadopoulos & Boskou, 1991). These authors showed that the active phenols were mainly *o*-diphenols such as hydroxytyrosol, protocatechuic, caffeic, and syringic acids. Hydroxy derivatives of cinnamic and benzoic acids were also reported to posses antioxidant activity (Pokorný, 1991).

The antioxidant activity of natural phenolic compounds is related to their free radical scavenging properties, particularly their ability to donate a hydrogen atom to the lipid radical (Chimi *et al.*, 1991; Shahidi *et al.*, 1992). The propagation phase, consisting of radical chain reactions, is blocked by phenolics and the development of rancidity is

delayed (Cuvelier *et al.*, 1992).

The phenol moiety itself is considered inactive as an antioxidant. Antioxidative potency is related to the delocalization of unpaired electrons around the aromatic nucleus of the aroxyl radicals (Chimi *et al.*, 1991). At least two and/or three neighbouring phenolic hydroxy groups (catechol or pyrogallol structure) and a carbonyl group in the form of an aromatic ester, lactone, chalcone, flavanone, or flavone are considered the essential molecular features required to achieve a high level of antioxidant activity (Dziedzic & Hudson, 1984a).

The substitution of hydrogen atoms in the *ortho*- and *para*-positions with alkyl groups increases the electron density of the hydroxyl moiety, and thus enhances its reactivity toward lipid radicals (Shahidi et al., 1992). Cuvelier *et al.* (1992) found that the *ortho*-diphenols, such as protocatechuic and caffeic acids, were more effective than the respective monophenols *p*-hydroxybenzoic and *p*-coumaric acids. Gallic acid with three hydroxy groups was more active than protocatechuic acid. Therefore, the antioxidative efficiency of monophenols increased substantially when one or two methoxy substitutions at the *ortho*-position in the phenol structure are present. The authors also found that the double bonds in the *p*-position in cinnamic acids ensures even better efficiency than the carboxyl group in benzoic acid.

The antioxidant properties of phenolics may be affected

by the medium. Marinova & Yanishlieva (1992) measured the antioxidative activity of phenolic acids in lipid systems with different degree of unsaturation. Methyl esters of sunflower oil, when fortified with *p*-hydroxybenzoic, vanillic, syringic and *p*-coumaric acids at concentrations ranging from 0.02 to 0.20%, showed no antioxidative effect. These same phenolics, except for *p*-hydroxybenzoic acid, showed antioxidant activity in methyl esters of lard. In both lipid systems, the derivatives of benzoic acid had weaker inhibitory properties than had corresponding analogues of cinnamic acid.

The mono, di, and triphenolic antioxidants present during the oxidation of fats and oils may undergo degradation, which could affect the antioxidant activity of the phenolic compounds. Generally, antioxidant dimers are the most common breakdown products. These dimers may be produced by the formation of aroxyl radicals, followed by radical rearrangement and a coupling reaction with another radical. Moreover, most oxidation products of phenolics retain antioxidant activity, which may influence the effect of the parent antioxidant during the course of its degradation (Shahidi *et al.*,1992).

2.3.7. Pigments

Vegetable oils and fats have a specific colour due to the presence of natural pigments, as well as to products of their decomposition and their accompanying substances. Most of the

colour is given by chlorophylls, carotenes, xanthophylls, and other related substances. The major pigments found in commercial vegetable oils are chlorophylls and carotenoids (Niewiadomski *et al.*, 1965). These pigments belong to the group of isoprenoid plant lipids known as phenyl lipids (Daood *et al.*, 1989).

2.3.7.1. Chlorophylls and derivatives

Chlorophylls *a* and *b* (Fig. 2.3.7.1.1) and their derivatives are naturally present in several oilseed plants. The amount of these components in the mature fruit or seed is related to agronomic and climatic conditions (Mínguez-Mosquera *et al.*, 1990).

Chlorophylls and their derivatives in some vegetable oils are the main pigments responsible for a greenish colour. Except for virgin olive oil, where a greenish colour is acceptable, an excessive amount of chlorophylls in oils is considered highly undesirable (Minguez-Mosquera et al., 1991). The amount of chlorophylls *a* and *b* in oil, as well as their decomposition products, is dependent on the processing of the oil (Schwartz & Lorenzo, 1990) and biological factors (Niewiadomski *et al.*, 1965). During oil processing stages, such as extraction, degumming, and bleaching, the composition of these chlorophyll pigments is changed (Mínguez-Mosquera *et al.*, 1990; Endo *et al.*, 1992; Suzuki & Nishioka, 1993; Tautorus & Low, 1994).

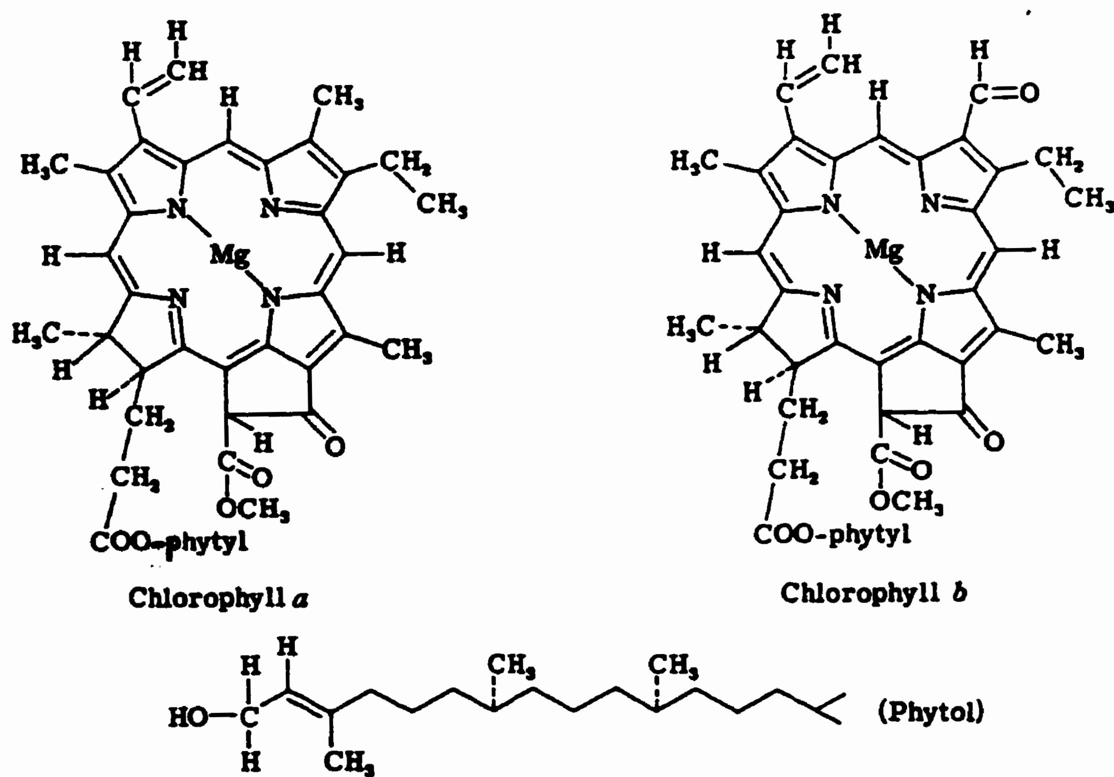


Fig. 2.3.7.1. Structure of Chlorophylls (Vernon & Seely, 1966).

Chlorophylls undergo decomposition by losing magnesium (Mg^{++}) or phytol groups from their structure, yielding chlorophyllides and pheophytins. Further chemical changes may generate pheophorbides and other decomposition products (Fig. 2.3.7.1.2) (Schwartz & Lorenzo, 1990). Even during oil storage in refrigerated conditions changes in the total amount and composition of chlorophylls have been observed (Ward *et al.*, 1994b).

Daun & Thorsteinson (1989) found pheophytin *a* to be the major chlorophyll derivative present in crude canola oil, and a small amounts of pheophytin *b* were also observed. Similar results were reported for virgin olive oil (Rahmami & Csallany, 1985; Rahmani & Csallany, 1991; Mínguez-Mosquera *et al.*, 1992), and crude rapeseed oil (Niewiadomski *et al.*, 1965). Crude soybean oils showed negligible amounts of pheophytin and chlorophyll pigments (Daun & Thorsteinson, 1989), and traces of pheophytin and pyropheophytin (Rahmami & Csallany, 1985; Fraser & Frankl, 1985). Pheophorbides *a* and *b* were detected in 10 kinds of commercial edible oils, including soybean, canola, cottonseed, safflower, corn, sunflower, olive and rice bran oils (Usuki *et al.*, 1984a). Methylpheophorbide *a* and pyropheophytins *a* and *b* were found in canola oil (Endo *et al.*, 1992). Ward *et al.* (1994b) found that type *a* of chlorophyll derivatives comprised more than 81% of the total chlorophyll pigments in commercially extracted crude canola oil.

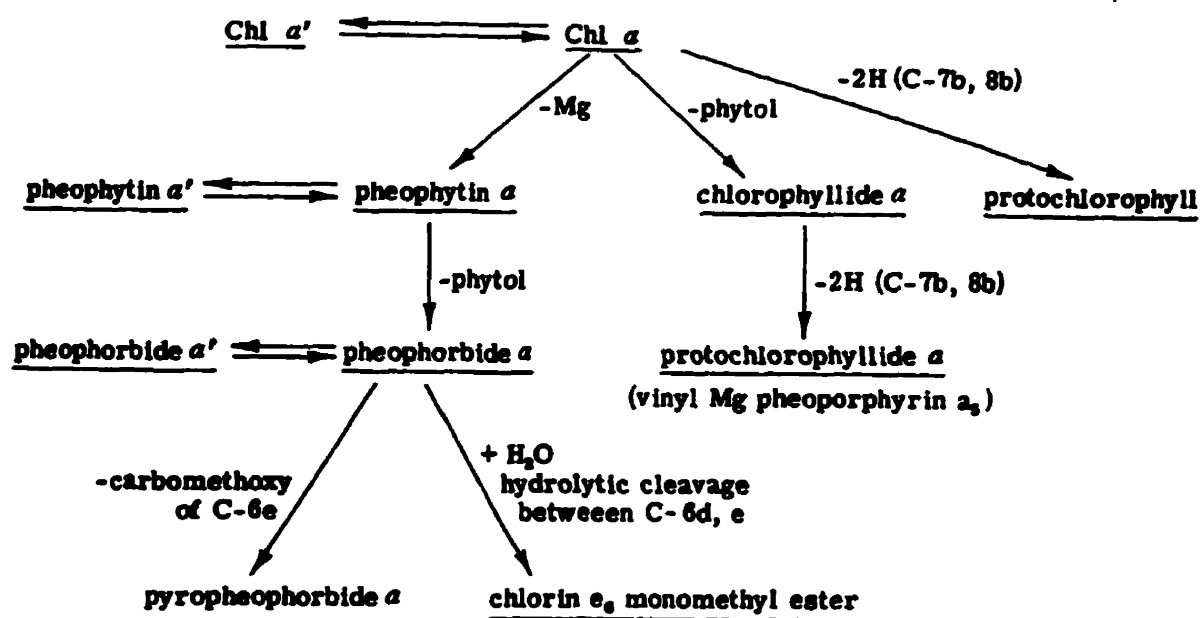


Fig. 2.3.7.2. Relationship of chlorophyll to some of its derivatives (Vernon & Seely, 1966).

The oxidative stability of vegetable oils is greatly affected by the presence of chlorophylls and their derivatives, mainly in the presence of light. Chlorophylls and their derivatives have the ability to transfer light energy to chemical molecules of some compounds. Therefore, they are potential photo-sensitizers generating singlet oxygen, which can react 1500 times faster with lipids than can triplet oxygen (Bradley & Min, 1992; Gutiérrez-Rosales et al., 1992).

Several investigations have showed the pro-oxidative effect of chlorophylls and their derivatives in vegetable oils exposed to light (Bradley & Min, 1992). Chlorophylls added to purified olive oil decreased oxidative stability, acting as a pro-oxidant under light storage. When a greater amount of chlorophyll was added, a lower oxidative stability of oils was achieved (Fakourelis *et al.*, 1987). Similar results were found using purified soybean oil, corn oil and in synthetic methyl linoleate systems (Endo *et al.*, 1984a,b; Usuki *et al.*, 1984a,b; Fakourelis *et al.*, 1987).

The presence of some chlorophyll derivatives may be as important as chlorophylls in the photo-oxidation of vegetable oils (Endo *et al.*, 1984a). Tautorus & Low (1993, 1994) showed that canola oil extracted from immature seeds had a lower stability then oil extracted from high-grade seeds. The authors proposed that chlorophylls and pheophytins could be converted to compounds that are capable of being pro-oxidants.

Usuki *et al* (1984b) found that both chlorophyll and pheophytin tended to promote photo-oxidation over a wide range of wavelengths, due to their similarity in the absorption spectra. They also found that the pro-oxidant activity of pheophytin was higher than that of chlorophyll, and chlorophyll *b* and pheophytin *b* accelerated to a higher degree the oxidative deterioration of vegetable oils than the counterparts of *a* derivatives. Endo *et al.* (1984b) also found that pheophytin and pheophorbide exhibited stronger pro-oxidant activities than chlorophylls when analysed in a methyl linoleate system.

Even though chlorophylls and their derivatives are considered pro-oxidants in the presence of light, these compounds may not always act as pro-oxidants, but may prevent oxidative deterioration if the oil is not exposed to light (Endo *et al.*, 1985a,b; Fakourelis *et al.*, 1987).

Virgin olive oils containing chlorophylls showed higher stabilities when stored without light than did the same samples without chlorophyll (Gutiérrez-Rosales *et al.*, 1992). Endo *et al.*(1985b) showed that chlorophyll and chlorophyll derivatives had antioxidative activity during the initial stage of autoxidation of methyl linoleate without light, when the hydroperoxides content was low. Similar results were observed when refined and purified rapeseed and soybean oils were analysed (Endo *et al.*, 1985a). Based on these observations, Endo *et al.* (1985a) proposed a mechanism for the

antioxidant activity of chlorophylls and their derivatives during storage without light. Chlorophylls and their derivatives might react with the peroxy radical produced in the initial stage of oxidation and transform them into a ¶-cation radical. Those cation radicals of chlorophylls interact with the negative charged peroxy-radical to form charge-transferred complexes. Furthermore, the charge-transferred complexes might react with another peroxy-radical, leading to inactivate products stopping the chain reaction involving free radicals. Holasová *et al.*(1989) proposed that hydroperoxides may destroy pheophytin. Once pheophytin is destroyed by hydroperoxides, some effect on lipid oxidation might be expected.

2.3.7.2. Carotenoids

Carotenoids are naturally occurring pigments that posses Vitamin *A* activity, specially β-carotene, which is converted by enzymes in the gastrointestinal tract into vitamin *A*.

Chemically, carotenoids are conjugated hydrocarbons that may be further classified into two classes: carotenes which do not contain an oxygen molecule, and xanthophylls which have one or more oxygen molecules (Tan, 1989; Ong & Tee, 1992).

The main carotenoids found in vegetable oils are β-carotene, lutein and α-carotene (Fig. 2.3.7.2) (Tan, 1989; Ranalli, 1992; Ong *et al.*, 1995).

Carotenoids have been found to act as potent protectors

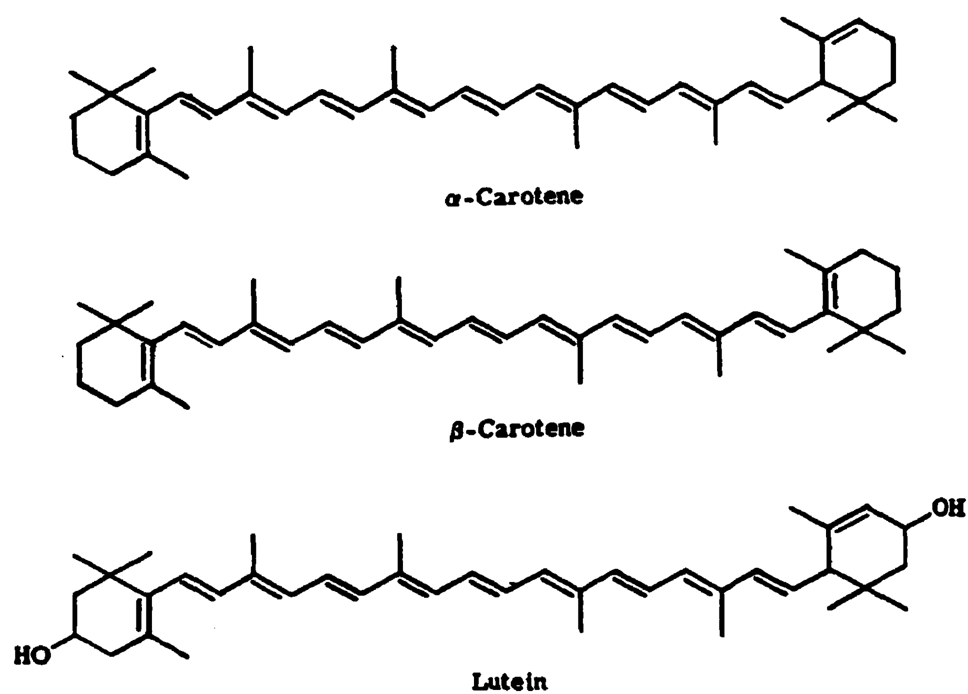


Fig. 2.3.7.2. Structure of some carotenoids commonly found in vegetable oils (Tannembaum *et al.*, 1985).

against photo-sensitized reactions, acting as singlet oxygen ($^1O_2$) quenchers (Bradley & Min, 1992; Palozza & Krisky, 1992). The physical quenching mechanism of carotenes is based on their low singlet energy state, which facilitates the acceptance of energy from singlet oxygen. The carotenoid triplet formed transfers acquired energy as heat and returns to its original state (Foote *et al.*, 1970).

The rate of singlet oxygen quenching is related to the number of conjugated double bonds present in the polyene chain. It was found that the rate of singlet-oxygen quenching increased when the number of conjugated double bonds of the carotenoids increased. Canthaxanthin with 13 conjugated double bonds had the greatest antioxidant activity, followed by β-carotene with 11 conjugated double bonds, then by β-apo-8'-carotenal with 10 conjugated double bonds. Zeaxanthin, lycopene and isozeaxanthin with 11 double bonds were similar in activity to β-carotene (Lee & Min, 1990).

Lee & Min (1988) showed that β-carotene when added at levels of 5-20ppm had a high potency in protecting purified soybean oil containing chlorophyll against deterioration under exposure to light. This protective effect was not observed during storage without light, consequently, β-carotene was regarded as a very effective singlet oxygen quencher. Similar results were found by Carlsson *et al*. (1976), Kiritsakis & Dugan (1985), Warner & Frankel (1987), Lee & Min (1990), Jung & Min (1991) and Lee & Kim (1992).

The presence of carotenoids in edible oils may also help to protect against the formation of singlet oxygen by blocking light transmission through the oil (Fakourelis *et al.*, 1987; Pokorný, 1991). It was suggested that these pigments function as a natural filter for light, effective through absorption of light with wavelengths between 400nm and 500nm (Fakourelis *et al.*, 1987; Frankel, 1991). Fakourelis *et al.* (1987) found that the presence of β-carotene decreased the photo-oxidation of purified olive oil when sensitizers such as chlorophylls were not present. The results suggested that the reduction in the rate of oxidation by adding β-carotene during storage under light might be due to an action other than singlet oxygen quenching. Consequently, the effect was attributed to the fact that purified olive oil containing β-carotene would derive less energy from a light source.

Although carotenes are efficient compounds protecting against photo-oxidation, degradation by lipid peroxides is expected due to the oxidative susceptibility of hydroxy groups and the conjugated system of double bonds in the carotenoid molecule (Frankel, 1991; Haila & Heinonen, 1994). Haila & Heinonen (1994) found that β-carotene acted as an antioxidant in an oxygen limited system, but it was not efficient when the amount of oxygen was unlimited in purified rapeseed oil during storage under light. Kiritsakis and Dugan (1985) reported that the addition of 4 to 6ppm of β-carotene to olive oil decreased peroxide formation only in the early stages of

photo-oxidation. The authors concluded that carotenes either acted as singlet oxygen quenchers or were oxidized, thus protecting the oil until all carotenes were destroyed by oxidation. Some studies suggest that β-carotene may act as an effective inhibitor of photo-oxidation in vegetable oil only when tocopherols are present (Warner & Frankel, 1987).

Other studies suggest that β-carotene may act as a pro-oxidant during storage without light. Lee & Kim (1992) reported that β-carotene and their oxidation products acted as pro-oxidants during the autoxidation of purified soybean oil. They found that the pro-oxidant activity of carotenes increased as their concentration increased from 50 to 200ppm when oil was stored without light at 60^{0}C.

2.3.8. Sterols

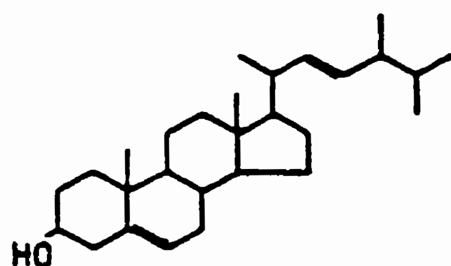
Sterols comprise the major portion of the unsaponifiable matter of most vegetable oils (Mordret *et al.*, 1985). They are collectively known as phytosterols, existing mainly as free sterols and sterols esters of fatty acids (Kochhar, 1983). In olive oil a very small portion of the total sterols present are esterified, while in others, such as sunflower, canola and corn oils, almost half of the sterols are present as esterified compounds (Johansson & Appelqvist, 1978; Dimitrios & Ioanna, 1986).

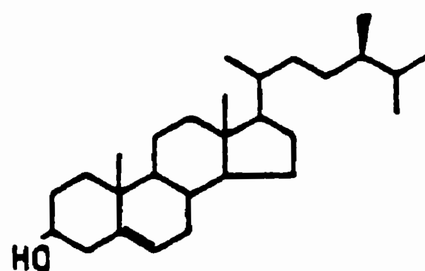
Generally there is a reduction in the amount of total and individual sterols during degumming, neutralization,

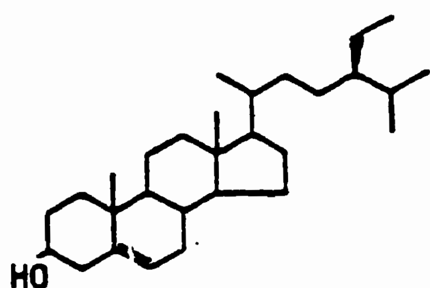
bleaching, hydrogenation, deodorization and steam refining compared to the initial content in crude oil. In common refined oils they range from trace amounts to about 1% of total oil composition (Kochhar, 1983).

Brassicasterol, campesterol, β-sitosterol and stigmasterol are the major sterols that have been identified in vegetable oils (Fig. 2.3.8) (Itoh *et al.*, 1973; Johansson & Appelqvist, 1978; Kochhar, 1983; Mordret *et al.*, 1985). Minor sterols may be present in some oils, such as Δ^5- and Δ^7-avenasterols, Δ^7-stigmasterol, Δ^7-campesterol, 28-iso avenasterol, fucosterol, ergosterol, and dermosterol (Itoh *et al.*, 1973; Johansson, 1979; Bianchini *et al.*, 1985; Holen, 1985). Other related compounds may also be present in oils, for example triterpene alcohols in olive oils, ferulate and *p*-coumarate esters of dimethyl sterols in corn oils, and oryzanols in rice bran oils (Cortesi *et al.*, 1987; Chryssafidis *et al.*, 1992; Rogers *et al.*, 1993; Diack & Saska, 1994; Norton, 1995). Cholesterol has been found in trace amounts in coconut, palm, palm kernel and linseed oils (Itoh *et al.*, 1973).

There is evidence that some naturally occurring phytosterols may improve the stability during heating of cooking oils at temperatures of about 180°C (Sims *et al.*, 1972; Boskou & Morton, 1975a). Some studies reported that the level of sterols decreased when oils were heated at 180°C, which could indicate the participation of sterols in







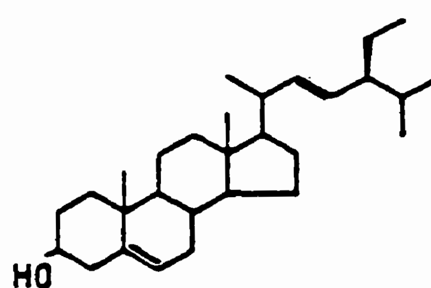


Fig. 2.3.8. Structure of some common phytosterols (Kochhar, 1980).

oxidation. Boskou & Morton (1975a,b) found that the total sterol content, measured by gas chromatography, was reduced about 30% after heating olive oil at 180°C. The authors found that among sterols, Δ^5-avenasterol appeared to be the most susceptible to air oxidation, or more vulnerable to heating at 180°C. Studies with synthetic and natural sterols present in soybean oil also indicated that large quantities of these sterols were lost when the system was exposed to high temperatures for a long period of time (Daly *et al.*, 1983; Ghavami & Morton, 1984).

Unsaponifiables, rich in sterols, acted as antioxidants when added to vegetable oils heated to frying temperatures. Sims *et al.* (1972) reported that the addition of olive, *Vernonia anthelmintic* and corn oil unsaponifiables, protected safflower oil from oxidative polymerization during heating at frying temperatures. The rate of destruction of polyunsaturated fatty acids by oxidative polymerization was measured by iodine value. The authors also reported that vernosterol, Δ^7-avenasterol and fucosterol were effective when added at a concentration of 0.2%, while ergosterol, lanosterol, β-sitosterol, stigmasterol and cholesterol were ineffective at the same concentration.

Boskou & Morton (1976) found that Δ^5-avenasterol and fucosterol had positive effects in retarding the oxidation of refined cottonseed oil. The authors suggested that the sterol ethylidene group, which is characterized by a double bond

between carbon atoms C_{24} and C_{20}, might have contributed to the stability of the oil (Boskou & Morton, 1975a, 1976).

A similar effectiveness of Δ^5-avenasterol and fucosterol as antioxidants was reported when they were added at concentrations of 0.01% to 0.1% into synthetic triglycerides (Gordon & Magos, 1983). These authors hypothesized that lipid free-radicals react rapidly with sterols that have unhindered allylic carbon atoms such as the ethylidene group. Isomerization could produce a stable allylic tertiary free-radical which interrupts the oxidation chain.

White & Armstrong (1986) also found that fractions isolated from oat oil containing Δ^5-avenasterol acted as antioxidants for soybean oil heated at 180^{0}C.

Hudson & Ghavami (1984) isolated and separated into individual classes the unsaponifiable matter from soybean and rapeseed oils. Those fractions containing sterols showed no antioxidant activity when tested in vegetable oils. The pro-oxidant effect of some sterols have also been demonstrated. Yanishlieva & Schiller (1984) found that the addition of 5% sitosterol caused a two-fold increase in the oxidation rate of tristearin heated at 120^{0}C.

2.3.9. Metals

The presence of metals in vegetable oils is due to their presence in the starting raw materials and because of contamination from manufacturing or storage equipment

(Ringkasan, 1982). Edible fats and oils are frequently subjected to processing, including refining, bleaching and deodorization, which inevitably makes the oil come in contact with metallic surfaces, often at elevated temperatures (Patterson, 1989). Heating vegetable oils may release catalytically active metals of oil oxidation from complexes with proteins and other oil components (Ringkasan, 1982; Ohlson, 1992). Nickel may be introduced as a contaminant during the hydrogenation and/or from equipments during the processing of vegetable oils. This metal is commonly used as a catalyst during hydrogenation process, and its complete removal from oil by filtration is not always possible (Roden & Ullyot, 1984).

Many reports have described the deleterious effect of trace amounts of metals (M) on the oxidative stability of oils. The general chemical mechanism for metal catalysis of lipid oxidation may follow different pathways (Nawar, 1985). Specifically iron (Fe), copper (Cu), and nickel (Ni) behave as direct initiators by electron transfer, or indirect initiators by oxidation and/or reduction reactions (Fig. 2.3.9.1). The hydroperoxide decomposition products formed increase the rate of chain re-initiation or propagation (Schaich, 1992).

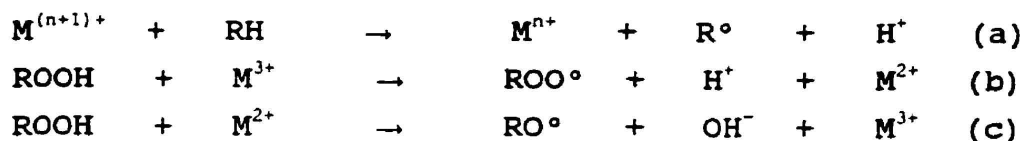


Fig. 2.3.9.1. Mechanism of metal catalyses: a) direct; b,c) indirect (Schaich, 1992).

Another possible pathway is to initiate fatty acid oxidation through the reaction of metals with triplet oxygen (Fig. 2.3.9.2). The superoxide anion radical (O^{-}_{2}) produced can either lose an electron to give singlet oxygen ($^{1}O_2$) or react with a proton to form a hydro-peroxyl radical (HOO°). Both radicals have high reactivity; therefore, they may be involved as oxidation initiators (Nawar, 1985).

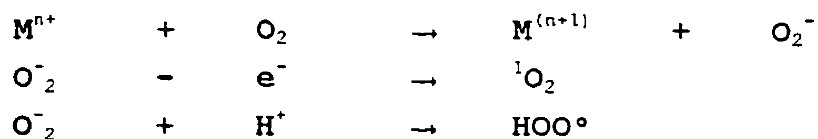


Fig. 2.3.9.2. Metal catalyses by reaction with oxygen (Nawar, 1985).

Metal reactions have proven to be complex and dependent on a number of factors such as the specific metal complex, valence state of the metal, concentration of the metal, oxygen tension of the system, types of lipids, and the presence of contaminating preformed hydroperoxides (Schaich, 1992).

The transition metals, which contain two or more valence states, have the highest oxidation potentials. These metals include iron, copper and nickel, as well as others of minor importance such as cobalt (Co) and chromium (Cr) (Labuza, 1971).

Copper and iron, in particular, greatly reduce the oxidative stability of unsaturated lipids and accelerate the development of rancidity in edible oils and fats at concentrations of a few ppm (Gapor & Ong, 1982; Benjelloun *et al.*, 1991). Metals affect not only the rate of initiation and total extent of lipid oxidation, but also the degree of chain reaction branching, secondary reactions, and the nature of termination reactions (Nawar, 1985).

Ringkasan (1982) found that increasing the content of iron from 1.5 to 15ppm in palm oil greatly increased the oil oxidation during storage at ambient temperature. The same author reported that palm oil in contact with traces of copper at 80^{0}C the residual colour readily increased from 2.0 to 4.8 units on Red Lovibond scale.

Martín-Polvillo *et al.*(1994) analysed the stability of sunflower and olive oils stored at 40^{0}C for 30 days when solid sheets of Fe, Cu, Ni, Al, Cr and Pb metals were added to the oil. They found that samples with higher acidity showed higher contents of Fe after the storage period. Samples with a higher content of Fe also showed the greatest loss in stability.

Angerosa & Di Giacinto (1993) found that the addition of 50ppb of nickel decreased the stability of virgin olive oil stored at room temperatures.

Abdel-Aal & Abdel-Rahman (1986) studied the action of some metals on the stability of cottonseed, sunflower and soybean oils during storage at ambient temperature. They found that copper and iron were the strongest pro-oxidant when added at levels of 5-10ppm. The effect of added metals on the rate of peroxide development was the greatest for Cu, followed by Fe and Ni.

Traces of Cu and Fe (0.1ppm) were also found to lower the flavour stability of margarines stored at ambient temperature (Mertens *et al.*, 1973).

Copper was also found to have a greater effect on the oxidation rate of rapeseed and palm oils than was found with iron (Jacobsberg *et al.*, 1978; Gordon *et al.*, 1994).

2.4. Measurement of oil stability

The length of time that an oil or fat can resist changes in its acceptable characteristics can be monitored by conducting stability tests. Although testing stability under ambient conditions may approximate real storage conditions of an oil, changes are too slow to be of practical value. Furthermore, under slow oxidation, the reproducibility of results is compromised by many variables that are difficult to control over prolonged storage periods (Frankel, 1993). For

this reason the stability of edible oils and fats is commonly determined using accelerated storage tests conducted at elevated temperatures and/or with or without the presence of light. The results obtained are often extrapolated to provide guidance on the probable behaviour of the products when stored at ambient temperatures (Dziedzic & Hudson, 1984b; Rossel, 1992).

2.4.1. Accelerated stability tests

To estimate the stability or susceptibility of a fat or oil to oxidation, the sample is usually subjected to an accelerated oxidation test under standardized conditions, and a suitable endpoint is chosen to determine signs of oxidative deterioration. The time required to reach an endpoint of oxidation corresponds to either a level of detectable rancidity, or a sudden change in the rate of oxidation (Ragnarsson & Labuza, 1977; Frankel, 1993).

There are a great variety of methods that can be applied to monitor the degree of deterioration of fats and oils, including chemiluminescence methods, storage under light, storage without light, storage at ambient temperatures, and storage at high temperatures. Since the rate of a reaction increases exponentially with the absolute temperature, this parameter is usually singled out to speed up oil oxidation (Ragnarsson & Labuza, 1977; Cash *et al.*, 1988).

Accelerated stability tests include the Active Oxygen

Method (AOM), Rancimat, Oxygen Uptake, Schaal Oven Test, and methods which expose samples to fluorescent light.

High temperature tests including the Active Oxygen Method (98^{0}C), Rancimat (100-140^{0}C), and Oxygen Uptake (80-100^{0}C) are considered unreliable, because the mechanism of lipid oxidation changes significantly at elevated temperatures. In addition, side reactions such as polymerization and cleavage become significant and undermine the reliability of the results (Ragnarsson & Labuza, 1977; Warner *et al.*, 1989; Frankel, 1993).

The Active Oxygen Method, designed to measure the peroxides formed by the reaction of oxygen with oil, may also be of questionable validity, because at 100^{0}C the rate of oxidation is highly dependent on oxygen solubility (Ragnarsson & Labuza, 1977). At this temperature the determination of a peroxide value becomes unreliable, because the primary hydroperoxides formed are unstable and decompose readily to form more stable secondary products (Frankel, 1993).

Oxygen Absorption Methods have limited sensitivity and require an excess of oxygen, and high levels of oxidation as the endpoint (Frankel, 1993).

The Rancimat is an automated instrumental test that measures the conductivity of low molecular weight fatty acids produced during the autoxidation of oils and fats (Wan, 1995). Levels of volatile acids measured are only significant at elevated temperatures, and therefore may not be relevant to

normal storage conditions (Rossell, 1992; Frankel, 1993).

A more reliable accelerate test to determine edible oil stability is the modified Schaal Oven Test. The Schaal Oven Test, originally developed for testing lard was conducted at 63^{0}C. It is considered the most reliable method to speed up oil oxidation since the endpoint represents a lower degree of oxidation and the results correlate well with evaluations of actual shelf-life temperatures (Ragnarsson & Labuza, 1977; Malcolmson *et al.*, 1994). At temperatures between 60-65^{0}C a number of side reactions are minimized; therefore, the possibility of misleading results are lower (Frankel, 1993). The quality of the oil sample may be examined periodically by chemical, physical and sensory tests (Robards *et al.*, 1988; Wan, 1995). Tests reported under these conditions showed that the flavour scores of oils aged four days at 60°C were equivalent to scores for oils aged four months at ambient temperature (Evans *et al.*, 1973).

There is no official method for light exposure testing, but usually these tests are performed in opened containers at 30-35^{0}C under fluorescent light (Wan, 1995). These tests are frequently used to evaluate the photo-oxidative stability of samples that naturally contain pigments which absorb energy from light (Bekbölet, 1990). They are very useful to predict storage stability and the selection of packaging material for oils and fats to prevent photo-oxidation (Robards *et al.*, 1988).

Besides temperature, the surface area and sample volume may have detrimental effects on oil oxidation rates. Variations in the surface area at a constant volume was found to be more influential on oxidation rate than the same volume with a constant surface area. A sample held in a 13-mm diameter container remained unaltered after 100 hours of heating, whereas that held in an 83-mm diameter container underwent intense oxidation (Garcia-Mesa *et al.*, 1993). Malcolmson *et al.* (1994) related the work done for other research, which measured the relation between the surface available for oxygen absorption and oil sample volume. The author found that when the ratio of volume to surface increased, oxidation rate decreased. This negative change in oxidation rate was directly related to oxygen partial pressure in the system.

2.4.2. Methods to assess oil stability

Lipid oxidation is a complex process involving numerous reactions that result in the formation of several different compounds, and many of them suffer further breakdown during the oxidation process (Labuza, 1971). There is no single method to measure all these compounds, due to the complexity of oxidized compounds present throughout the entire course of oxidation (Gray, 1978). Therefore, the use of more than one testing method is recommended to evaluate the oxidative stability of lipids.

Several methods are available to assess vegetable oil oxidation either by analytical procedures or sensory evaluation.

Numerous chemical and physical methods have been developed to quantitate oxidative deterioration in oils and fats. Each method gives information about particular stages of the oxidative process. Some are more suitable for measuring the primary products of oxidation, while others measure secondary products of lipid oxidation (Gray, 1978; Frankel, 1993). Among the most commonly used methods are peroxide value, conjugated dienoic acids, thiobarbituric acid value, anisidine value and volatiles.

Peroxide value determination is a valuable measure of the early stages of oxidation occurring under low temperatures (Hahm & Min, 1995; Gray, 1978) since at elevated temperatures, peroxides and hydroperoxides may decompose (Frankel, 1993). There are numerous procedures for determining peroxide value, but methods based on iodometric titration, which measure the iodine produced from potassium iodide by the hydroperoxides present in the oil, are the most common (Robards *et al.*, 1988; Hahm & Min, 1995; AOCS Official procedure Cd 8-53). Although they are simple to perform, these methods are considered highly empirical and accuracy depends on standardisation of the experimental conditions and operator (Robards *et al.*, 1988). The absorption of iodine by unsaturated bonds of fatty acids may affect results, and the oxygen present in the medium

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may facilitate the liberation of iodine from potassium iodine (Hahm & Min, 1995). The iodometric method for determination of peroxide value has shown good correlation with total volatiles, conjugated dienes, and flavour scores (Fioriti *et al.*, 1974; Snyder & Mounts, 1990; Hahm & Min, 1995).

A method to measure conjugated dienes was first developed to analyse the conjugation of unsaturated fatty acids in dehydrated oils (AOCS Ti-1a 64). Despite this fact, this procedure can be used successfully to measure oxidation of oils with unsaturated fatty acids (White, 1995). During the oxidation of polyunsaturated fatty acids, peroxide formation is accompanied by diene conjugation, which strongly absorbs energy at a wavelength of 232 to 234nm. The magnitude of changes is not readily related to the degree of oxidation, but the changes in absorption can be used as a relative measurement of oxidation (Gray, 1978). On the other hand, the magnitude of the value can not be compared between oils especially when large differences in the fatty acid composition are present (White, 1995). Positive correlations were found for conjugated dienes and peroxide values (St. Angelo, 1975).

The Thiobarbituric Acid Value (TBA) involves the reaction of thiobarbituric acid with monoaldehydes in the sample to produce a chromogen that absorbs energy in the visible spectrum (Robards *et al.*, 1988). The test is sensitive and precise, but the information provided can be misleading. The

TBA values may overestimate the extent of oxidation since other substances may react with TBA to yield coloured species (Gray, 1978; Frankel, 1993).

The anisidine value results from the reaction of aldehydes with *p*-anisidine, and the pigment formed is measured at a wavelength of 350nm. The test is particularly useful for abused oils with a low peroxide value (Robards *et al.*, 1988). The anisidine value serves as a useful indicator of oil quality, but the method is not sensitive enough, and it is not particularly suited for predicting off-flavours in oils (White, 1995).

Volatiles determined by gas chromatography is well adapted to the measurement of oxidation as it combines high sensitivity with the ability to separate complex mixtures (Robards *et al.*, 1988). This method can also provide useful data on the origin of flavour and odour volatiles and their precursors (Frankel, 1993). The variety of compounds produced appears to depend on the type of oxidation mechanism, the hydroperoxides formed, and the nature of the fatty acids involved (Przybylski & Eskin, 1995). In general the concentration of individual and total volatiles, which are secondary oxidation products, increases as the oxidation of vegetable oil increases (Fritsch & Gale, 1977; Snyder & Mounts, 1990). Several workers have reported a high correlation between the amount of individual and total volatiles and flavour scores, and between the amount of

volatiles and peroxide value (Jackson & Giacherio, 1977; Min, 1981; Snyder *et al.*, 1985; Warner & Frankel, 1985; Snyder & Mounts, 1990).

Sensory evaluation is widely used to monitor lipid oxidation for off-flavour formation. Flavour and odour assessments by a trained panel provides the most reliable and useful information related to consumer acceptability (Eskin et *al.*, 1996). However, sensory evaluation requires a long period of time to train panels (Gray, 1978) and the diversity of sensory vocabulary used by different investigators has led to controversies in vegetable oil oxidation studies (Frankel, 1991). High correlations have been achieved between flavour scores, volatiles and peroxide values (Fioriti *et al.*, 1974; Warner & Frankel, 1985).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Oils

Thirty-three vegetable oils with different origin and degree of processing were selected for the study (Table 3.1.1). These oils represented a wide variability in chemical composition related to fatty acid composition and minor components.

Fresh oils were obtained directly from processors, and stored at -40^{0}C in the absence of light until analysed. Oils contained no citric acid or other additives. All oils were packed in opaque plastic containers with capacity of one, four or twenty litters.

Golden palm (PLG) oil is also known as red palm oil. Olive pomace (OPR) oil is the oil obtained by treating olive pomace with solvents or the oil comprising the blend of refined olive pomace oil and virgin olive oil (International Olive Oil Council, 1995).

Table 3.1.1. Vegetable oils[1] used in the study.

Code	Oils	Industry
BOR	Borage[2]	Bior. Food & Sci.Co., Saskatoon,Canada
CAN	Canola	Canbra Foods Ltd., Lethbridge, Canada
CAO	Canola[3]	Canbra Foods Ltd., Lethbridge, Canada
CAR	Low Linolenic Canola	Cargill Foods, Idaho Falls, USA
CAS	Canola	CanAmera Foods, Toronto, Canada
CHO	High Oleic Canola	Cargill Foods, Idaho Falls, USA
COL	High Oleic Low Linolenic Canola	Cargill Foods, Idaho Falls, USA
COR	Corn	Archer Daniels Midland, Decatur, USA
CRN	Corn	Quincy, Charlotte, Canada
CRW	Corn[4]	Archer Daniels Midland, Decatur, USA
COC	Coconut[2]	Prem. Edible Oils, Corp., Portland, USA
COT	Cottonseed	Archer Daniels Midland, Decatur, USA
CTO	Cottonseed	Quincy, Charlotte, Canada
EPR	Evening Primrose[2]	Bior.Foods & Sci.Co., Saskatoon, Canada
FCO	Flax	Bior.Foods & Sci.Co., Saskatoon, Canada
FLL	Low Linolenic Flax	Prot.Oilseeds Starch, Saskatoon, Canada
OEV	Extra Virgin Olive[2]	Int. Olive Oil Council, Madrid, Spain
OPR	Olive Pomace	Int. Olive Oil Council, Madrid, Spain
ORF	Olive	Int. Olive Oil Council, Madrid, Spain
PAL	Palm[5]	Prem. Edible Oils Corp., Portland, USA
PLG	Golden Palm[2]	Global Palm Products, Johor, Malaysia
PLK	Palm Kernel[5]	Prem. Edible Oils Corp., Portland, USA
PEA	Peanut	Archer Daniels Midland, Decatur, USA
PNT	Peanut	Quincy, Charlotte, Canada
RBO	Rice Bran	Irgovel, Pelotas, Brazil
RIO	Rice Bran	Rito Inc., Stuttgart, Arkansas, USA
RWO	Rice Bran[4]	Rito Inc., Stuttgart, Arkansas, USA
SHO	High Oleic Sunflower	Cargill Foods, Idaho Falls, USA
SUN	Sunflower	CanAmera Foods, Toronto, Canada
SUR	Sunflower	Cargill Foods, Idaho Falls, USA
SOY	Soybean	Sadia, Tres de Maio, Brazil
SYB	Soybean	Quincy, Charlotte, Canada
SYS	Soybean	CanAmera Foods, Toronto, Canada

[1]refined, bleached and deodorized oils; [2]cold pressed oil; [3]refined, bleached and deodorized oil produced from grade III seeds; [4]refined, bleached, dewaxed and deodorized oils; [5]refined oil.

3.1.2. Chemicals and standards

TABLE. 3.1.2. Chemicals and standards used in the study.

Analyse(Company): standards
Fatty acids (Nu-Chek-Prep, Elysian, USA): Standard mixtures of methyl esters of fatty acids containing methyl hexanoate, octanoate, decanoate, dodecanoate, tri decanoate, tetradecanoate,9-tetradecenoate, pentadecanoate, hexadecanoate, 9-hexadecenoate, 9-transhexadecenoate, hepta decanoate, 10-heptadecanoate, octadecanoate, nonadecanoate, 6-octadecenoate, 9-octadecenoate, 11-octadecenoate, 9,12-octa decadienoate, 9,12,15-octa decatrienoate, 6,9,12-octadeca trienoate, eicosanoate, 5-eicosenoate, 8-eicosenoate, 11-eicosenoate, 11,14-eicosadienoate, docosanoate, 13-doco senoate, 11,14,17-eicosatrienoate, homo-gamma linolenate, eico sanoate, 13,16-docosadienoate, 13,16,19-docosatrienoate, 7,10, 13,16-docosatetraenoate, tetracosanoate, 15-tetracosenoate and methyl docosahexaenoate.
Triacylglycerols (Nu-Check-Prep, Elysian, USA): Trilaurin, tripalmitolein, tristearin, triolein, trilinolein, trilinolenin, tri-homogama linolenin and trinervonin.
Lipid classes (Sigma Chemical Co., St.Louis, USA): Sterylester (SE), triacylglycerol (TAG), 1,2 diacylglycerol (1,2DAG), 1,3 diacylglycerol (1,3DAG), free fatty acids (FFA), monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (MGMG), phosphatidic acid (PA), phosphatidyl inositol (PI), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), lyso-phosphatidyl ethanolamine (LPE), phosphatidyl choline (PC) and lyso-phosphatidyl choline (LPC).

TABLE. 3.1.2. (Cont.)

Analyse(Company): standards
Phenolic acids (Sigma Chemical Co., St.Louis, USA, and Fluka Chemica, Switzerland): 3,4,5-trihydroxy benzoic (gallic), *p*-hydroxybenzoic, 4-hydroxy-3-methoxybenzoic (vanillic), 2,5-dihydroxybenzoic (gentisic), 2-hydroxycinnamic (*o*-coumaric), 3,4-dihydroxybenzoic (protocatechuic), 4-hydroxycinnamic (*p*-coumaric), *p*-hydroxyphenylacetic, 4-hydroxy-3,5-dimethoxy benzoic (syringic), 3,4-dihydroxyphenylacetic (homo protocatechuic), 4-hydroxy-3-methoxycinnamic (ferulic), 3,5-dimethoxy-4-hydroxycinnamic (sinapic), 3,4-dihydroxycinnamic (caffeic) and 3-phenylpropenoic (*trans*-cinnamic) acids.
Tocopherols (Sigma Co., St.Louis, USA): Standards mixtures containing α-, β-, γ-, and δ-tocopherols.
Pigments (Sigma Chemical Co. St.Louis, USA): Chlorophylls *a* and *b*, lutein, α- and β-carotenes.
Phytosterols (Sigma Chemical Co., St.Louis, USA): 24-methyl-5,22-cholestadien-3βol (brassicasterol), 3β-hydroxy-24-ethyl-5,22cholestadiene(stigmasterol),24-methyl-5-cholesten -3βol (campesterol) and 24β-ethylcholesterol (β-sitosterol).
Volatiles (Bedoukian Research Inc., Danbury, USA): Pentane, hexane, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, 2,4-dodecadienal, 2-pentenal, 2-hexenal, 2-heptenal, 2-octenal, 2-nonenal, 2-decenal, 8-undecenal, 2,4-hexadienal, 2,4-heptadienal, 2,4-octadienal, 2,4-nonadienal, 2,4-decadienal, 2-butenal, 1-octen-3ol, 2-pentylfuran, 2-propenal and 2-methylpyrazine.

3.2. Evaluation of oil composition

3.2.1. Fatty acid composition

Fatty acid compositions (FA) of the oils were determined by modified acid-catalysed esterification and trans esterification of free fatty acids and glycerides, respectively, using AOCS Method Ce 1-62 (AOCS, 1991). Approximately 80mg of oil was dissolved in 1mL of petroleum ether, followed by the addition of 12mL of a 0.5N methanolic-hydrochloric acid solution. Samples were placed in an oven at 65^{0}C for 1 hour, periodically agitated, cooled, and then 6mL of water and 5mL of isooctane were added to the samples. The samples were shaked, and a part of the organic layer was taken to determine the fatty acid composition by gas chromatography (GC). The methylated samples were placed in an autosampler (Hewlett Packard model 7673, Avondale-USA), and 0.5μL of sample was injected into the gas chromatograph (Hewlett Packard model 5890A, Avondale-USA) equipped with a split injector and a flame ionization detector (FID). A capillary column, 30m x 0.25mm i.d., coated with 0.2μm of cyanopropyl phenyl-bicyanopropyl polysiloxane phase (Restek, Bellefonte, USA) was used. An initial column temperature of 130^{0}C was used for one min, then programed to 145^{0}C at the rate of 3^{0}C/min, then to 165^{0}C at the rate of 1.2^{0}C/min, and finally to 220^{0}C at the rate of 2.5^{0}C/min. At each stage of programing, the temperature was held for 1, 1.5, 0.5 and 1.5 min, respectively. The injector and detector were held at 250^{0}C.

The relative percentage (area %) of the fatty acids was determined using a reference mixture of methyl esters of fatty acids. The analyses were carried out in duplicate.

3.2.2. Triacylglycerol species

Triacylglycerol molecular species were determined using a modified procedure of Bergqvist & Kaufmann (1993). Oils were diluted in ethanol:isooctane (3:1, v/v) at a concentration of 1% (w/v), centrifuged and injected (10μL) in a liquid chromatograph (HPLC). The HPLC used was a Shimadzu SCL-10A (Shimadzu, Japan) equipped with auto injector SIL-10A, system controller LC-10AD, and an on-line degassing (Degassex DG440, Phenomenex, USA). Two reverse phase columns were used in series: Waters (USA) Nova-Pak C_{18}, 30cm x 3.9mm i.d. (pore size 60Å, particle size 4μm), and SGE (Australia) Exsil ODS-2 (2.5cm x 4mm i.d., particle size 3μm, and pore size 80Å). Both columns were held in a water jacket at 30°C. The detector was an evaporative light scattering detector (ELSD), Sedex 45 (SEDERE, France). The detector nebulizer was set at 40psi, gain 6, and held at 50°C. The solvents, acetonitrile (A), and a mixture(B) of ethanol:isooctane:acetonitrile- 2:2:1 (v/v/v) were used at a flow rate of 0.8mL/min. The gradient started at 100%A and increased to 50%B in 5min, continuing to 100%B in 60min, then was held for 10min, and programed back to 100%A in 20min. An additional 10min was used to re-equilibrate the columns.

The identification of triacylglycerol molecular species was made by comparison with retention data of the standards, and by the calculation of theoretical carbon numbers (TCN) of triacylglycerols as proposed by El-Hamdy & Perkins (1981) and Dotson *et al.* (1992). The reproducibility of the method was evaluated by running random samples in triplicate.

3.2.3. Lipid classes

Lipid classes, including non-polar fraction (NL), free fatty acids (FFA) and polar fraction (PL), were separated using solid-phase extraction SPE cartridges packed with amino propyl phases (Waters, Missassagua Sep-Pak cartridges) (Pietsch & Lorenzo, 1993). The aminopropyl cartridges were activated with 8mL of hexane prior to separation. Oils (25mg) were dissolved in chloroform:isopropanol (2:1, v/v) and applied on activated cartridges. The non-polar fraction (TG, 1,2DAG, 1,3DAG, SE) was eluted with 10mL of chloroform: isopropanol (2:1, v/v), FFA by 8mL of diethylether:acetic acid (95:5,v/v), and finally 10mL of methanol:hydrochloric acid (98:2, v/v) was used to elute the polar fraction (GL, PL). The solvent from fractions was evaporated at 50^0C under nitrogen, and the residue dissolved in 100-500μL of chloroform:methanol (4:1, v/v).

Further separations of the non-polar and polar fractions with quantification of the individual lipids were done by thin-layer chromatography with a flame ionization detector

(TLC/FID) (St. Angelo & James-Jr., 1993). An Iatroscan TH-10 analyser model MK-II (Iatron, Japan) was used. The FID was operated with a hydrogen gas and air flow at 185mL/min and 2L/min, respectively. The scan speed used was 3.1 mm/sec. Silica-coated Chromarods S-III (Iatron, Japan) were used for separation. The rods were initially cleaned in 50% of sulfuric acid, throughly washed with distilled water, dried at 120^{0}C, and scanned to burn off impurities. The rods were routinely burned before and after samples application.

The non-polar, free fatty acids and polar fractions were spotted at 2-20μg in the TLC rods. The non-polar and free fatty acid fractions were separated by developing for 1 hour in the solvent mixture of 1,2-dichlorometane:chloroform: acetone:acetic acid (59:10:1.4:0.4, v/v/v/v). The polar fraction was developed sequentially with partial scan and in two solvent systems. Initially for 25 min in acetone:acetic acid:water (70:1.6:1.5, v/v/v), then the rods were scanned to about the half of length to determine glycolipids. Secondly, the set of rods was developed for 1 hour in chloroform: methanol:water:acetic acid (50:27:2:0.3, v/v/v/v) to separate phospholipids.

The individual lipids from those fractions were identified by comparison with retention data of pure standards. For quantitative determination of the individual lipid components, standard solutions containing from 0.1μg to 20μg of each component were applied. A standard curve of peak

area versus lipid concentration was obtained for each individual component. Samples were spotted in TLC rods in duplicate.

3.2.4. Tocopherols and tocotrienols

Tocopherols and tocotrienols were analysed by HPLC with a fluorescence detector (Chase *et al.*, 1994). A HPLC Shimadzu System was used, equipped with a Hewlett Packard (HP-1046A) programable fluorescence detector set at 290nm excitation and 330nm emission. A 5μm silica column, 2.50cm x 3.2mm i.d. (Prodigy, Phenomenex, Torence, CA) was used. A mobile phase of hexane:isopropanol (99.5:0.5, v/v) at a flow rate of 0.7mL/min was employed.

Samples (1g) were diluted in 10mL of hexane, centrifuged and 35μL injected on HPLC.

Standards mixtures containing α-, β-, γ-, and δ-tocopherols were used for peak identification. Tocotrienols were further identified by mass-spectrometry after collection of their peaks. Tocopherols and tocotrienols were quantified based on a calibration curve made for α-tocopherol.

3.2.5. Phenolic compounds

The phenolic compounds were extracted from oils using a modification of the method described by Akasbi *et al.* (1993). Ten grams of oil was dissolved in 15mL of hexane, and extracted three times with 9mL of methanol:water (60:40, v/v).

The mixture was shaken for two minutes and allowed to separate, then the lower layer was collected. The excess of solvent was evaporated under reduced pressure at 40^{0}C. The residue was dissolved in 0.5mL of methanol:water (60:40, v/v) and 50μL injected onto the HPLC.

The separation on HPLC followed the modified method described by Diack & Saska (1994). The HPLC system was equipped with a reverse phase column Ultrasphere ODS-5μ, 25cm x 4.6mm i.d. (Beckman, USA). A gradient elution with water:acetic acid (99:1, v/v)(A) and methanol(B) was used at a flow rate of 1mL/min. During the first minute of run the gradient was held at 5%B, increasing consecutively to 20%B at 8min and to 40%B at 25min. The mobile phase composition was held at 40%B for a further 10min, then returned to the starting mobile phase in 5min, and finally equilibrated for 10min. A UV detector set up at 280nm was used.

Standards were used for peak identification and to calibrate for quantification of individual phenolic compounds. Solutions containing gallic and *p*-coumaric acids were used for recovery studies. A 100mL solution containing 2.5mg of gallic and *p*-coumaric acids was prepared in methanol:ethanol (4:1, v/v). Volumes of 1, 2 and 3mL of the solution were transferred to three sets of 10g of peanut oil (PEA), which did not contain gallic acid and only traces of *p*-coumaric acid. The samples were agitated and the extraction procedure previously described applied. From the final solution 10-20μL

were injected on HPLC. The obtained recoveries were 98.7% (standard deviation 0.89) and 99.4% (standard deviation 1.27) for gallic and *p*-coumaric acids, respectively.

3.2.6. Pigments

Total chlorophylls were determined by AOCS Tentative Method Cc 13d-55 (AOCS, 1991). The measurements were made with samples prepared as 10% solution (w/v) in isooctane: ethanol (3:1, v/v) using a Varian DHS 200 UV-Visible spectrophotometer (Palo Alto, CA). The slit was set at 2.0 and the scan rate at 20nm/sec. The presence of carotenoids was analysed scanning the samples at 400-500nm at a scan rate of 50nm/sec.

Individual chlorophylls, chlorophyll derivatives and carotenoids were qualitatively determined by HPLC (Daun & Thorsteinson, 1989). Samples (1.25g) were diluted in 5mL of isooctane:ethanol (3:1, v/v) and 50μL injected into HPLC. The HPLC system consisted of three Waters pumps model 510, a 715 Ultra Wisp Waters autosampler and a Waters 994 Programable Photodiode Array detector. A 5μm reverse phase column, 25cm x 4.6mm i.d. (Pierce Chemical Co., Rockford- USA), packed with ODS-2 was used. A mobile phase consisted of three solvents: (A)acetonitrile, (B)isooctane:methylene chloride (1:1, v/v) and (c)water:methanol (1:9, v/v) were used with a flow rate of 1mL/min. The gradient was programed as followed: 100% of C was held for 6min and then linearly reduced to 45% in 18min,

while A and B increased to 40% and 15%, respectively. Solvent A was linearly increased to 80% and C reduced to 5% in 28min. The column was cleaned between samples by running each solvent for 5min.

A modified procedure was used for the quantification of pigments, with the following modification of the mobile phase and gradient: Solvent C was changed to 100% of methanol. The gradient changed linearly from 100%C to 60%C, 30%A and 10%B for 20min, then to 60%A, 20%B and 20%C for 30min, and finally to 60%A, 30%B and 10%C for 35min at a flow rate of 0.9mL/min. A Shimadzu HPLC system was used. Chlorophyll *b*, lutein, α- and β-carotenes were measured at 450nm, chlorophyll *a* and pheophytin *b* at 432nm, and pheophytin *a* at 408nm. An Ultrasphere ODS 5μm column, 25cm x 4.6mm i.d. (Beckman, USA) was used.

Standards of chlorophylls *a* and *b*, lutein, and α- and β-carotenes were used for identification and calibration curves of individual pigments. Pheophytins *a* and *b* were prepared by acidification of the respective chlorophylls (Canjura & Schwartz, 1991). Hydrochloric acid solution (1N) was added dropwise to chlorophylls in a separatory funnel. The mixture was shaken until the colour changed from green to olive brown, then 10mL of diethyl ether was added. The excess of acid was removed by washing the solution 4 times with 5mL of water. The organic layer was dried over anhydrous sodium sulphate and evaporated under nitrogen at 35^{0}C. The residue was dissolved

in isooctane:ethanol (3:1, v/v). The analyses for samples containing pigments were carried out in duplicate.

3.2.7. Sterols

Oils were saponified with an ethanolic solution of potassium hydroxide (KOH) (Lozano *et al.*, 1993). Samples (0.5g) were weighted in screw-cap tubes, dissolved in 10mL of KOH (1.0N), flushed with nitrogen and while protected from light were kept overnight at ambient temperature. Then 10mL of water was added to the mixture, and extracted three times with 10mL of diethylether. The organic layers were combined and washed twice with water, then the excess of solvent was removed under vacuum at 40^{0}C. The residue was dissolved in 4mL of methanol:ethanol (1:1, v/v) and analysed by HPLC.

The sterols were separated on HPLC using the modified method described by Indyk (1990). A sample of 30μL was injected into the Shimadzu HPLC system equipped with a reverse phase column Exsil ODS-2 (SGE, Australia), 25cm x 4mm i.d., particle size 3μm and pore size 80Å. A UV detector was used set at 205nm. An isocratic elution with methanol at a flow rate of 0.7mL/min was used. Standards were used for peak identification and calibration for individual sterols.

Solutions of stigmasterol and β-sitosterol were used for recovery studies. A solution containing stigmasterol and campesterol was prepared by adding 1.0mg of each sterol in 10mL volumetric flask, and the volume was completed with

methanol:ethanol (1:1, v/v). Volumes of 1, 2 and 3mL of solution were transferred to three vials containing 0.5g of borage (BOR) oil, which did not contain stigmasterol and only a small amount of β-sitosterol. The saponification was carried out as previously described, and 20-40μL of the final solutions were applied on HPLC. The recovery was 98.7% (standard deviation 1.37) and 99.5% (standard deviation 0.91) for stigmasterol and β-sitosterol, respectively.

3.2.8. Metals

Iron (Fe), copper (Cu) and nickel (Ni) were measured in oils by Atomic Absorption Spectrophotometry using a graphite furnace, following modified AOCS Official Method Ca 18-79 (AOCS, 1991). Samples and standard mixture were dissolved in methylisobutyl ketone (MIBK), with the detection limit of 0.020ppm. The analyses were performed at the Grain Research Laboratory, Winnipeg, Manitoba.

3.3. Oxygen consumption

The oxidative stability based on measurement of oxygen consumption by oils was carried out by a headspace method modified from that described by Jung *et al.* (1989, 1991). A Shimadzu chromatograph (Series 14, Columbia, MD) equipped with a thermal conductivity detector was used. A stainless steel column, 2m x 1.25mm i.d., packed with molecular sieve 5A 80/100 mesh (Supelco Inc., USA) was used for gas analysis.

Helium at a flow rate of 10mL/min was used as carrier gas and a detector current of 180mA was applied. The column temperature was kept at 85°C while injector and detector were set at 90°C.

Samples, 10g of oil, were placed in 60mL clear serum bottles (5.6cm x 3.7cm i.d.), providing a ratio of surface area to volume of 1.0. The bottles were sealed air tight with Teflon-coated rubber septa and aluminium caps. An additional 5mL of air was added to each bottle to avoid too low an internal pressure during analyse.

Two sets for each oil were prepared for storage. One set was stored in the absence of light, where each bottle containing oil was covered with aluminium foil as protection from light exposure. Samples were randomly placed in an oven (Fisher Scientific, model 655F) and stored at 65°C. The other set of samples was stored under light exposure by placing the bottles randomly placed in an oven (provided with air circulation) and storing at 35°C under fluorescent light (4500 lux). All samples were periodically agitated during the storage period.

Measurements performed, in duplicate, were taken every 12 hours during 14 days of storage by injecting 20μL of headspace into the GC. The oxygen content in the headspace was expressed as a percentage (w/w) of the oxygen in the headspace.

To calculate the relative percent of oxygen present,

calibration curves for oxygen and nitrogen were prepared. The calculation was based on the Universal equation of ideal gases (PV=nRT), assuming that air contains 20.946% oxygen, and that 1mol of air occupy volume of 22.4L at 1atm and 273K. Therefore, 1mL of air contains 0.20946 mL of O_2. Substituting this volume in the equation PV=nRT gives 9.3567×10^{-6} moles of oxygen, or 9.3567 μmoles of oxygen/mL of air. The oxygen measurement was carried out in duplicate.

3.4. Conventional storage of oils

The storage of oils was performed using the modified procedure described by Przybylski *et al.* (1993). Selected oils, 20g, were placed in clear jars (8cm x 5cm i.d.) giving a ratio of surface area to volume equal to 1.0. Two sets of each oil were prepared for each storage condition. Two sets were stored in the absence of light at 65°C, and another two sets were stored in the presence of light at 35°C, at the same conditions previously described in 3.3. The jars were not covered during storage.

For both storage conditions, two jars of each oil were randomly removed at each storage interval for both storage conditions. The oil from the two jars were combined, flushed with nitrogen, and stored at -40°C until analysed. Oils stored in the absence of light were sampled at 2, 4, 6, 10 and 16 days of storage, while oils stored under light were sampled at 1, 3, 5, 8 and 12 days of storage.

To evaluate the reproducibility and accuracy of measurements two oils were randomly selected and stored in duplicate for each type of storage condition. The oils selected were CHO and SYS for storage in the absence of light, and CAS and SUR for storage in the presence of light.

3.4.1. Measurement of oil stability

3.4.1.1. Peroxide value (PV)

Peroxide values were determined by AOCS Method Cd 8-53 (AOCS, 1991). Analyses were performed in duplicate.

3.4.1.2. Conjugated dienoic acid (CLA)

The conjugated dienes were determined by spectrophotometric measurement at 233nm, AOCS Method Ti 1A-64 (AOCS, 1991). Analyses were performed in duplicate.

3.4.1.3. Volatiles

The determination of volatiles was carried out using the method described by Przybylski *et al.* (1993). Oils (50 to 100μL) were placed into 15cm glass inserts packed with 1-2cm of cleaned glass wool. An internal standard, dodecane, at 187ng was added to the glass wool as an oil solution. The glass insert with sample was placed into the injection port of a gas chromatograph (Perkin Elmer 8500, Norwalk, Connecticut) and purged for 15min with carrier gas (He). A precolumn cooled with liquid nitrogen was used as a trap for volatiles.

After purging, the volatiles were transferred to a capillary column RTX-5 (95% dimethyl and 5% diphenol polysiloxane), 60m x 0.32mm i.d. and 1μm phase coating (Restek, Bellefonte, USA). For separation, the column temperature was consecutively programmed from 45^{0}C to 85^{0}C at the rate of 2^{0}C/min, 85^{0}C to 125^{0}C at the rate of 3^{0}C/min, 125^{0}C to 205^{0}C at the rate of 4^{0}C/min, and finally from 205^{0}C to 240^{0}C at the rate of 5^{0}C/min. Lower, intermediate and upper programming temperatures were held for 2, 0.1, 0.1 and 4.5 min, respectively. The injector and detector temperatures were set at 125^{0}C and 250^{0}C, respectively. Standards were used for peak identification. Random samples were run in triplicate to determine the reproducibility of the method.

3.4.2. Measurement of oil composition

To evaluate the changes in oil components during storage, analyses of fatty acids, triacylglycerol species, tocopherols, tocotrienols, phenols, chlorophylls, carotenoids and sterols were performed, as previously described.

3.5. Statistical analysis

Analyses of variance (ANOVA and MANOVA) were performed to evaluate the differences between the rate of oxidation of oils, and changes in composition during storage periods. Duncan's test was performed when statistical significance was shown at $P<0.05$. Cluster analyses were performed to classify

oils with similar oxidation. The statistical analyses were performed using Statistica software (StatSoft, 1994).

To compare data obtained by the oxygen consumption method, linear regression analysis was applied for each curve obtained by plotting time versus oxygen (%) in the headspace. The induction period was calculated at the point in which the slopes of the sigmoidal curves were intercepted. It was assumed that $y_a = \beta_0 + \beta_1 x_a$ the equation that represented the first slope of the sigmoidal curve (initiation period), and $y_b = \gamma_0 + \gamma_1 x_b$ as the equation for the second slope (propagation period). On the intersection of the two slopes $y_a = y_b$, where y_a and y_b represent the % of oxygen in the headspace. Reorganizing the equations, the intersection point is displayed as $x_a = (\beta_0 - \gamma_0)/(\gamma_1 - \beta_1)$, where x_a represents the induction period in hours, and β_0, β_1, γ_0 and γ_1 as the constants obtained from the regression equations.

Artificial Neural Networks (ANNW) was used to predict the stability of the oils based on their composition. The oils were separated into two data sets, where one set was used as training data, and another set was used to predict values. Correlation coefficients (r^2) and standard error of the estimate (SEE) were calculated between predicted and experimental values. This analysis was performed using the program Brain Maker (California Scientific Software, CA).

4. RESULTS

4.1. Oil composition

4.1.1. Fatty acid composition

The fatty acid composition of the oils is provided in Table 4.1.1. Canola oils (CAN, CAO, CAS, CHO and CAR) were the major source of unsaturated fatty acids, presenting the lowest content of saturated fatty acids (6.7% to 7.9%) of all the oils. Palm oils, PLG and PAL, showed a similar levels of saturated and unsaturated fatty acids. These oils contained 40-50% saturated fatty acids, where palmitic acid, with a contribution of 33.9% and 42.3%, respectively was the major component (Appendix I). Coconut (COC) and palm kernel (PLK) oils showed the lowest proportion of unsaturated fatty acids. Saturated fatty acids contributed 80-90% of total acids, while oleic acid was the main unsaturated fatty acid. Lauric acid was the major component, with levels of 45.5% and 46.1% in COC and PLK, respectively (Appendix I).

Genetically modified canola oils: high oleic (CHO), low linolenic (CAR), and high oleic low linolenic (COL) fatty acid varieties, showed higher amounts of oleic acid when compared to conventional canola varieties. Among canola oils, low linolenic canola oil (CAR) had a level of linolenic acid of

2.3% compared with levels of 7.9 to 10.1% in regular canola oils, while the oleic acid contribution increased in a similar proportion. The reduction in linolenic acid in high oleic low linolenic canola oil was 3.2%, while in high oleic canola it was 4.9%.

A similar trend was observed with high oleic sunflower oil (SHO), which presented 76.3% oleic acid, while the conventional lines (SUN and SUR) showed 15.3 to 16.9%. The high proportion of oleic acid in SHO was related to a similar reduction of linoleic acid content, and a smaller decrease in stearic and palmitic acids. The high oleic acid varieties contained oleic acid at levels similar to olive oils (OEV, OPR and ORF).

Conventional flax oil (FCO) contained linolenic acid (54.2%) as more than half of total fatty acids, while in genetically modified low linolenic flax oil (FLL) the linolenic acid content was reduced to less than 2.0%. The reduction in linolenic acid in the latter oil was compensated for by an increase in linoleic acid in a similar proportion. FLL contained a similar amount of linoleic acid (about 70%) as evening primrose (EPR) and regular sunflower oils (SUN and SUR).

Borage (BOR) and evening primrose (EPR) oils were distinguished from others due to the content of γ-linolenic acid (18:3 *n*-6). These oils contained 98-99% of the linolenic acid presented in table 4.1.1 as γ-isomers (Appendix I).

Fatty acids with more than 18 carbon atoms were found in only small amounts for most oils. Canola and peanut oils contained about 3% behenic acid, and peanut oils contained about 2% lignoceric acid. Borage (BOR) oil contained 4, 2, and 2% of eicosenoic, erucic and brassidic fatty acids, respectively.

Table 4.1.1. Fatty acid compositions (area %).

OILS[1]	SFA	$C_{18:1}$[2]	$C_{18:2}$[3]	$C_{18:3}$[4]	MUFA	PUFA
BOR	12.96	16.52	38.47	22.75[5]	25.47	61.56
CAN	6.98	62.41	20.12	8.37	64.41	28.61
CAO	6.73	59.31	21.00	10.10	62.08	31.19
CAS	7.92	63.06	18.93	7.91	65.16	26.93
CAR	7.94	67.59	20.45	2.26	69.27	22.78
CHO	6.84	77.00	9.02	4.90	79.19	13.97
COL	8.44	74.44	12.17	3.24	76.03	15.53
COR	13.86	24.23	60.38	0.99	24.77	61.37
CRN	13.47	25.54	59.27	1.07	26.09	60.44
CRW	13.50	24.31	60.60	0.95	24.83	61.68
COC	90.69	7.45	1.80	-	7.51	1.80
COT	25.73	16.61	56.35	0.33	17.50	56.78
CTO	26.20	17.37	54.78	0.53	18.34	55.38
EPR	8.12	7.50	74.00	9.76[5]	7.99	83.90
FCO	8.16	21.42	15.18	54.24	22.02	69.81
FLL	10.72	16.73	70.29	1.86	17.04	72.24
OEV	12.98	76.34	8.64	0.75	77.43	9.59
OPR	13.53	74.00	10.33	0.77	75.37	11.10
ORF	15.27	75.55	7.01	0.66	77.00	7.73
PAL	49.49	39.37	10.62	0.21	39.67	10.83
PLG	39.74	46.71	12.85	0.32	47.01	13.25
PLK	80.34	16.46	2.76	-	16.91	2.76
PEA	18.38	48.66	31.01	0.23	50.37	31.24
PNT	19.18	49.04	29.45	0.23	51.03	29.79
RBO	20.69	40.50	35.69	2.11	41.40	37.91
RIO	18.05	43.87	36.28	0.99	44.67	37.27
RWO	17.44	44.41	35.91	1.39	45.25	37.30
SHO	9.16	76.33	12.56	0.96	77.19	13.64
SUN	12.35	15.26	71.17	0.45	15.94	71.72
SUR	11.93	16.86	70.69	0.27	17.11	70.96
SOY	15.10	21.35	56.02	7.15	21.72	63.17
SYB	15.44	23.44	52.84	7.60	24.01	60.56
SYS	15.01	24.30	52.80	7.51	24.68	60.31

SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; [1]abbreviations see Table 3.1.1; [2]oleic acid, [3]linoleic acid; [4]γ-linolenic acid for BOR and EPR and α-linolenic acid for other oils.

4.1.2. Triacylglycerol composition

Conventional flax, which contained 54% linolenic acid, showed that about 58% of all triacylglycerols had at least two

linolenic acids' moities attached to the glycerol molecule, in the following triacylglycerides: LnLnLn, LnLnL, LnLnO and LnLnP (Table 4.1.2.1).

Borage (BOR) oil, with 22% linolenic acid, showed a similar distribution of triacylglycerols, where 48% contained at least one linolenic acid residue attached to the glycerol molecule, LnLnL, LnLnO, LnLO, LnLP and LnOO. But, in BOR the linolenic acid attached to the glycerol molecule was a γ-isomer.

Oils with a high content of linoleic acid, EPR, FLL, SUN and SUR, contained 42-58% glycerides as LLL molecular species, and about 80% triacylglycerols as LLL, LLO and LLP. Among the above mentioned oils, evening primrose (EPR) had the highest content of linoleic acid (74%), and 58% of it was esterified in the form of LLL molecular specie. Also, EPR was richer in linolenic acid than SUN, SUR and FLL, which was esterified as LnLnO (15%).

Table 4.1.2.1. Triacylglycerol species (area %).

OILS[1]	LnLnLn	LnLnL	LnLnO	LnLnP	LLL	LnLO	LnLP	LLO	LnOO	LLP
FCO	23.37	12.98	16.46	5.45	0.26	7.69	6.64	1.94	10.36	1.31
BOR	0.00	10.76	11.97	0.00	7.76	13.34	9.06	18.02	3.10	11.65
EPR	0.76	0.00	15.35	0.00	58.24	2.00	1.31	9.44	0.00	9.52
SUN	0.00	0.00	0.00	0.00	42.32	0.00	0.02	27.76	0.00	11.04
SUR	0.00	0.00	0.00	0.00	45.10	0.00	0.00	28.65	0.00	9.88
FLL	0.00	0.00	0.04	0.00	48.20	0.35	0.13	21.23	0.00	12.25

[1]Abbreviations see Table 3.1.1; Ln= Linolenic acid; L= Linoleic acid; O= Oleic acid; P= Palmitic acid; TAG species which were present at levels higher than 5% in at least one oil.

Genetically modified canola (CHO and COL) and sunflower (SHO) oils, contained more than 65% of their oleic acid as trioleate (OOO)(Table 4.1.2.2). Regular canola oils, which contained lower amounts of oleic acid, showed a reduction in the contribution of trioleate when compared to the high oleic canola oils. But, the proportion of molecular species LnLO, LLO, LnOO and LOO increased due to their higher content of linoleic and linolenic fatty acids. Olive oils, OEV, OPR and ORF, high in oleic acid content, showed about 10% less trioleate than in CHO, COL and SHO oils. The amount of palmitodioleate contained in CAR, CHO, COL and SHO was 15 to 20% less than the amount contained in olive oils. For oils with high oleic acid content, more than 86% of triacylglycerols were present in the form of OOO, LOO, LnOO and POO.

Table 4.1.2.2. Triacylglycerol species (area %).

OILS[1]	LnLL	LnLO	LLO	LnOO	LOO	OOO	POO
CHO	1.12	1.16	3.31	4.22	16.78	65.06	4.11
COL	1.08	1.07	3.83	3.90	17.33	65.03	4.81
CAR	1.35	1.08	7.52	1.33	27.63	52.24	3.40
SHO	0.03	0.00	2.41	0.18	13.17	67.74	5.51
OEV	0.00	0.00	0.37	0.14	13.79	55.49	22.53
OPR	0.00	0.02	1.47	0.31	12.11	53.25	21.78
ORF	0.00	0.01	0.36	0.23	8.44	53.26	25.98
CAS	1.12	3.90	9.80	8.06	29.43	38.25	3.29
CAN	1.07	3.53	10.14	8.78	29.82	38.81	2.41
CAO	2.28	6.79	8.23	10.06	27.42	34.17	2.81

[1]Abbreviations see Table 3.1.1; Ln= Linolenic acid; L= Linoleic acid; O= Oleic acid; P= Palmitic acid; TAG species which were present at levels higher than 2% in at least one oil.

Palm oils, PAL and PLG, which contained high amounts of saturated fatty acids, especially palmitic acid, showed more than 85% of triacylglycerols containing at least one molecule of this acid (Table 4.1.2.3). PLG showed a higher content in POL and POO than did PAL, because of PLG's higher oleic acid content. However, PAL showed a higher contribution of dipalmitoleate because of its higher content of palmitic acid.

Corn and soybean oils had very similar compositions of saturated and oleic acids, while corn oils contained 4 to 8% more linoleic acid, and soybean about 7% more linolenic acid. These differences were well expressed in the glyceride species. Both oils had very similar compositions of LLL, LLP, LOO and POL, while corn oils had about 8% more dilinoleate and 5% less LnLL than soybean oils.

Peanut and rice bran oils showed similar fatty acid

composition as well as similar triacylglycerol species. Peanut oils contained higher amounts of oleic acid and a higher content of OOO and LOO glycerides species than did rice bran oils. However, rice bran oils showed higher proportions of glycerides species with palmitic acid, POO and POL, due to their higher content in this fatty acid (about 15%) than peanut oils (about 9.5%).

Table 4.1.2.3. Triacylglycerol species (area %).

OILS[1]	LnLL	LLL	LLO	LLP	LOO	POL	PPL	OOO	POO	POP
COR	0.08	26.64	30.45	15.91	11.59	9.95	0.56	1.87	1.40	0.11
CRN	0.08	24.13	30.27	15.59	12.87	10.88	0.58	2.23	1.51	0.11
CRW	0.07	25.84	30.76	16.17	11.93	10.03	0.54	1.85	1.28	0.10
COT	0.00	16.01	10.47	37.95	1.65	15.56	13.60	0.14	0.97	1.48
CTO	0.00	16.55	10.50	37.67	1.79	15.10	13.17	0.21	0.99	1.51
PAL	0.00	0.00	0.10	0.64	0.63	8.58	7.74	2.12	28.53	42.14
PLG	0.00	0.00	0.10	1.34	1.24	16.85	8.61	3.85	42.45	18.85
PEA	0.00	0.94	12.18	2.67	28.59	12.93	0.70	24.41	8.31	0.46
PNT	0.00	0.29	12.36	1.47	31.61	12.55	0.40	25.33	9.05	0.29
RBO	0.05	1.69	13.24	8.46	20.99	23.06	3.95	10.95	14.00	1.96
RIO	0.00	2.57	14.93	7.05	23.04	20.04	3.90	12.66	12.31	1.35
RWO	0.03	2.74	15.44	7.36	22.34	20.27	4.84	13.39	11.04	1.02
SOY	5.39	23.63	22.91	15.68	9.45	9.39	1.84	1.06	1.90	0.10
SYB	5.28	21.73	21.59	15.60	8.78	9.27	1.23	1.90	1.42	0.19
SYS	4.00	23.35	23.57	15.49	9.85	9.94	0.82	2.21	1.39	0.11

[1]Abbreviations see Table 3.1.1; Ln= Linolenic acid; L= Linoleic acid; O= Oleic acid; P= Palmitic acid; TAG species which were present at levels higher than 3% in at least one oil.

Palm kernel (PLK) and coconut (COC) oils had a high content of low molecular weight fatty acids such as capric, caprylic and lauric fatty acids. These fatty acids formed mainly glycerides with equivalent carbon number (ECN) between

30 to 40. Due to the low ECN, these triacylglycerols were very difficult to fully separate. They eluted early, and even two columns in series were not sufficient to separate some critical pairs, which are molecular species that had the same ECN. A poor separation for compounds with an ECN lower than 36 was observed. This is the group containing lauric, caprylic and capric acids. Due to this fact, the triacylglycerols of PLK and COC are presented as groups with the same equivalent carbon number, instead of individual triacylglycerol species as presented for other oils (Table 4.1.2.4).

Table 4.1.2.4. Relative percentage (area %) of glyceride species with same ECN.

Oils[1]	ECN									
	30	32	34	36	38	40	42	44	46	48
COC	0.92	14.66	18.38	22.83	20.01	13.71	7.16	2.34	-	-
PLK	0.15	4.67	6.17	29.93	22.94	15.32	8.34	8.12	1.90	2.44

[1]Abbreviations see Table 3.1.1; ECN= CN-2*n*, where CN equals the actual carbon number and *n* equals the number of double bounds per molecule.

These oils had a similar composition of triacylglycerols with an ECN of 38 to 42, because of their similar content of lauric, myristic and palmitic acids. Coconut contained a higher contribution of glycerides with an ECN of 32 and 34 as a result of the elevated content of capric and caprylic acids. However, in PLK, where the content of oleic and linoleic acids

was higher than in COC, the content of triacylglycerols with an ECN of 44 to 48 was higher.

4.1.3. Lipid classes

Triacylglycerols (TAG) were the major lipid components present in oils (Table 4.1.3.1), making up to 97.51% (PLK). Cold pressed olive oil (OEV) had a lower content of triacylglycerols (94.79%) than refined, bleached and deodorized oils of the same genetic background (OPR and ORF). Oils that were dewaxed, RWO and CRW, showed higher levels of TAG than corresponding oils of the same genetic background (RIO, RBO and COR, CRN).

The amount of 1,3- and 1,2-diacylglycerols (DAG) was usually related, because the increased amount of one compound was accompanied by an increased amount of the other. The content of 1,2 DAG was higher than 1,3 DAG in CHO, SOY and SYS, while for all other oils the amount of 1,3 DAG was higher than the amount of 1,2 DAG.

Esterified sterols (SE) amounts were higher than 0.1% in rice bran oils (RBO, RIO and RWO), while for other oils the amount was below 0.04%.

The free fatty acid content was relatively high for BOR (1.59%) and PAL (0.36%) oils compared to other oils, where levels were below 0.05% for most oils.

Table 4.1.3.1. Neutral lipids (w/w %).

OILS[1]	TAG	1,3 DAG	1,2 DAG	SE	FFA
BOR	95.32	0.44	0.29	tr	1.59
CAN	97.23	0.40	0.32	0.03	0.03
CAO	97.14	0.42	0.27	0.04	0.03
CAR	96.86	0.41	0.36	0.02	0.03
CAS	97.02	0.45	0.37	0.03	0.04
CHO	96.93	0.34	0.36	0.02	0.03
COL	96.90	0.32	0.23	0.04	0.04
COR	95.75	0.55	0.34	0.04	0.03
CRN	96.23	0.54	0.37	0.04	0.03
CRW	96.78	0.53	0.31	0.03	0.05
COC	96.85	0.53	0.23	0.02	0.05
COT	96.77	0.47	0.35	tr	0.03
CTO	96.52	0.58	0.38	tr	0.05
EPR	97.29	0.26	0.23	0.03	0.11
FCO	96.55	1.12	0.42	0.02	0.10
FLL	96.86	1.09	0.47	0.01	0.06
OEV	94.79	1.05	0.51	0.04	0.15
OPR	96.69	1.15	0.68	0.03	0.05
ORF	96.92	1.02	0.38	0.02	0.04
PAL	94.04	2.30	1.11	0.02	0.36
PLG	94.89	1.72	0.89	0.02	0.14
PLK	97.51	0.25	0.22	tr	0.03
PEA	96.86	0.40	0.24	0.04	0.04
PNT	96.84	0.45	0.27	0.01	0.05
RBO	95.34	1.40	0.79	0.18	0.04
RIO	96.40	1.19	0.58	0.17	0.03
RWO	96.87	1.23	0.62	0.13	0.03
SHO	96.70	0.37	0.31	0.01	0.03
SUN	96.79	0.35	0.30	0.03	0.03
SUR	96.71	0.36	0.27	0.03	0.02
SOY	96.26	0.24	0.26	0.01	0.03
SYB	96.20	0.25	0.23	0.01	0.03
SYS	96.59	0.23	0.24	0.01	0.04

[1]Abbreviations see Table 3.1.1; TAG= triacylglycerol; 1,3DAG= 1,3diacylglycerol; 1,2DAG= 1,2diacylglycerol; SE= esterified sterols; FFA= free fatty acid; tr= traces.

The polar fraction was comprised mainly of phospholipids (PL) (Table 4.1.3.2) present in the oils at concentrations below 0.68%. Among them, phosphatidyl choline (PC) was the major phospholipid present in all oils. Among glycolipids

(GL), only monogalactosyl monoglyceride (MGMG) and digalactosyl diglyceride (DGDG) were identified in small amounts, whereas in olive, palm and rice bran oils the contribution of these components was just over 0.05%.

Table 4.1.3.2. Polar lipids (w/w %).

OILS[1]	PL							GL
	PI	PA	PE	LPE	PC	LPC	TOTAL	
BOR	-	0.01	0.02	0.01	0.18	-	0.23	0.04
CAN	0.03	0.02	0.08	tr	0.17	0.01	0.32	0.03
CAO	0.01	0.01	0.04	0.02	0.15	tr	0.23	0.03
CAR	0.01	0.02	0.08	0.02	0.25	0.02	0.40	0.03
CAS	tr	0.02	0.06	0.02	0.18	0.01	0.29	0.03
CHO	0.01	0.01	0.03	0.04	0.22	0.01	0.32	0.03
COL	tr	0.03	0.03	0.01	0.24	0.02	0.34	0.03
COR	0.02	tr	0.04	0.04	0.17	0.03	0.29	0.02
CRN	0.01	tr	0.08	0.07	0.33	0.03	0.52	0.02
CRW	0.01	tr	0.04	0.01	0.36	0.04	0.46	0.02
COC	-	0.01	tr	tr	0.17	0.01	0.20	0.02
COT	-	0.04	0.05	0.02	0.25	0.03	0.39	0.02
CTO	-	0.02	0.02	0.02	0.37	0.03	0.45	0.02
EPR	0.01	0.02	0.12	0.04	0.18	0.13	0.41	0.01
FCO	0.01	0.02	0.02	tr	0.09	0.18	0.32	0.02
FLL	0.01	0.01	0.03	0.04	0.21	0.16	0.47	0.02
OEV	0.01	0.01	0.01	0.01	0.27	0.02	0.33	0.08
OPR	0.01	tr	0.03	0.02	0.11	tr	0.17	0.07
ORF	0.01	tr	0.03	0.01	0.14	tr	0.19	0.05
PAL	tr	0.02	0.02	0.04	0.26	0.01	0.35	0.08
PLG	tr	0.02	0.01	0.04	0.44	0.17	0.68	0.07
PLK	tr	0.02	0.12	0.01	0.17	0.05	0.27	0.02
PEA	0.01	0.02	0.14	0.02	0.28	0.02	0.36	0.04
PNT	0.01	0.01	0.01	0.02	0.15	0.03	0.23	0.03
RBO	0.05	0.01	tr	0.03	0.24	0.02	0.35	0.09
RIO	0.09	tr	tr	0.05	0.34	0.01	0.49	0.06
RWO	0.08	0.01	tr	0.03	0.26	0.01	0.39	0.06
SHO	0.03	tr	tr	0.02	0.24	0.01	0.30	0.02
SUN	0.02	0.04	tr	0.01	0.21	0.03	0.32	0.03
SUR	0.01	0.03	tr	0.02	0.19	0.01	0.26	0.03
SOY	0.05	0.03	0.03	0.14	0.38	0.03	0.66	0.03
SYB	0.06	0.02	0.02	0.08	0.19	0.03	0.40	0.03
SYS	0.06	0.02	0.02	0.11	0.15	0.03	0.37	0.03

PL= phospholipids; PI= phosphatidyl inositol; PA= phosphatidic acid; PE= phosphatidyl ethanolamine; LPE= lisophosphatidyl ethanolamine; PC= phosphatidyl choline; LPC= lisophosphatidyl choline; GL= glycolipids; tr= traces, [1]abbreviations see Table 3.1.1.

4.1.4. Tocopherols and tocotrienols

Alpha and γ-tocopherols were the major tocopherols present in most oils (Fig. 4.1.4.1). β-tocopherol was present in small amounts, below 30ppm in only a few oils, while δ-tocopherol was detected at concentrations over 30ppm only in borage and soybean oils. Genetically modified oils, CHO, COL and CAR, showed a higher amount of total tocopherols than regular oils: CAO, CAS and CAN. The same was observed with SHO compared to SUR and SUN, but not with FLL compared with FCO.

Tocotrienols were present only in a few oils, where α-tocotrienol was observed in coconut (COC), palm (PAL, PLK and PLG) and flax (FCO and FLL) oils, and β- and δ-tocotrienol in palm (PLG and PAL) and rice bran oils (RIO, RWO and RBO) (Fig. 4.1.4.2). The amount of tocotrienols in PLG was more than 3 times higher than in any other oil analysed, and about 50% of the total amount of tocotrienols in PLG was β-isomer. Low linolenic flax oil (FLL) had a lower content of γ-tocopherol than conventional flax oil, but this oil had a higher amount of α-tocotrienol, and higher amounts of total tocopherols and tocotrienols than were found in conventional flax oil.

Coconut (COC) and palm kernel (PLK) oils had only small amounts of tocopherols and tocotrienols; the total amount of tocotrienols was lower than 35ppm.

Soybean (SOY, SYS and SYB), borage (BOR), palm goldem (PLG) and corn (CRW, CRN and COR) oils were distinguished from

others due to their high content of total tocopherols and tocotrienols. These oils had amounts of total tocopherols or tocotrienols two to three times higher than the rest of the oils.

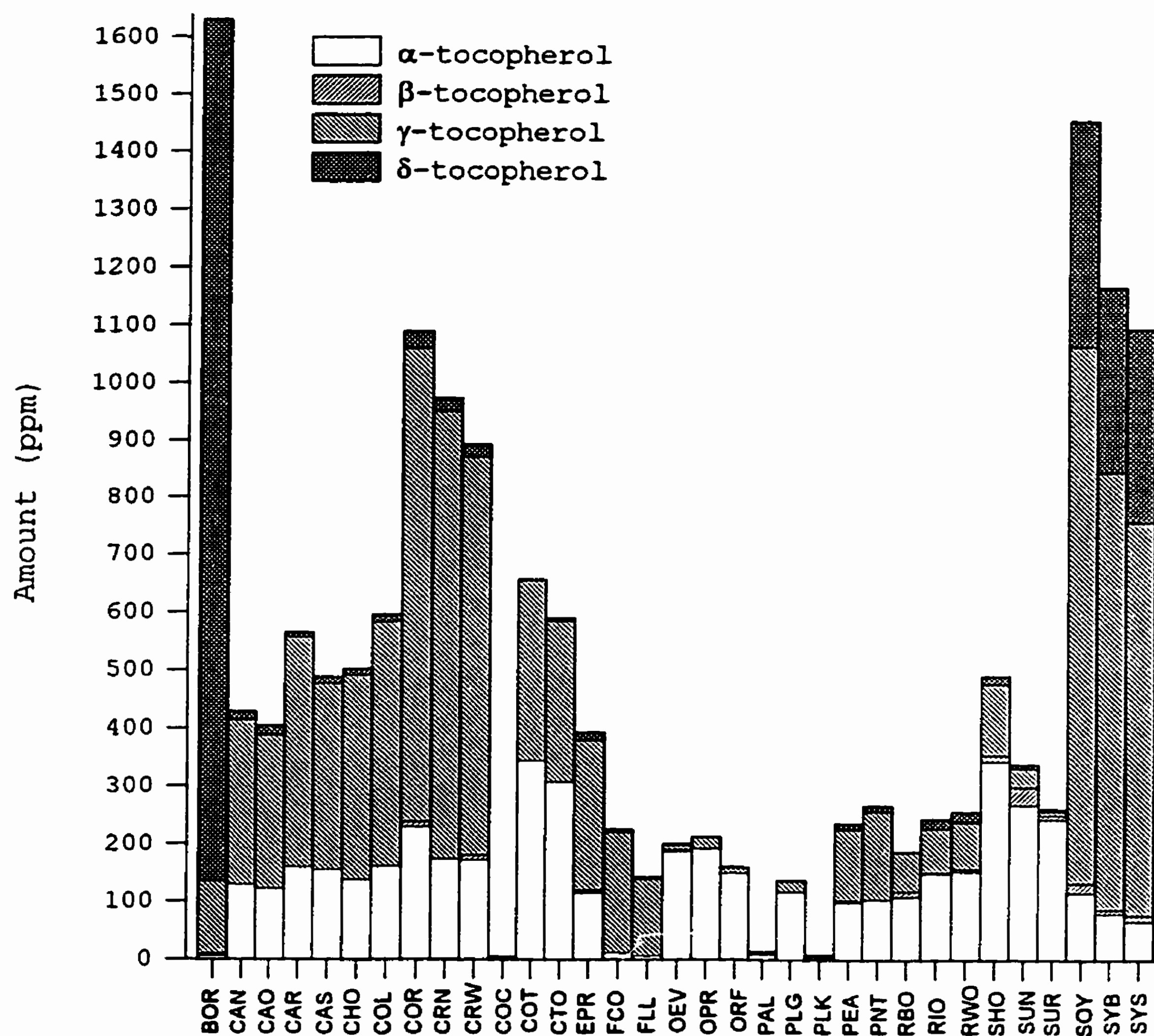

Fig. 4.1.4.1. Content of tocopherols in oils (for abbreviations see Table 3.1.1).

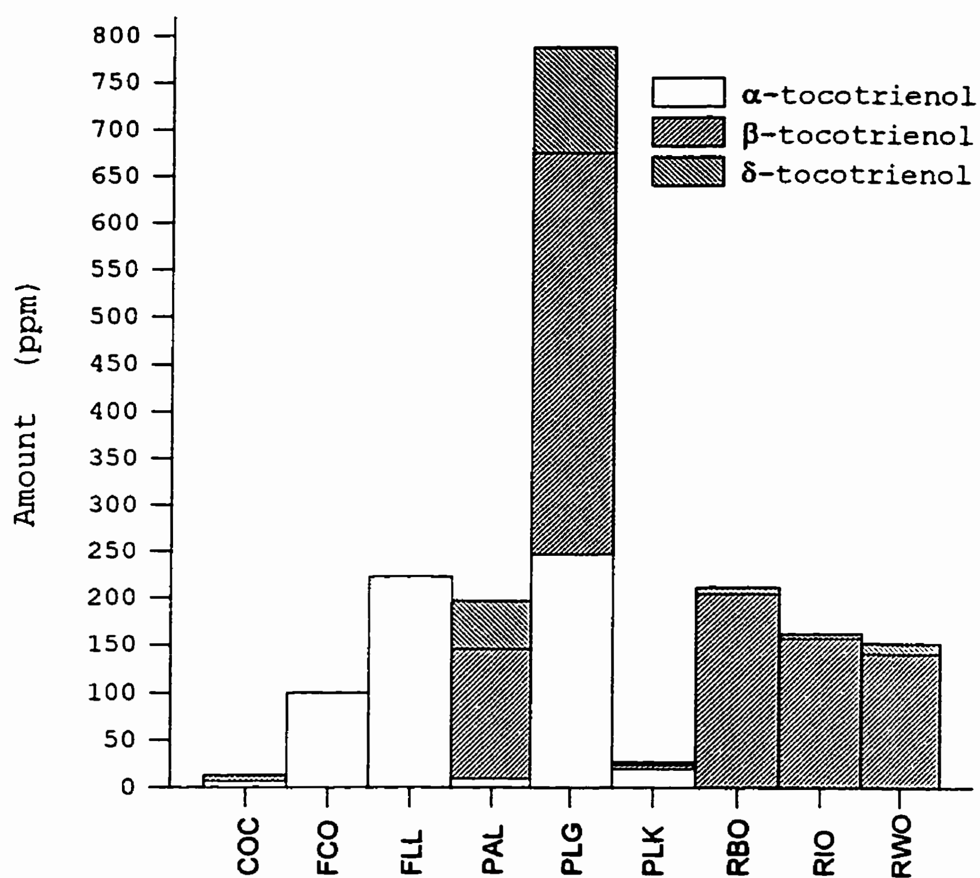

Fig. 4.1.4.2. Content of tocotrienols in oils (for abbreviations see Table 3.1.1).

4.1.5. Phenolic compounds

The total amount of phenolic compounds present in the oils is provided in Fig. 4.1.5. Cold pressed olive oil (OEV) had the highest amount of phenols, where gentisic (72%), *o*-coumaric (15%) and protocatechuic (9%) acids were the major compounds. Refined, bleached and deodorized olive oils (OPR and ORF) showed a reduction to 72 to 84% of the total phenolic content when compared with OEV. Despite this reduction in the total content of phenolic compounds, OPR and ORF still had amounts more than 10 times higher than other oils, with the exception of BOR which contained 4 to 5 times less phenolics these olive oils.

The amount of total phenolic compounds in other oils was below 0.5ppm, whereas BOR contained 1.25ppm. Small amount of gallic, gentisic, *p*-hydroxybenzoic, protocatechuic, vanillic, siringic, ferulic, *p*-coumaric, sinapic and *o*-coumaric acids were found in several other oils.

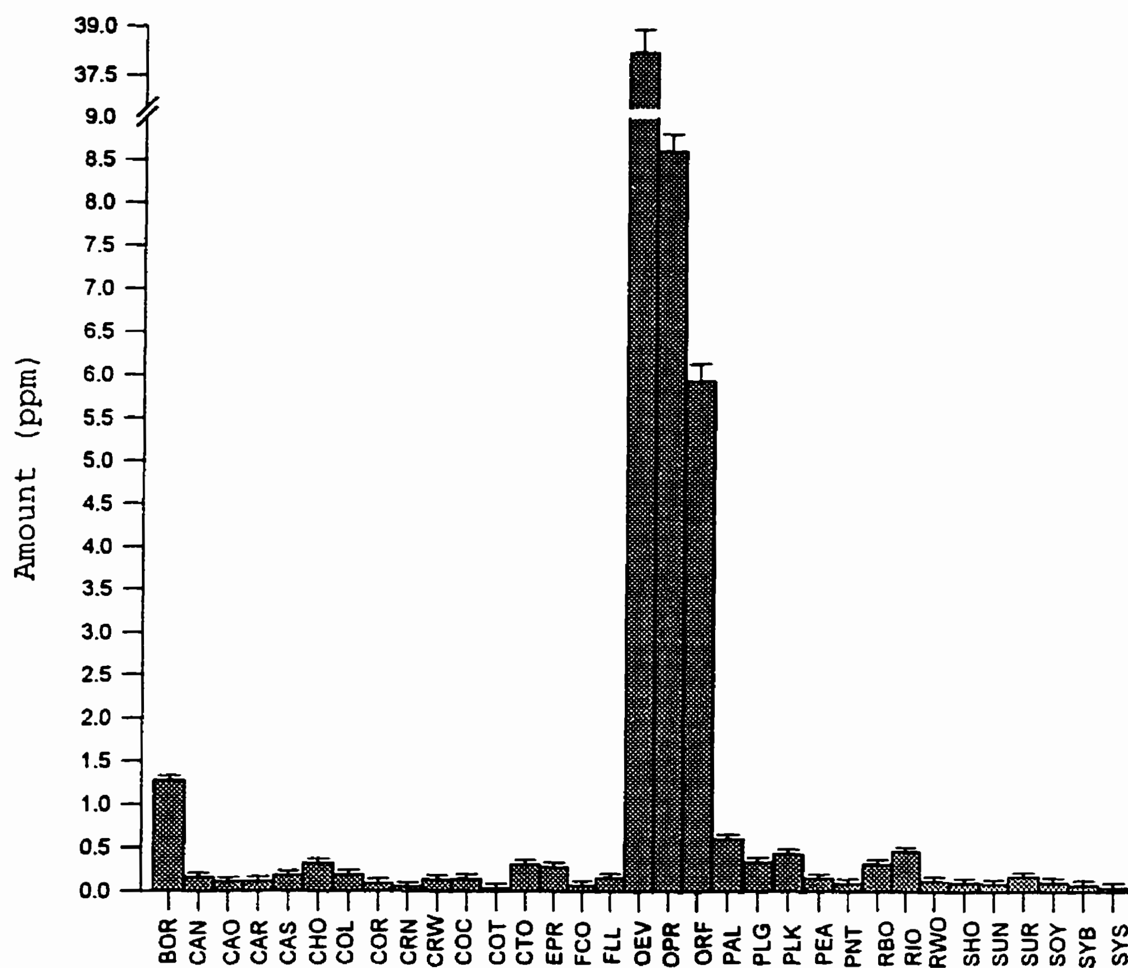

Fig. 4.1.5. Total amount of phenolic compounds (error bars represent standard error, and for abbreviations see Table 3.1.1).

4.1.6. Pigments

Chlorophylls and their derivatives were measured by the HPLC method were only detected in the cold pressed olive oil (OEV). Pheophytin *a* was the major derivative present (83.10%), followed by pheophytin *b* (9.49%), and small amounts of chlorophylls *a* (4.88%) and *b* (2.53%). The total contribution of *a* isomers comprised more than 85% of the total pigments present in the oil.

The oils were also analysed by the spectrophotometric method, which showed a higher sensibility and a lower detection limit than the HPLC method. Spectrophotometrically chlorophyll was detected in OEV, OPR, CAO, SOY, BOR and COT oils (Fig. 4.1.6.1). The total amount of chlorophylls and their derivatives detected by HPLC in cold pressed olive oil was 1.85 times higher than the content of chlorophyll detected by the spectrophotometric method in the same oil.

Carotenoids were detected in cold pressed olive (OEV) and palm golden (PLG) oils, either by the HPLC (Fig. 4.1.6.2) or the spectrophotometric methods. In cold pressed olive oil the major carotenoid detected was lutein, which comprised 64% of the total carotenoids, followed by β-carotene (23%) and α-carotene (13%).

Palm golden oil (PLG) had a very high concentration of α- and β-carotenes when compared with OEV oil, in which β-carotene comprised 63% and α-carotene 37% of the total carotenoids identified. Several other small peaks (14) were

also detected in the HPLC chromatogram of PLG. But the amount of these carotenoids was not at measurable levels.

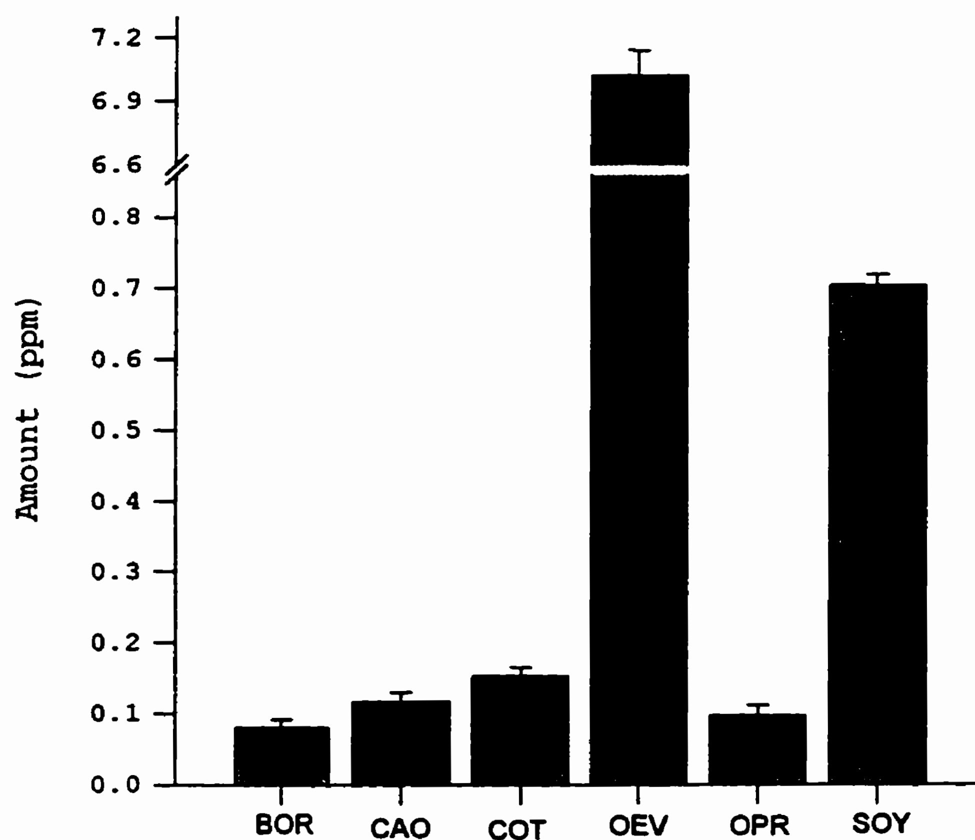

Fig. 4.1.6.1. Chlorophyll content determined by AOCS method (error bars represent standard error, and for abbreviations see Table 3.1.1).

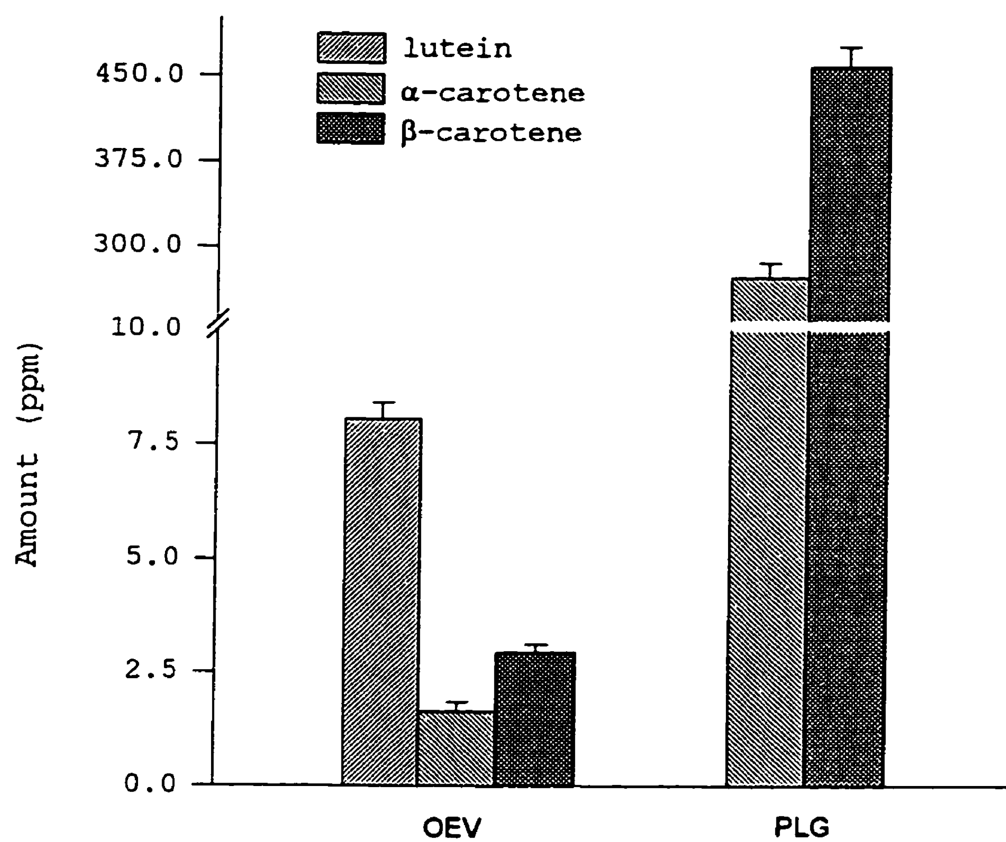

Fig. 4.1.6.2. Content of carotenoids determined by HPLC in olive (OEV) and palm golden (PLG) oils (error bars represent standard error).

4.1.7. Sterols

Evening primrose (EPR) and corn (CRN, COR and CRW) oils had the highest levels of total sterols, followed by rice bran (RBO, RWO and RIO), and canola oils (CAN, CAR, CAO, CHO, COL and CAS), while coconut (COC) and palm oils (PAL, PLG and PLK) showed the lowest content (Fig. 4.1.7).

Phytosterols were comprised mainly of β-sitosterol, campesterol and stigmasterol, while brassicasterol was present at the lowest levels in almost all oils. Evening primrose (EPR) and canola (CAN, CAS, CAO, CHO, COL and CAR) oils were distinguished from others due to the relatively high content of brassicasterol.

In borage (BOR) oil more than 70% of the total sterols was campesterol, while β-sitosterol was the major phytosterol present in almost all oils.

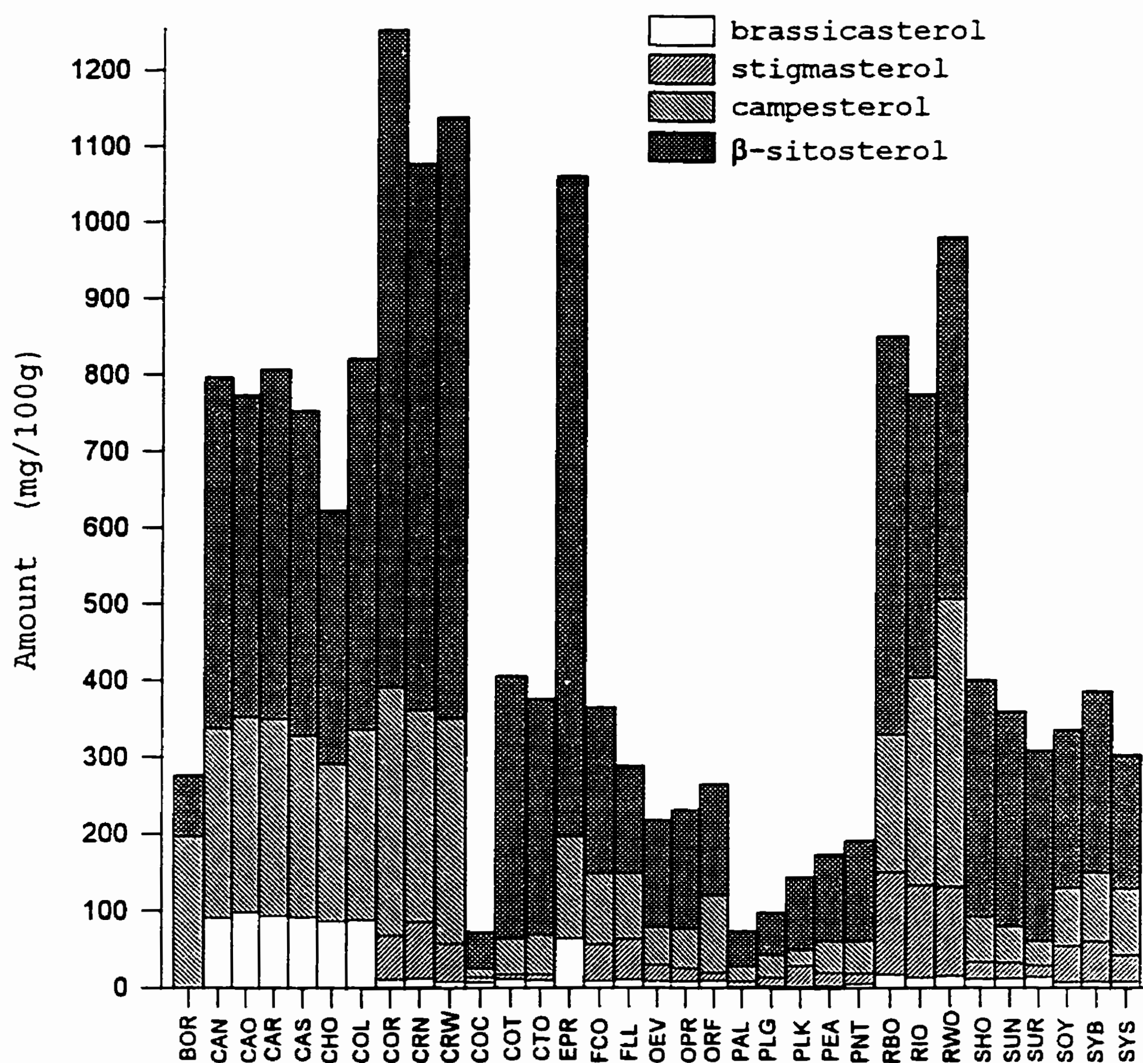

Fig. 4.1.7. Content of phytosterols in oils (for abbreviations see Table 3.1.1).

4.1.8. Metals

The metal content in the analysed oils was at levels bellow 0.10ppm in most oils (Fig. 4.1.8). Nickel was present only in palm oil (PAL), while copper was present in evening primrose (EPR), olive pomace (OPR) and borage (BOR) oils. Regular canola (CAO), PAL, COT and CTO also contained copper, but at levels close to the detection limit (0.020ppm). Iron was the most common metal present in the oils, with palm oil (PAL) showing the highest concentration (1.24ppm). The concentration present in the other oils was below 0.15ppm.

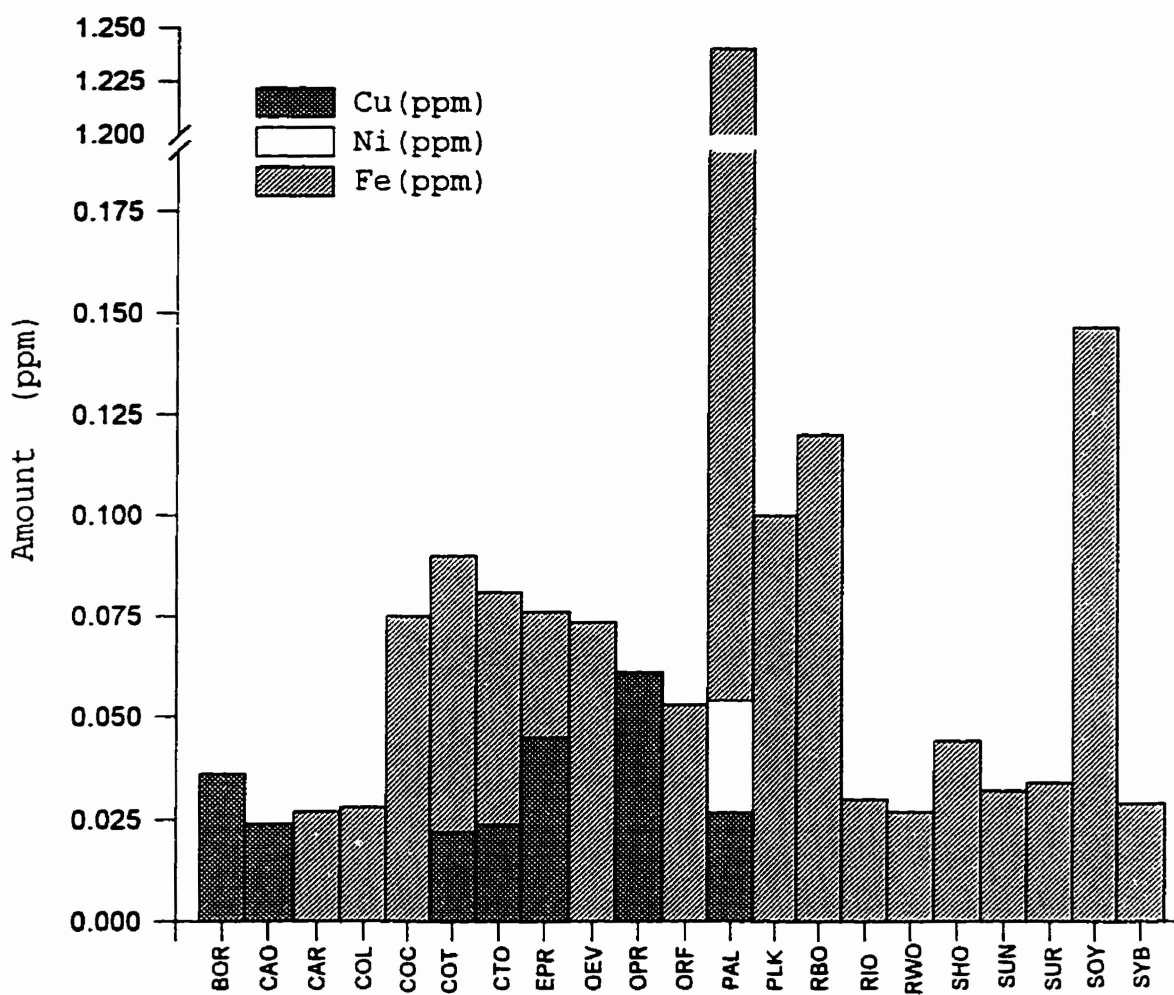

Fig. 4.1.8. Content of metals in oils (for abbreviations see Table 3.1.1).

4.1.9. Peroxide value (PV)

Peroxide values in fresh oils is presented in Fig. 4.1.9. Only palm oils showed PV values close to 10, where PAL and PLG had PV 12 and 9, respectively. Cold pressed olive (OEV) oil had PV 2.7, but according to the International Olive Oil Council (1995), virgin olive oil could have PV values up to 10 to still qualify as prime quality (International Olive Oil Council, 1995). The elevated PV value in PAL and PLG is an indication that these oils were partially oxidized, probably due to manipulation and transportation, but PV values up to 3.5 are still considered normal for these oils (Ringkasan, 1982; Tan, 1989).

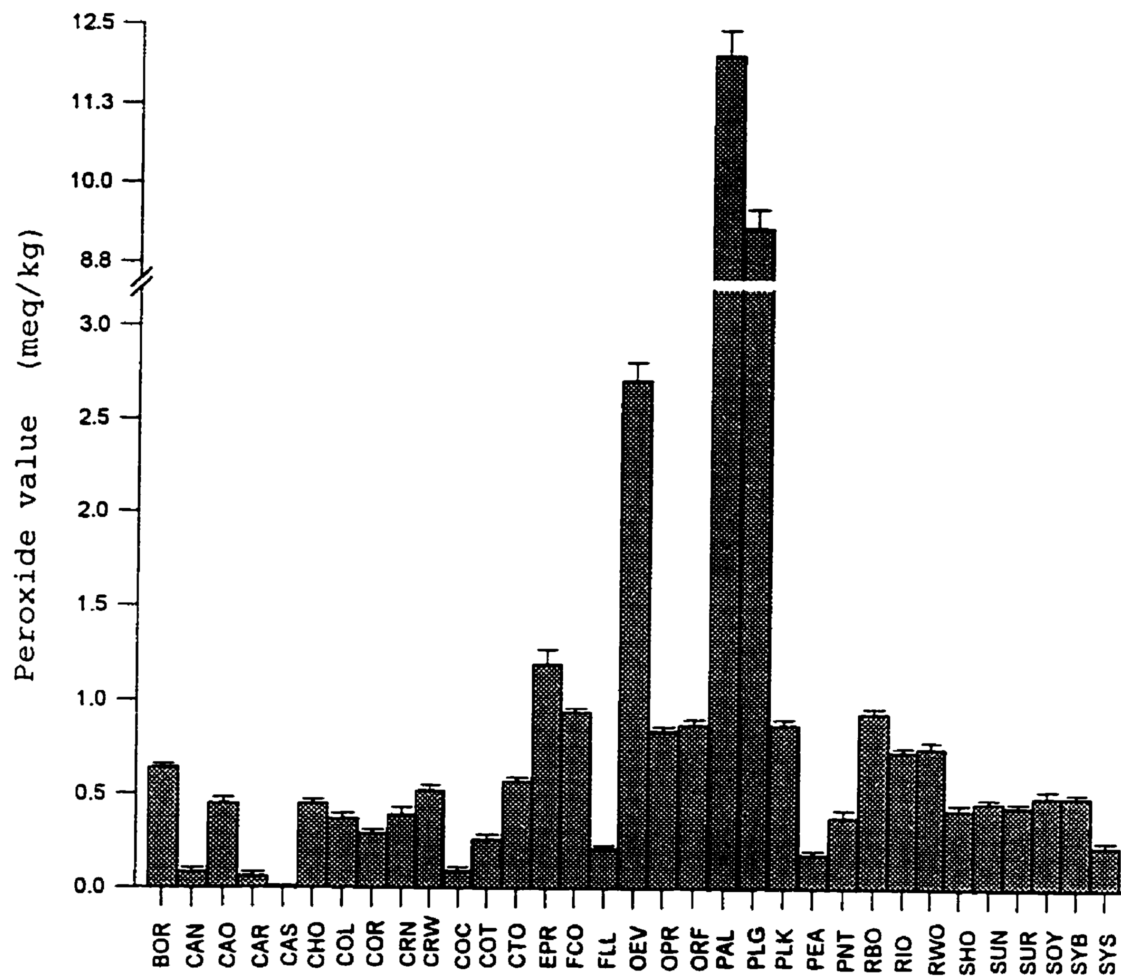

Fig. 4.1.9. Peroxide value in fresh oils (error bars represent standard error, and for abbreviations see Table 3.1.1).

4.2. Oxygen consumption

The oxygen consumed during oil storage is associated with the oxidative stability of the oil. A faster rate of oxygen decrease in the headspace during storage implies a faster rate of oxidation of the oil (Mistry & Min, 1987; Jung *et al.*, 1989).

4.2.1. Oils with high linolenic acid content

4.2.1.1. Canola oils

The oxygen consumption for canola oils stored in the presence and absence of light is presented in Fig. 4.2.1.1.1 and 2.

All canola oils stored without light showed an initial resistance to oxidation, while the same oils stored with light exposure started to take up oxygen at faster rates from the beginning of storage.

Regular canola oil (CAO), obtained from grade III seeds, showed the fastest rate of oxygen uptake in both storage conditions, which was significantly different from other regular canola oils (Table 4.2.1.1). Among canola oils, CAO showed a similar fatty acid composition as CAS and CAN, and a higher content in linolenic acid than genetically modified canola oils (Table 4.1.1). Even though CAO presented a similar fatty acid composition to other regular canola oils, CAO showed slight differences in fatty acid distribution in the triacylglycerol molecules. CAO showed a lower content in

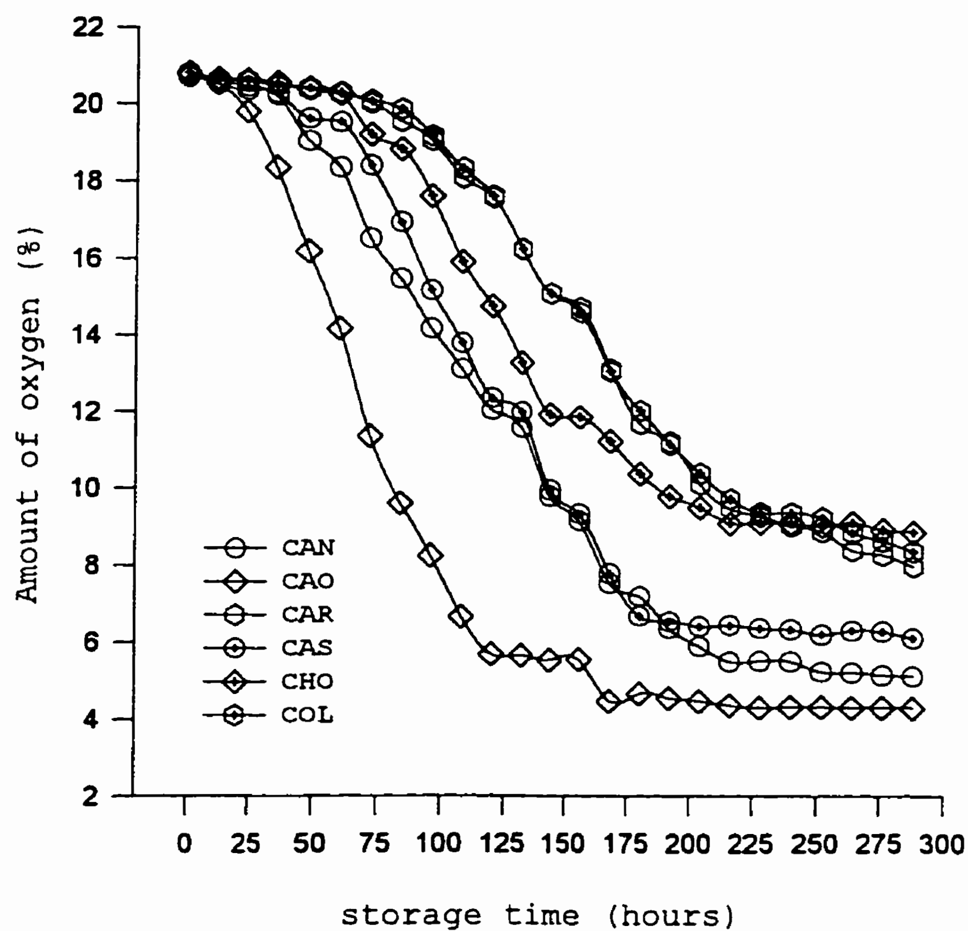

Fig. 4.2.1.1.1. Oxygen uptake during storage withou light at 65°C (for abbreviations see Table 3.1.1).

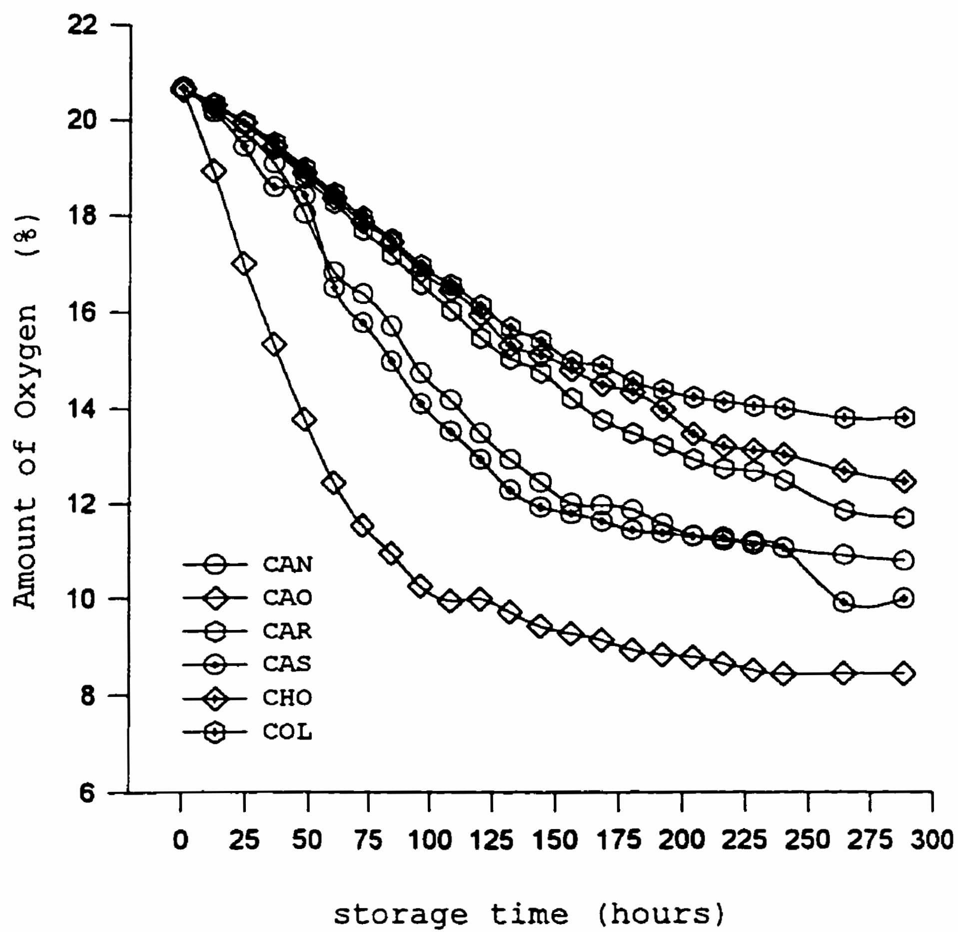

Fig. 4.2.1.1.2. Oxygen uptake during storage with light at 35°C (for abbreviations see Table 3.1.1).

Table 4.2.1.1. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines.

Oils[1]	Absence of light[2]			Light exposure[3]
	k_1	k_2	ip(hr)	k_2
CAS	-0.012[a]	-0.107[ad]	46.9[a]	-0.068[a]
CAN	-0.015[a]	-0.095[ac]	39.6[a]	-0.063[a]
CAO	-0.041[b]	-0.165[b]	25.0[b]	-0.138[b]
CAR	-0.009[c]	-0.085[c]	86.8[c]	-0.045[c]
COL	-0.008[c]	-0.084[c]	87.1[c]	-0.040[c]
CHO	-0.007[c]	-0.116[d]	72.7[d]	-0.042[c]

[a-d]Values with different letters in each column are significantly different by Duncan's multiple range test at $P<0.05$; [1]abbreviations see Table 3.1.1; [2]65°C; [3]35°C.

OOO and LOO, and a higher content in LnLO and LnOO molecular species than did CAN and CAS (Table 4.1.2.2). However, CAO was the only canola oil that contained chlorophyll (0.12ppm), traces of copper (0.024ppm) and also the lowest content of tocopherols (404.4ppm). Although there was no significant difference in the oxygen uptake between CAS and CAN for both storage conditions, they showed slight differences in induction period (ip) during storage without light (Table 4.2.1.1). CAS showed more resistance to oxidation during the initial 46 hours (ip= 46.9), but once the oxidation started, CAS showed a slightly faster oxygen uptake (k_2= -0.107) than did CAN (k_2= -0.095). These two oils showed a similar composition, with CAN presenting an amount of tocopherols 18.0ppm less than did CAS. Both oils showed no contamination

by metals, nor the presence of pigments. Genetically modified oils (CAR, COL and CHO) showed significantly larger induction periods than regular canola oils (CAO, CAS and CAN) during storage without light, and significantly slower rates of oxidation during storage with light exposure (Table 4.2.1.1). As the linolenic acid content decreased in these oils, their oxidative stability improved. Oxidation rates were not significantly different between these oils (CHO, CAR and COL) when stored in the presence of light. But, when stored without light, CHO showed a significantly shorter induction period (72.7) and a significantly faster oxygen uptake during the propagation period (k_2= -0.116) than did COL (p_{ip}=0.00002; p_{k2}=0.0069) and CAR (p_{ip}=0.00002; p_{k2}=0.0075). CHO contained a difference of 2.0 to 2.5% more linolenic acid content than did COL and CAR, but both CAR and COL contained a small amount of iron (Fig. 4.1.8). Also, CAR and COL had a higher content of tocopherols (Fig. 4.1.4.1) and sterols (Fig. 4.1.7) than did CHO.

4.2.1.2. Soybean, flax and non-conventional oils

Oxygen uptake data for soybean oils (SOY, SYS and SYB) stored without and with light is presented in Fig. 4.2.1.2.1 and 2. These oils showed no significant differences in oxygen uptake during storage period without light (Table 4.2.1.2), but in storage with light exposure, SOY showed a significantly faster oxidation rate than SYB (p_{k2}=0.00001) and SYS

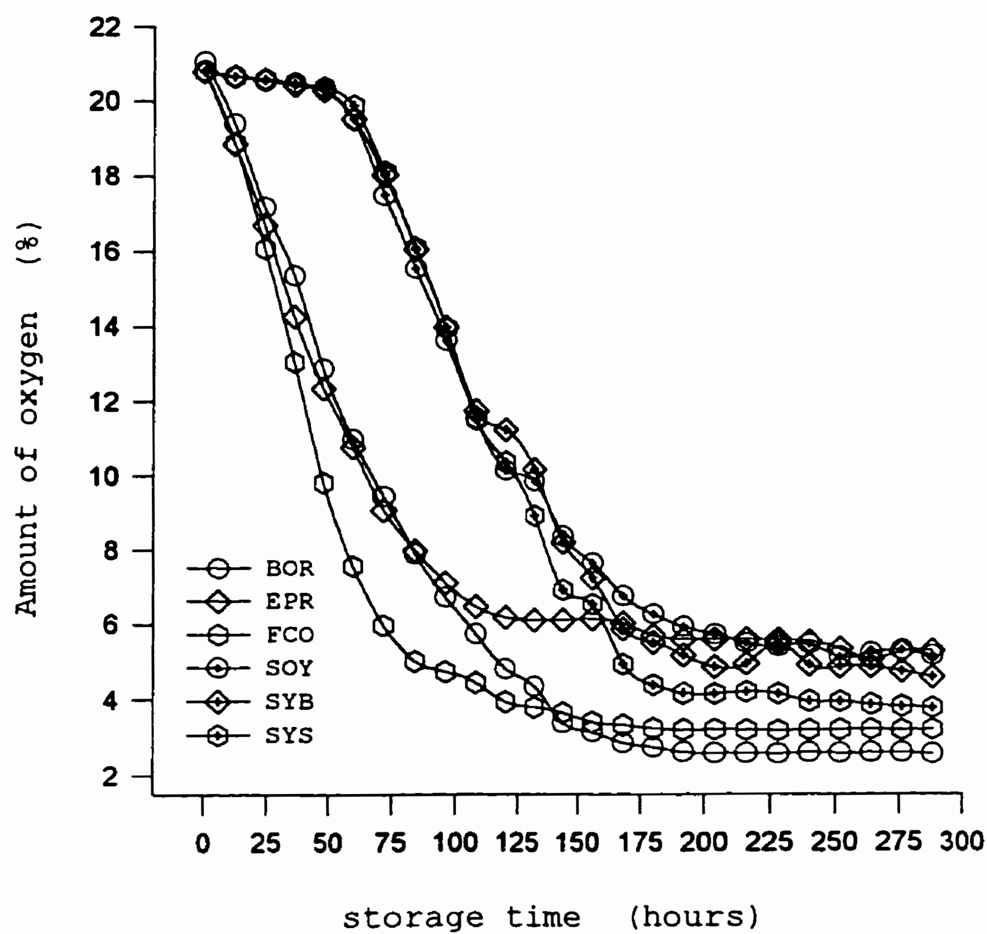

Fig. 4.2.1.2.1. Oxygen uptake during storage without light at 65°C (for abbreviations see Table 3.1.1).

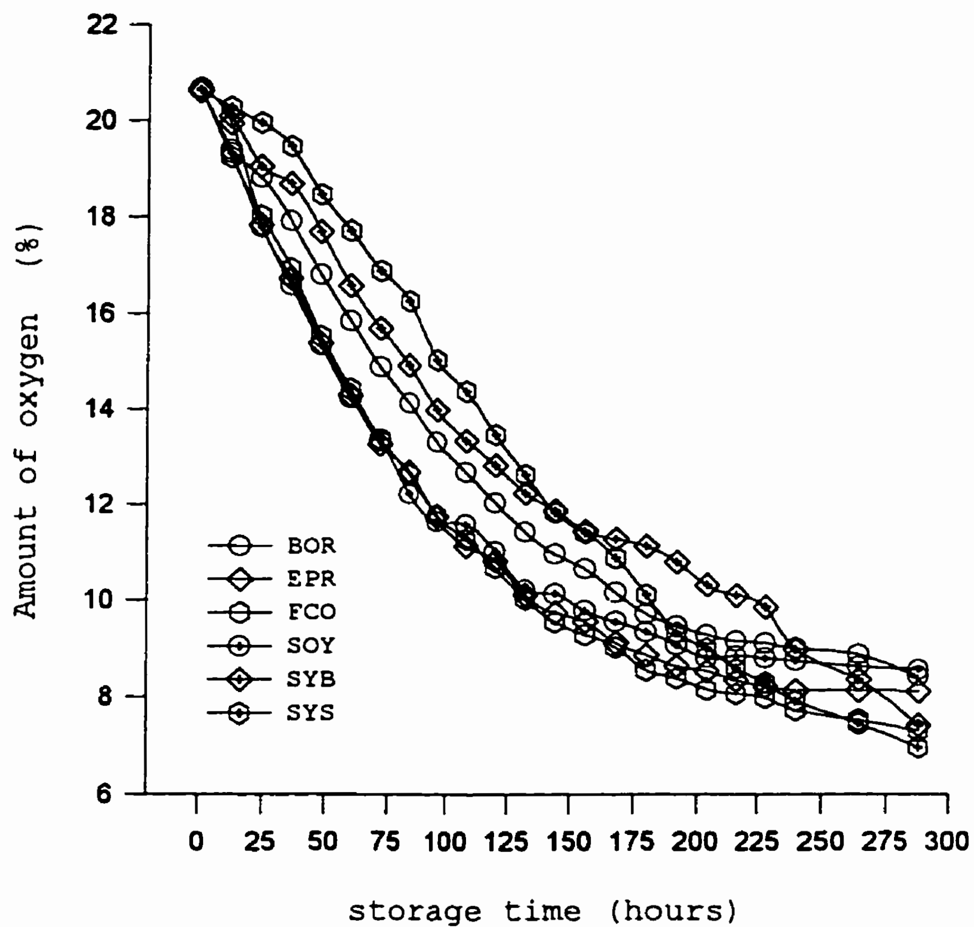

Fig. 4.2.1.2.2. Oxygen uptake during storage with light at 35°C (for abbreviations see Table 3.1.1).

Table 4.2.1.2. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines.

Oils[1]	Absence of light[2]			Light exposure[3]
	k_1	k_2	ip(hr)	k_2
SOY	-0.011[a]	-0.130[a]	49.2[a]	-0.099[a]
SYS	-0.009[a]	-0.136[a]	51.5[a]	-0.070[b]
SYB	-0.011[a]	-0.129[a]	50.0[a]	-0.072[b]
EPR	-	-0.159[b]	-	-0.103[a]
BOR	-	-0.162[b]	-	-0.077[b]
FCO	-	-0.218[c]	-	-0.101[a]

[a-c]Values with different letters in each column are significantly different by Duncan's multiple range test at $P<0.05$; [1]abbreviations see Table 3.1.1; [2]65°C; [3]35°C.

(p_{k2}=0.00001). During storage with light, SYB and SYS showed no significant differences in oxidation rate (p_{k2}=0.0538), but slight differences in oxygen uptake between these two oils were observed. SYS showed a slightly higher resistance to oxidation than did SYB, mainly during the first 120 hours of storage. SOY was the only soybean oil which exhibited chlorophyll (0.70ppm). Similarly, as observed in CAO which also contained chlorophyll, SOY showed lower stability than other soybean oils when stored with light exposure. SOY had the highest content of iron (0.150ppm) among the soybean oils analysed, and also the highest content of tocopherols (1458.3ppm) and phospholipids (0.66%). SYB contained a lower

amount of iron (0.029ppm) and a higher amount of sterols (Fig. 4.1.7) and tocopherols (Fig. 4.1.4.1) than SYS. During storage without light, conventional flax (FCO) showed the fastest oxygen uptake (k_2= -0.218), which was significantly higher than borage (BOR) (p_{k2}=0.00006) and evening primrose (EPR) (p_{k2}=0.00005) oils. The oxidation rate of BOR was not significantly different from EPR (p_{k2}=0.7815), despite the finding that BOR contained two times more linolenic acid (Table 4.1.1). During storage with light exposure, FCO and EPR were not significant different in oxygen uptake (p_{k2}=0.8838), although BOR was significantly more stable than EPR (p_{k2}=0.00002) and FCO (p_{k2}=0.00002) oils (Table 4.1.1). Among these three oils, FCO contained the highest amount of linolenic acid (54.2%). EPR, with only 9.8% linolenic acid, showed the same oxidation rate as FCO. However, if the total amount the linolenic and linoleic acids are taken into account, FCO contained 69.8% while EPR had 83.9%. Taking into account the fact that linolenic acid oxidized easier and faster than linoleic acid (Frankel, 1980), these results indicate that the fatty acid composition does not explain the finding that same oxidation rate was found between these two oils. BOR was the only oil that contained chlorophyll (0.08ppm), but even the presence of chlorophyll and a higher linolenic acid content than EPR is not sufficient to explain why both oils oxidized at the same rate during storage without light and why BOR had a lower oxidation rate than EPR during

storage with light. BOR oil had the highest content of tocopherols (1631.2ppm) among all the oils analysed, and phenolic compounds (1.5ppm) were also detected. EPR contained metals at levels twice as high as those in BOR, while in FCO metals were not detected (Fig. 4.1.8).

4.2.2. Oils with high linoleic acid content

Regular sunflower oils, SUN and SUR, showed no significant difference in oxygen uptake during storage without and with light exposure (Fig. 4.2.2.1 and 2; Table 4.2.2). The similarity in oxidation was a result of these two oils having similar compositions. During storage without light, these oils showed an initial resistance to oxidation until about 28 hours of storage.

The reduction to less than 2% linolenic acid in low linolenic flax (FLL) oil improved resistance to oxidation in both storage conditions when compared to regular flax oil (FCO). FLL had a fatty acid composition similar to regular sunflower oils (Table 4.1.1); however, FLL had significantly better stability than SUN and SUR in both storage conditions (Table 4.2.2). These oils had similar fatty acid composition, but FLL had a higher proportion of LLL and a lower proportion of LLO molecular species than did SUN and SUR oils (Table 4.1.2.1). During storage without light, FLL showed a two times longer initiation time (ip= 55.7 hours) than did regular sunflower oils (ip≈ 28.0 hours). During storage with light

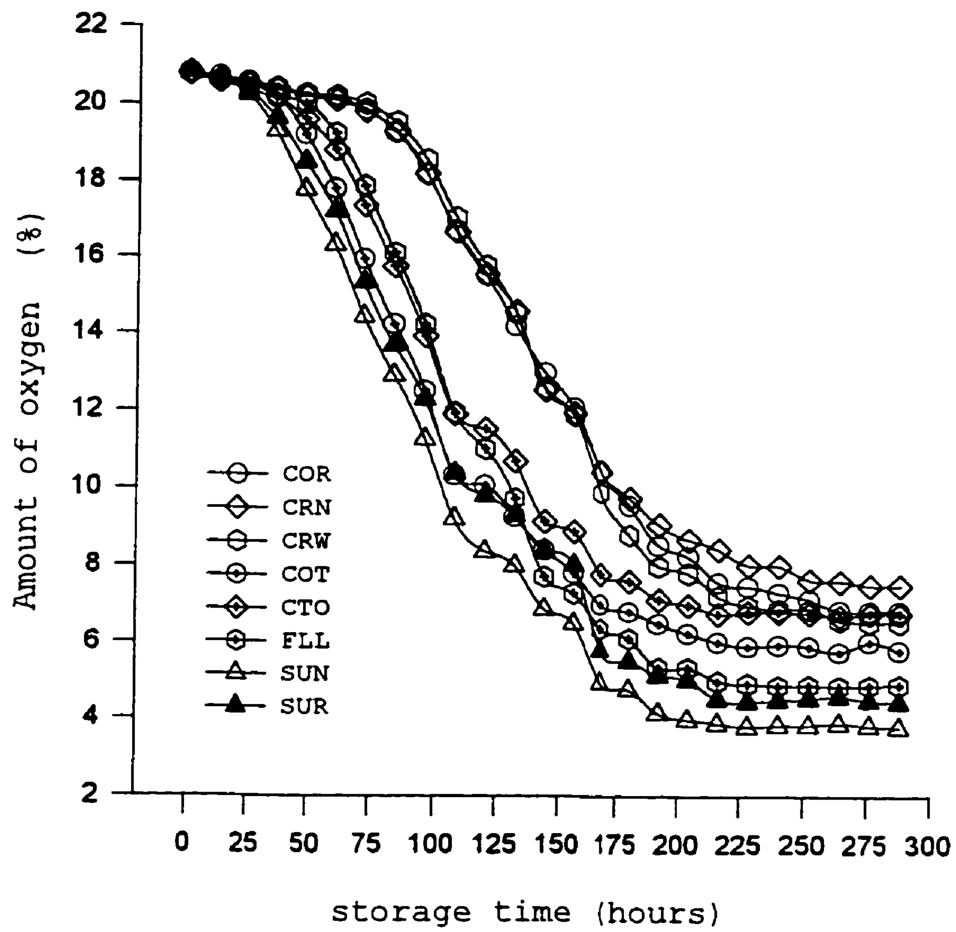

Fig. 4.2.2.1. Oxygen uptake during storage without light at 65°C (for abbreviations see Table 3.1.1).

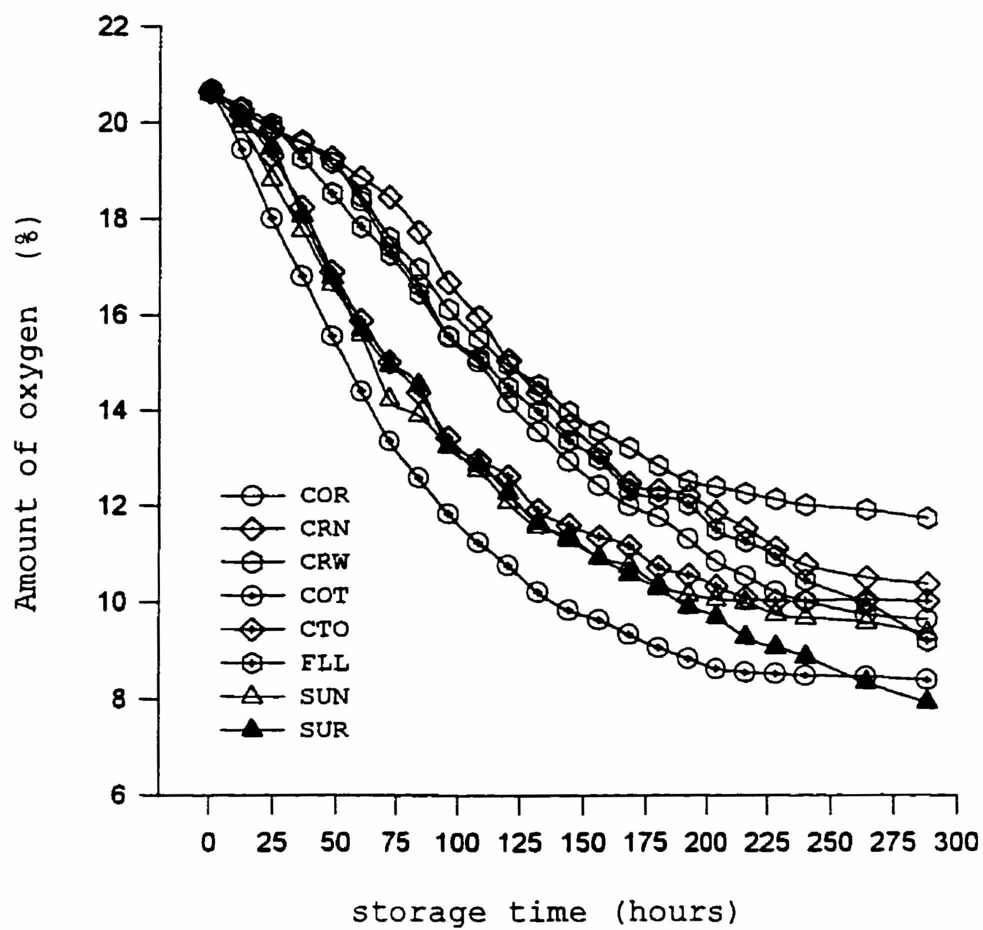

Fig. 4.2.2.2. Oxygen uptake during storage with light at 35°C (for abbreviations see Table 3.1.1).

Table 4.2.2. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines.

Oils[1]	Absence of light[2]			Light exposure[3]		
	k_1	k_2	ip (hr)	k_1	k_2	ip (hr)
SUN	-0.024[a]	-0.116[a]	27.6[a]	-	-0.083[ac]	-
SUR	-0.020[a]	-0.111[ae]	28.4[a]	-	-0.079[ae]	-
FLL	-0.017[ab]	-0.138[b]	55.7[be]	-	-0.052[b]	-
COT	-0.015[bd]	-0.126[bc]	37.5[ac]	-	-0.096[c]	-
CTO	-0.016[b]	-0.115[ac]	42.1[cb]	-	-0.082[a]	-
COR	-0.010[c]	-0.104[d]	77.9[de]	-0.030[a]	-0.061[de]	43.5[a]
CRN	-0.011[cd]	-0.106[de]	78.9[d]	-0.029[a]	-0.063[d]	57.3[b]
CRW	-0.010[c]	-0.109[de]	79.9[d]	-0.030[a]	-0.059[db]	44.8[a]

[a-e]Values with different letters in each column are significantly different by Duncan's multiple range test at $P<0.05$; [1]abbreviations see Table 3.1.1; [2]65°C; [3]35°C.

exposure, FLL oxidized at even slower rates than did regular sunflower oils (SUN and SUR) when compared to storage without light. The amount of oxygen depleted during the first 108 hours of storage without light was 27% for FLL and about 32% for both sunflower oils. In the same period during storage with light the amount of oxygen depleted was 42% for FLL and about 54% for both sunflower oils. Both regular sunflower oils contained iron (about 0.33ppm), which was not detected in low linolenic flax oil. But FLL had a higher amount of tocopherols and tocotrienols (Fig. 4.1.4.1 and 2) and phospholipids (Table 4.1.3.2) than did regular sunflower oils. The one sample of cottonseed (COT) oil showed no significant difference in stability compared with the other sample of

cottonseed (CTO) during storage without light (p_{k1}=0.0580; p_{k2}=0.3307; p_{ip}=0.512), but COT was significantly less stable than was CTO as determined by significant difference in k_2 (p_{k2}=0.00006) when stored with the presence of light. Small differences, not statistically significant, were observed during storage without light, where CTO had a slightly longer induction period (ip= 42.1) than did COT (ip= 37.5). Both oils contained iron and copper in different amounts (Fig. 4.1.8), and COT also had chlorophyll (0.15ppm). Again, even when COT contained chlorophyll its stability was lower than was CTO's during storage with light exposure. There was no significant differences among the corn oils (COR, CRN and CRW) in oxygen uptake during storage without light (Table 4.2.2). In this condition, these oils had the longest initiation period among oils from this group, about 79.6 hours. During storage with light exposure CRN showed a significantly longer induction period (57.3 hr) than did COR (43.5 hr) (p_{k2}=0.0509) and CRW (p_{k2}=0.0505). In the latter storage condition, the slopes of the initiation period (k_1) for these oils were about three times higher than their respective slopes during storage without light. These oils had similar fatty acid composition (Table 4.1.1) and triacylglycerol species (Table 4.1.2.3); however, CRN had a higher content of phospholipid than did CRW and COR (Table 4.1.3.2), and a higher content of tocopherols than did CRW (Fig. 4.1.4.1), and also had the lowest content in sterols (Fig. 4.1.7).

4.2.3. Oils with high oleic acid content

Olive oils (OEV, OPR and ORF) showed different characteristics in their rate of oxygen uptake during storage with and without light (Fig. 4.2.3.1 and 2). During storage without light ORF had significantly lower stability than did OPR (p_{k1}=0.00002; p_{k2}=0.0152) and OEV (p_{k1}=0.00001; p_{k2}=0.0612) when the slopes of initiation (k_1) and propagation (k_2) were compared (Table 4.2.3). The induction period for ORF was the longest. At the end of the induction period for ORF (236.4 hr), the amount of oxygen depleted was 32.1% compared to 21.8% for OPR and 16.4% for OEV. Even though OEV and OPR had no significant difference in relation to the initiation period (k_1)(p_{k1}=0.0502) and propagation period (k_2) (p_{k2}=0.6947) during storage without light, they showed slightly different behaviour. During the first 60 hours these oils had a similar amount of oxygen depletion, about 4.0%. However, from 60 to 195 hours of storage, OPR had higher stability than did OEV, while OEV showed higher stability than did OPR after this time. During storage with light, OEV showed significantly lower stability as measured by k_2 than did OPR (p_{k2}=0.00002) and ORF (p_{k2}=0.00000), and OPR had significantly lower stability than did ORF (p_{k2}=0.00001). Both OEV and OPR contained chlorophyll, but the content of chlorophyll in OEV was about 70 times higher than in OPR (Fig. 4.1.6.1). An elevated content of chlorophyll in olive oils was accompanied by a higher oxidation rate during storage with light; OEV

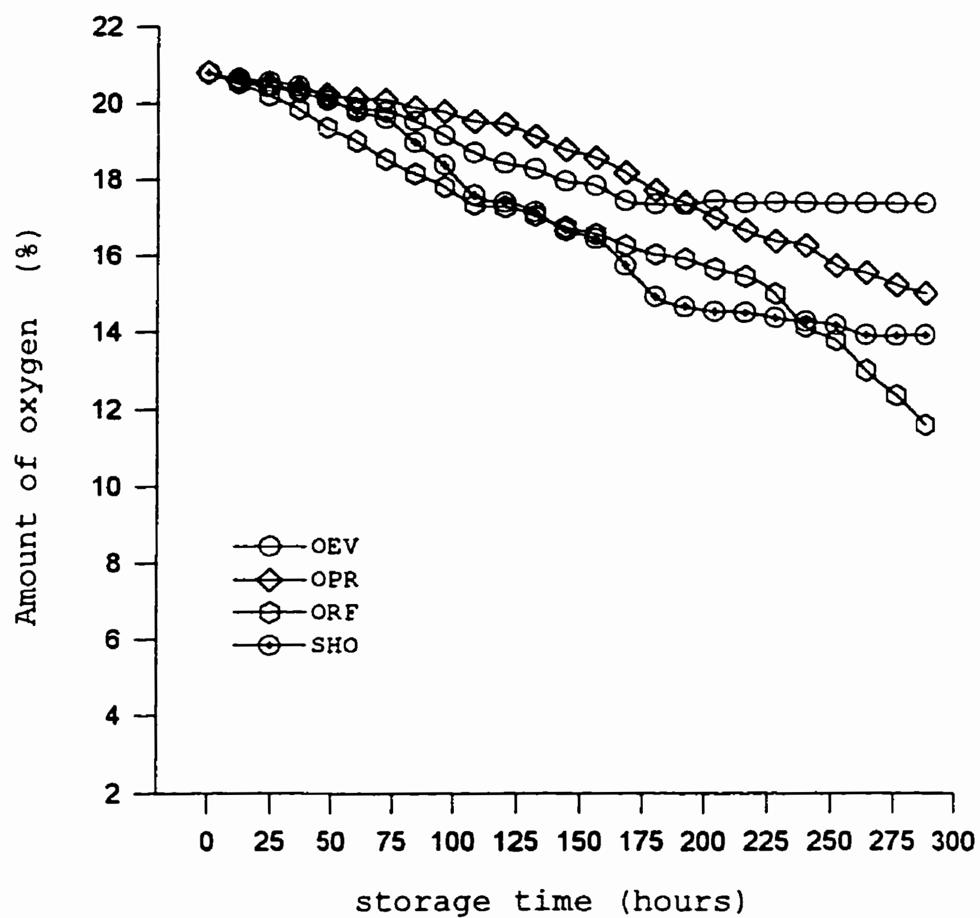

Fig. 4.2.3.1. Oxygen uptake during storage without light at 65°C (for abbreviations see Table 3.1.1).

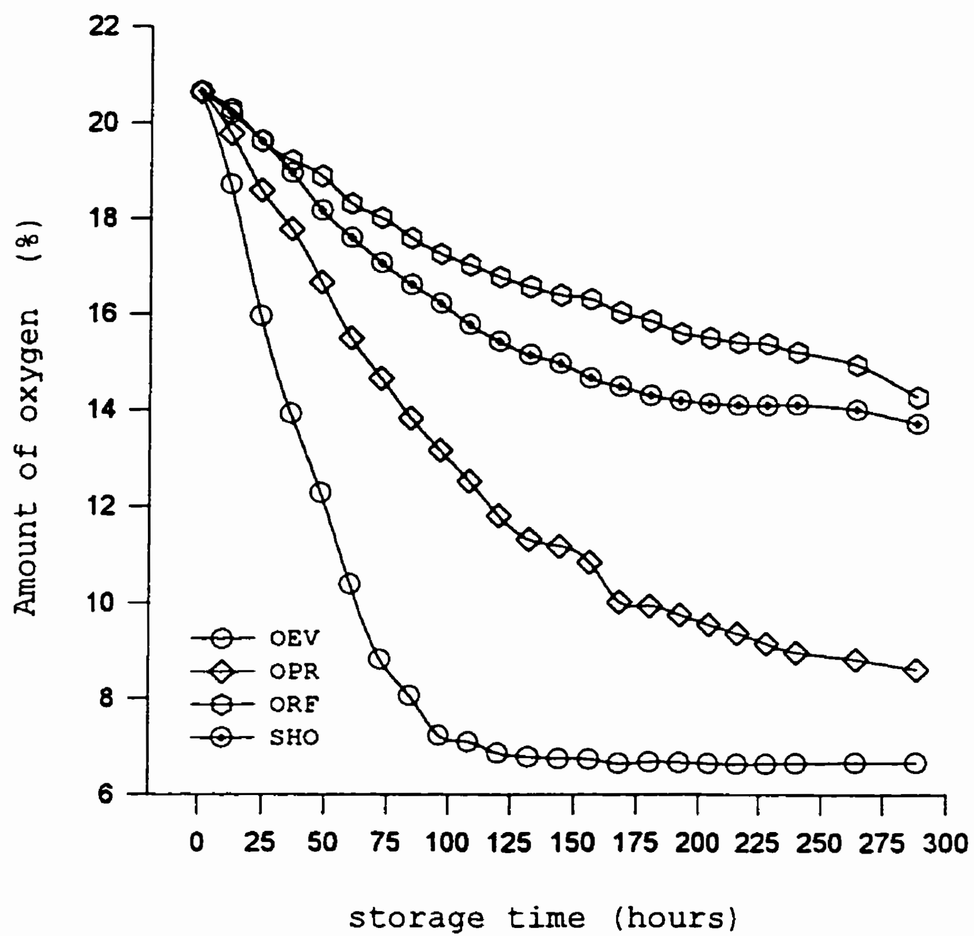

Fig. 4.2.3.2. Oxygen uptake during storage with light at 35°C (for abbreviations see Table 3.1.1).

Table 4.2.3. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines.

Oils[1]	Absence of light[2]			Light exposure[3]
	k_1	k_2	ip(hr)	k_2
OEV	-0.014[a]	-0.023[a]	79.1[a]	-0.173[a]
ORF	-0.026[b]	-0.054[b]	236.4[b]	-0.036[b]
OPR	-0.010[a]	-0.028[a]	114.2[c]	-0.080[c]
SHO	-0.010[a]	-0.039[c]	46.2[d]	-0.047[b]

[a-d]Values with different letters in each column are significantly different by Duncan's multiple range test at $P<0.05$; [1]abbreviations see Table 3.1.1; [2]65°C; [3]35°C.

oxidized faster than other olive oils, and OPR oxidized faster than did ORF. OEV contained 4 to 6 times more phenolic compounds than did the other two olive oils (Fig. 4.1.5); additionally, in this oil the presence of carotenoids was detected (Fig. 4.1.6.2). All olive oils contained metals, but OEV and ORF had iron while OPR had copper (Fig. 4.1.8).

High oleic sunflower oil (SHO) showed higher resistance to oxidation than regular sunflower varieties (SUN and SUR) during both storage conditions. Similarly to other genetically modified oils, when the amount of polyunsaturated fatty acids (PUFA) decreased, the stability of the oils increased. SHO also had a fatty acid composition similar to olive oils, but the difference in saturated fatty acid contribution was 4 to 6% lower than in olive oils. During the first 46 hours of storage without light, SHO showed the same

oxidative stability as OPR and OEV, when they consumed about 3.0% of the oxygen. In the same period SHO showed higher stability than ORF, which consumed 7.0% of the oxygen during the first 36 hours of storage. After this initial period, SHO consumed oxygen at faster rate than did OEV and OPR, but still slower than ORF until 120 hours of storage. SHO had higher amounts of tocopherols (491.1ppm) and sterols (400.4mg/100g), while it had a lower content of metals (0.044ppm) and phenolics (Fig. 4.1.5) than did olive oils. Also, a higher content of OOO and a lower content of POO triacylglycerols were observed in SHO than in olive oils (OEV, OPR and ORF)(Table 4.1.2.2).

4.2.4. Oils with similar oleic and linoleic acids content

Both peanut oils, PEA and PNT, showed no significant differences in oxygen uptake during storage under both conditions (Fig. 4.2.4.1 and 2; Table 4.2.4). Even though PEA and PNT showed no significant difference in the presence of light (p_{k2}=0.1910), PNT was oxidized slightly faster than was PEA during the initial 160 hours of storage. This could be observed, for example at 80 hours of storage, when the amount of oxygen depleted was 25.5% and 20.9% for PNT and PEA, respectively. The composition of these oils was similar, except that PEA contained a higher amount of phospholipids than did PNT (Table 4.1.3.2), and a lower contribution of triacylglycerols with one and two oleic acids in the molecule,

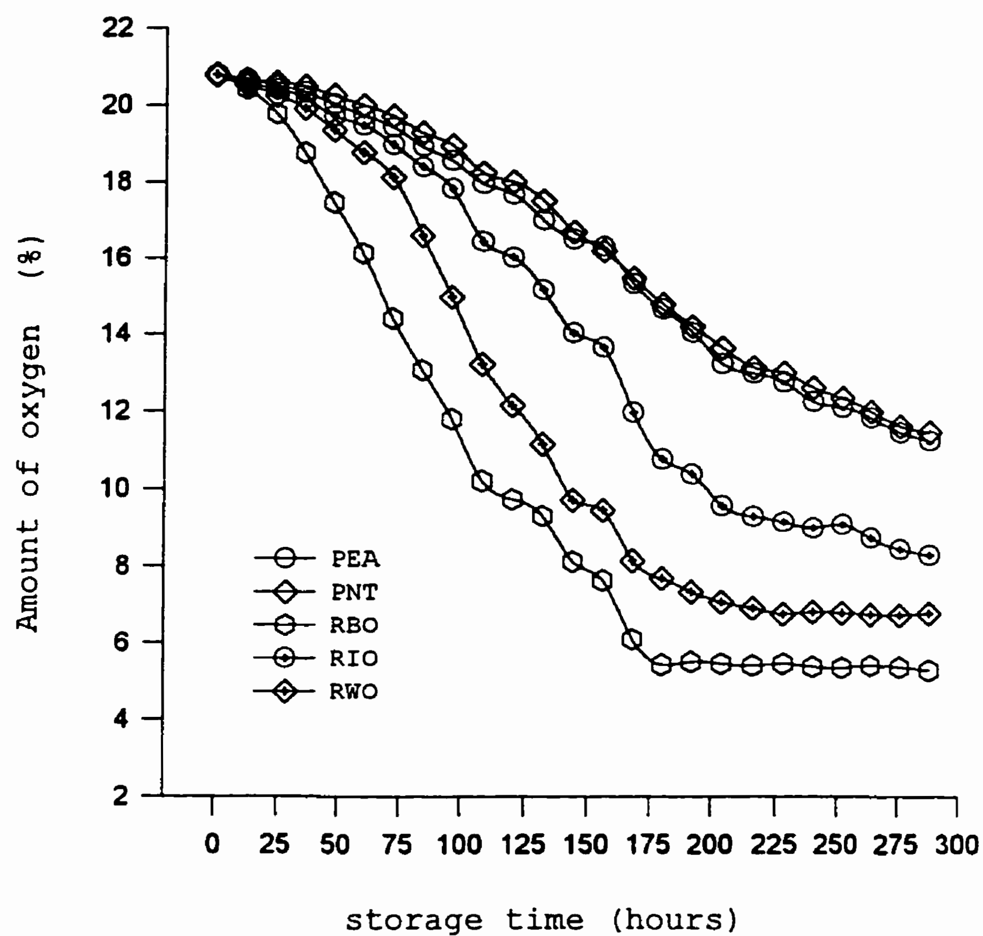

Fig. 4.2.4.1. Oxygen uptake during storage without light at 65°C (for abbreviations see Table 3.1.1).

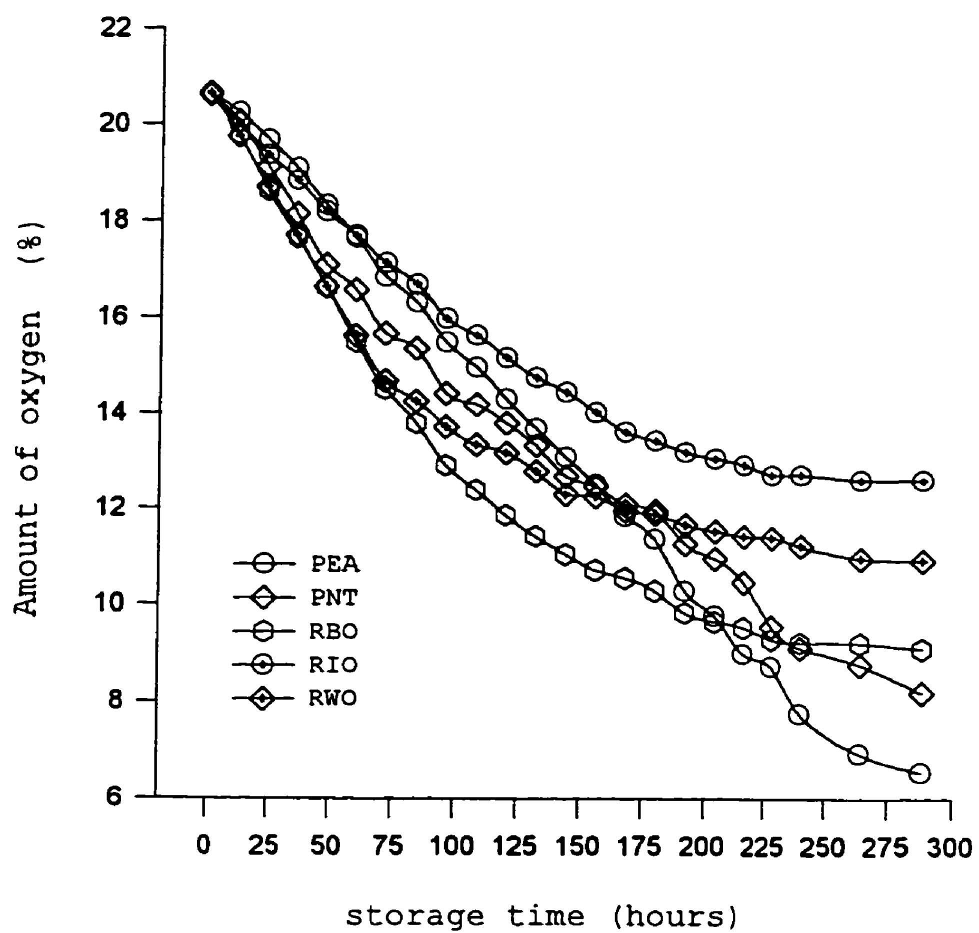

Fig. 4.2.4.2. Oxygen uptake during storage with light at 35°C (for abbreviations see Table 3.1.1).

Table 4.2.4. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines.

Oils[1]	Absence of light[2]			Light exposure[3]
	k_1	k_2	ip(hr)	k_2
RIO	-0.016[ac]	-0.075[a]	68.1[a]	-0.042[a]
RBO	-0.042[b]	-0.113[b]	27.3[b]	-0.079[b]
RWO	-0.021[d]	-0.121[c]	56.7[c]	-0.072[c]
PEA	-0.012[c]	-0.048[d]	78.1[ad]	-0.055[d]
PNT	-0.012[c]	-0.050[d]	80.4[d]	-0.054[d]

[a-d]Values with different letters in each column are significantly different by Duncan's multiple range test at P<0.05; [1]abbreviations see Table 3.1.1; [2]65°C; [3]35°C.

such as LOO, OOO and POO (Table 4.1.2.3).

Among rice bran oils, RBO oxidized significantly faster than did RWO and RIO during both storage conditions (Table 4.2.4). RWO oxidized significantly faster than did RIO during storage without light (p_{k1}=0.0006, p_{k2}=0.00008, p_{ip}=0.00011) and also during storage with light (p_{k2}=0.00001). All rice bran oils contained iron, but the amount of this metal in RBO was 4 times higher than in RWO and RIO (Fig. 4.1.8). RIO contained a higher amount of phospholipids (Table 4.1.3.2) and a lower amount of sterols (Fig. 4.1.7) than did RWO.

Peanut oils (PEA and PNT) had a similar fatty acid composition to rice bran oils (Table 4.1.1). The difference in the contribution of linoleic acid in peanut oils was 5 to

6% lower and in oleic acid was 5-8% higher than in rice bran oils. Rice bran oils had a significantly lower stability than peanut oils during storage in the absence of light (Table 4.2.4). During storage with light exposure RIO was significantly more stable than were PEA (p_{k2}=0.00002) and PNT (p_{k2}=0.00003) oils, while RBO and RWO were significantly less stable than peanut (PEA and PNT) oils (Table 4.2.4). Rice bran oils contained metals (Fig 4.1.8) and a higher concentration of tocotrienols (Fig. 4.1.4.2) and sterols (Fig. 4.1.7) than did peanut oils. RIO also had the highest content of polar lipids (Table 4.1.3.2). The peanut oils had higher amounts of LOO and OOO and lower amounts of LLP and POL glycerides compared to the rice bran oils (Table 4.1.2.3).

4.2.5. Oils with high content of saturated fatty acids

Coconut (COC) and palm kernel (PLK) oils, both highly saturated fats, showed no significant differences in oxygen uptake in both storage conditions (Fig. 4.2.5.1 and 2; Table 4.2.5). Both oils were very stable during storage without light. During storage with light, for both oils the initiation period ended after 160 hours of storage. Both oils showed no significant differences in oxygen uptake during the propagation period (k_2)(p_{k2}=0.0508) in storage with light, but PLK had slightly faster oxidation than did COC after 160 hours of storage. PLK contained about twice the unsaturated fatty acids of COC. Both oils contained a low amount of tocopherols

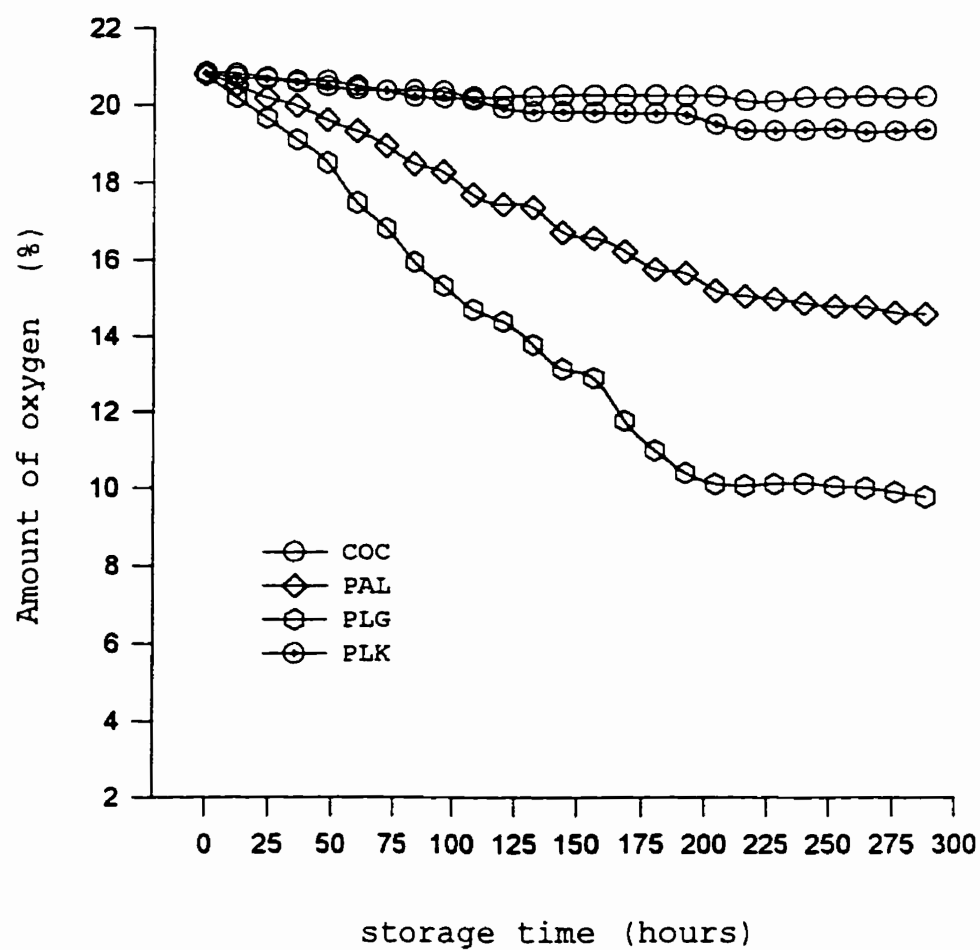

Fig. 4.2.5.1. Oxygen uptake during storage without light at 65°C (for abbreviations see Table 3.1.1).

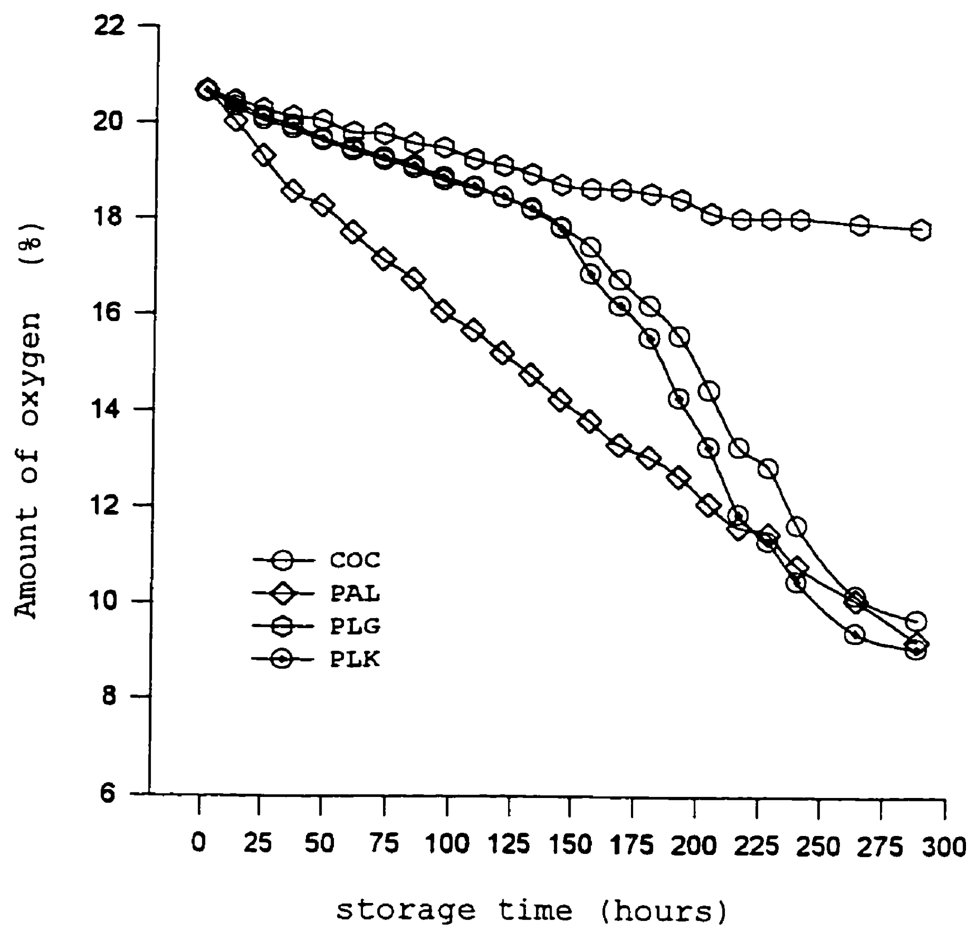

Fig. 4.2.5.2. Oxygen uptake during storage with light at 35°C (for abbreviations see Table 3.1.1).

Table 4.2.5. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines.

Oils[1]	Absence of light[2]			Light exposure[3]		
	k_1	k_2	ip(hr)	k_1	k_2	ip(hr)
COC	-0.003[a]	-	288.0[a]	-0.018[a]	-0.077[a]	162.6[a]
PLK	-0.004[a]	-	288.0[a]	-0.018[a]	-0.081[a]	159.2[a]
PAL	-	-0.026[a]	-	-	-0.039[b]	-
PLG	-	-0.054[b]	-	-	-0.012[c]	-

[a-c]Values with different letters in each column are significantly different by Duncan's multiple range test at P<0.05; [1]abbreviations see Table 3.1.1; [2]65°C; [3]35°C.

(Fig. 4.1.4.1), tocotrienols (Fig. 4.1.4.2) and sterols (Fig. 4.1.7), and neither oils had iron at a level higher than 0.10ppm (Fig. 4.1.8).

Palm oil (PAL) had significantly better stability than did palm golden oil (PLG) when stored without light (p_{k2}=0.0010) whereas PAL had a significantly lower stability than PLG when stored in the presence of light (p_{k2}=0.0117). Both palm oils had lower stability than coconut (COC) and palm kernel (PLK) oils during storage without light. PLG showed better stability than the other highly saturated (PLK and COC) oils when stored with light exposure. PLG contained a higher content in tocopherols (Fig. 4.1.4.1) and tocotrienols (Fig. 4.1.4.2) than did PAL, but this oil (PAL) showed a lower oxidation rate during storage without light. PLG had a high

content of carotenoids (Fig. 4.1.6.2), which were absent in PAL oil. Both palm oils (PAL and PLG) had a relatively high initial peroxide value as compared with other oils (Fig. 4.1.9).

4.2.6. Comparative stability between oils based on oxygen uptake

4.2.6.1. Oxygen uptake during storage without light

The cluster analysis performed on slope k_1 (initiation period), k_2 (propagation period) and ip (induction period) for oils stored without light is presented in Fig. 4.2.6.1 and Table 4.2.6.1. Nine groups were obtained from 33 oils evaluated at a linkage distance 1.18. These groups were denominated as #1-9 in order to facilitate their comparisons in Table 4.2.6.1.

Flax (FCO), borage (BOR) and evening primrose (EPR) oils formed one group (group #1). These oils showed no initiation time and the fastest oxidation rate among the oils analysed. A high rate of oxygen depletion implies fast deterioration of these oils. Conventional flax oil showed the fastest rate of oxidation among all oils, followed by BOR and EPR, and these three oils had a faster oxidation rate than did oils from all other groups (Table 4.2.6.1). Under this storage condition, the fastest rate of oxidation was directly related to the highest contents of linolenic acid at 54.2%, 22.8% and 9.8% for FCO, BOR and EPR, respectively. Evening primrose had a

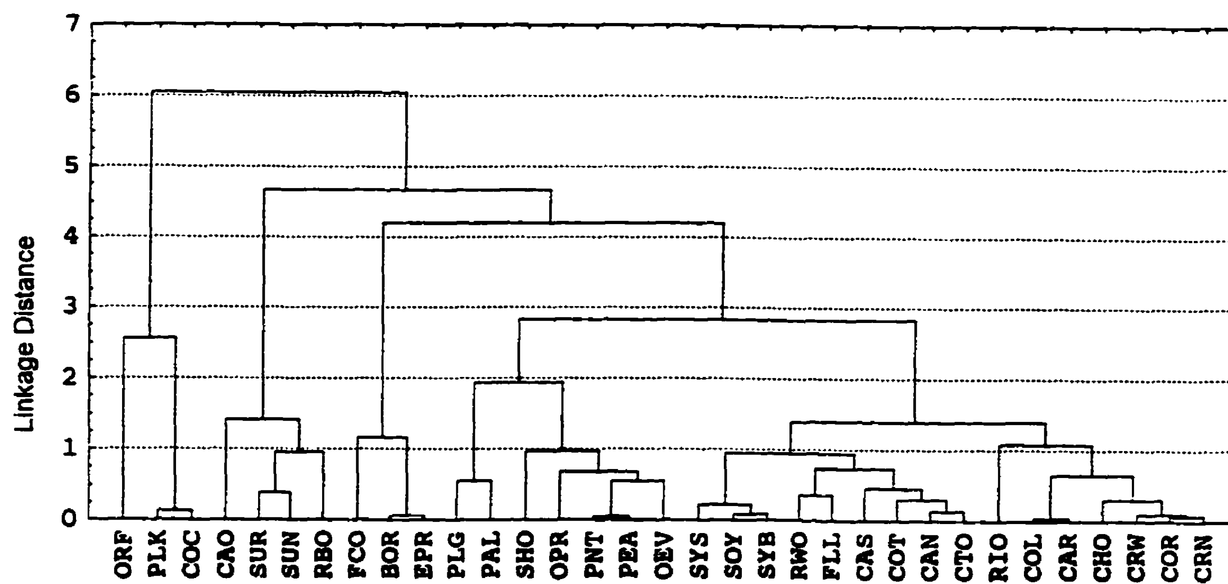

Fig. 4.2.6.1. Tree diagram for oils stored without light at 65°C, with complete linkage and Euclidean distances (for abbreviations see Table 3.1.1).

Table 4.2.6.1. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines during storage without light (65°C).

Group#	Oils[1]	k_1 (±STDx10^{-3})	k_2 (±STDx10^{-3})	ip(hrs)(±STD)
1	FCO	-	-0.218 (1.0)	-
	BOR	-	-0.162 (0.8)	-
	EPR	-	-0.159 (0.5)	-
2	CAO	-0.041 (0.3)	-0.165 (0.1)	25.0 (0.9)
3	SUN	-0.024 (0.7)	-0.116 (0.3)	27.6 (1.1)
	SUR	-0.020 (0.5)	-0.111 (1.1)	28.4 (0.7)
	RBO	-0.042 (0.6)	-0.113 (0.9)	27.3 (1.0)
4	SOY	-0.011 (0.8)	-0.133 (1.2)	49.2 (0.5)
	SYS	-0.009 (0.2)	-0.130 (1.3)	51.5 (1.3)
	SYB	-0.011 (0.1)	-0.129 (0.9)	50.0 (1.1)
	RWO	-0.021 (0.4)	-0.121 (1.0)	56.7 (1.5)
	FLL	-0.017 (0.5)	-0.138 (1.5)	55.7 (0.9)
	COT	-0.015 (0.3)	-0.126 (1.7)	37.5 (0.8)
	CTO	-0.016 (0.6)	-0.115 (0.6)	42.1 (1.2)
	CAN	-0.015 (0.9)	-0.095 (0.2)	39.6 (1.4)
	CAS	-0.012 (0.3)	-0.107 (0.5)	46.9 (1.7)
5	RIO	-0.016 (0.7)	-0.075 (1.0)	68.1 (2.1)
	COL	-0.008 (0.3)	-0.084 (0.7)	87.1 (1.3)
	CAR	-0.009 (0.1)	-0.085 (0.9)	86.8 (1.8)
	CHO	-0.007 (0.5)	-0.116 (1.5)	72.7 (2.5)
	CRW	-0.010 (0.4)	-0.102 (1.8)	79.9 (1.6)
	CRN	-0.011 (0.7)	-0.106 (0.5)	78.9 (2.3)
	COR	-0.010 (0.8)	-0.104 (0.3)	77.6 (0.8)
6	ORF	-0.026 (0.1)	-0.054 (0.6)	236.4 (3.1)
7	OEV	-0.014 (0.6)	-0.023 (0.8)	79.1 (2.1)
	OPR	-0.010 (0.2)	-0.028 (0.4)	114.2 (0.9)
	SHO	-0.010 (0.1)	-0.039 (1.2)	46.2 (0.7)
	PEA	-0.012 (0.2)	-0.048 (0.7)	78.1 (1.4)
	PNT	-0.012 (0.1)	-0.050 (0.6)	80.4 (1.8)
8	PLG	-	-0.054 (0.8)	-
	PAL	-	-0.026 (0.6)	-
9	PLK	-0.004 (0.7)	-	288.0 (0.0)
	COC	-0.003 (0.9)	-	288.0 (0.0)

STD= standard deviation; [1]abbreviations see Table 3.1.1.

similar amount of linolenic acid (9.76) to canola (CAO) (10.1%), but the excess of the percentage of PUFA in EPR was 52.6% over that in CAO. A similar finding applied to BOR and EPR. These two oils showed a similar oxidation rate, and BOR contained a higher amount of linolenic acid (22.7%) than did EPR (9.8%), but EPR had a higher amount of total PUFA (83.7%) than did BOR (61.6%).

Regular canola (CAO) oil formed an individual cluster (group #2). This oil showed an induction period of 25 hours, whereas the oils from group #1 had none. Due to its induction period CAO was more stable than were oils from the previous group. This could also be observed by the amount of oxygen consumed after 84 hours of storage, which was 75.89%, 62.51%, 61.53% and 53.82% for FCO, BOR, EPR and CAO, respectively. CAO had a lower content of linolenic acid than did FCO and BOR, but a similar content to EPR; however, EPR contained more than twice the total PUFA of CAO (Table 4.1.1). Again, the higher content of PUFA was related to the faster oxidation of these oils compared to CAO.

Another group was formed by regular sunflower (SUN and SUR) and RBO oils (group #3). RBO oil showed significantly lower stability in the initial 27-28 hours of storage than did SUN (p_{k1}=0.00005) and SUR (p_{k1}=0.00003). No significant differences in the oxidation rate of RBO were observed after this period in relation to SUN (p_{k2}=0.5697) and SUR (p_{k2}=0.9157). Regular sunflower oils contained about 71%

linoleic acid, while RBO contained only 36% linoleic and approximately 2% linolenic acids. Therefore, the content of unsaturated fatty acids in RBO was not alone responsible for the faster oxidation rate than was found in sunflower oils. These oils contained iron, but the amount present in RBO was 4 times higher than that in sunflower oils (Fig. 4.1.8). RBO also contained 1.5 times more tocopherols and tocotrienols (Fig. 4.1.4.1 and 2) and twice the amount of sterols (Fig. 4.1.7) compared to sunflower oils. Oils from this group (group #3) showed better stability than did CAO (group #2)(Table 4.2.6.1). RBO had a higher proportion of PUFA than did CAO (Table 4.1.1) and accordingly was more stable than that oil.

The biggest cluster was formed by 9 oils, SYS, SOY, SYB, RWO, FLL, COT, CTO, CAN and CAS (group #4). Oils from this group showed a lower oxidation rate than did oils from group #3. With the exception of RBO and FLL, the oils classified into group #3 contained a higher amount of PUFA than did the oils from the latter group (#4). In that group, oils showed slight differences in stability during the initiation and propagation steps. Soybean oils had the highest resistance to oxidation for the first 60 hours of storage, followed by low linolenic flax, regular canola, rice bran and cottonseed oils, with oxygen depletions of about 5.9, 7.7, 8.7, 9.6 and 12.0%, respectively. During further storage, 100-120 hours, all oils in this cluster demonstrated similar oxygen uptake, about 45%.

In the final period of storage (144 hours), two distinct groups were observed: (1) soybean and low linolenic flax oils, which had a similar oxygen uptake of about 61.5%, and (2) canola, rice bran and cottonseed oils with an oxygen consumption of about 54.5%. Soybean oils, with about 7.5% linolenic acid revealed better stability in the initial stages of storage than did those oils of group #4 which contained almost no linolenic acid. However the stability of soybean oils decreased at faster rates during storage than did those of canola, rice bran and cottonseed oils. In soybean (SOY and SYB), cottonseed and rice bran oils, the presence of metals was observed (Fig. 4.1.8). The total amount of tocopherols in soybean oils was at least twice that of the other oils from this group (Fig. 4.1.4.1). Still in the initial stages of storage, regular canola oils exhibited better resistance to oxidation than did cottonseed oils, even though regular canola oils included higher amounts of linolenic acid than cottonseed oils, 8.0% vs 0.5%. Cottonseed oils had a higher amount of tocopherols than did regular canola oils (Fig. 4.1.4.1), but at the same time they contained metals which were not present in CAS and CAN (Fig. 4.1.8). In the final stages of storage, rice bran, canola and cottonseed oils exhibited similar stability, in spite of their large differences in fatty acid composition. Canola oils had the lowest content of saturated fatty acids (Table 4.1.1), and rice bran oil contained metals, but at lower levels than did cottonseed oils (Fig. 4.1.8).

Another cluster (group #5) was formed by RIO, COL, CAR, CHO, CRW, COR and CRN oils. Oils in this group showed similar resistance to oxidation in the first 72 hours of storage, with an oxygen depletion of about 3.8%. An exception was the RIO which exhibited lower stability in the same period, with an oxygen consumption of 8.6%. After this time, RIO exhibited a lower rate of oxygen consumption than did other oils (k_2= -0.075), but in the final period of storage (144 hours) the stability of RIO was similar to CAR and COL oils, when the oxygen depleted was approximately 28.0% of the initial amount. In the last storage period mentioned above, these three oils (RIO, CAR and COL) showed better stability than CHO, COR, CRW and CRN oils, which depleted about 40% of the oxygen in 144 hours of storage. CHO and COL showed similar oxidation characteristics to corn oils during the initial storage period. All of these oils exhibited large differences in fatty acid composition. Genetically modified canola oils (CHO and COL) included higher amounts of linolenic and oleic acids, while corn oils contained higher amounts of linoleic and saturated fatty acids (Table 4.1.1). Even though COL had a lower content of saturated fatty acids than did corn oils, in the second half of the storage period this oil exhibited better stability than did corn oils. In this group, rice bran oil (RIO), which contained the highest amount of saturated fatty acids, demonstrated the fastest oxidation rate in the first stages of the storage period. RIO contained 0.030ppm of

iron and also had the lowest amount of total tocopherols and tocotrienols, 406.6ppm. Corn oils demonstrated a similar fatty acid composition to cottonseed oils (from group #4), which had an even higher content of saturated fatty acids than did corn oils. However corn oils showed much better stability than did cottonseed oils. When compared to cottonseed oils, corn oils did not contain metals (Fig. 4.1.8), but contained higher amounts of tocopherols and tocotrienols (Fig. 4.1.4.1 and 2) and sterols (Fig.4.1.7).

Olive (ORF) oil was classified as an individual cluster (group #6). This oil had lower stability during the first 72 hours of storage, with a depletion of 11.1% of the oxygen, compared to oils from group #5. But, after 72 hours of storage, ORF had better stability due to the lower oxidation rate of the propagation period (k_2= -0.054) when compared to other oils from group #5 (Table 4.2.6.1). ORF contained the lowest content of PUFA (Table 4.1.1), and also the highest content of total phenolics (Fig. 4.1.5) compared to the oils from the previous group.

Peanut (PEA and PNT), olive (OEV and OPR) and high oleic sunflower (SHO) oils formed another group (group #7). These oils showed better stability during storage than did oils from groups #1-6. Peanut (PEA and PNT) and SHO oils showed lower stability than did OPR and OEV oils during the whole storage period. The peanut oils showed better stability than ORF (from group #6) until 144 hours of storage, when ORF had

better stability than did peanut and SHO oils. High oleic sunflower (SHO) oil showed a similar fatty acid composition to olive oils, while peanut oils presented a higher content of linoleic acid than the other oils (Table 4.1.1). Olive oils were characterized by a higher content of phenolics (Fig. 4.1.5), while SHO presented a higher content of tocopherols (Fig. 4.1.4.1). Both SHO and olive oils contained metals, while peanut oils showed no metals (Fig. 4.1.8) and a lower content in sterols than did SHO and olive oils (Fig. 4.1.7).

Palm oils, PAL and PLG, formed another cluster (group #8). These oils showed no induction period, but the rate of oxidation during the propagation period was relatively low when compared with other oils (Table 4.2.6.1). These oils contained about 50% saturated fatty acids (Table 4.1.1), but they showed faster oxidation then several other oils with a lower content of saturated fatty acids. This could be seen at 180 hours of storage, when the amount of oxygen consumed was 13.0, 16.3, 23.1, 24.2, 28.8, 29.3, 42.1, 43.8 and 47.1% for OPR, OEV, ORF, PAL, PNT, PEA, COL, CAR and PLG, respectively. As discussed before, PAL contained the highest amount of metals (1.24ppm) and PLG the highest amount of carotenoids (731.9ppm). Also, these two oils showed PV values of about 10, while all other oils showed initial PV values lower than 1.5.

Palm kernel (PLK) and coconut (COC) oils formed the last cluster (group #9). They showed the highest stability among

all the analysed oils during the whole storage period. These oils contained the highest amount of saturated fatty acids (80.3 to 90.7%), the lowest content of total tocopherols and tocotrienols (17.3 to 33.9ppm), and also a low content of sterols (71.6 to 143.8mg/100g). Although containing iron (0.075 to 0.10ppm), these oils consumed only about 4% of the oxygen during the whole storage period (288 hours).

4.2.6.2. Oxygen uptake during storage with light exposure

The cluster analysis using the slopes of the initiation (k_1), propagation (k_2) and induction period (ip) of oxygen uptake for oils stored with light is presented in Fig. 4.2.6.2 and Table 4.2.6.2. Seven clusters were formed at a linkage distance 1.29, from 33 oils evaluated.

Virgin olive (OEV) and canola (CAO) oils (group #1) oils showed the fastest oxygen depletion among all oils during storage in the presence of light. OEV had a significantly higher rate of oxidation than did CAO (p_{k2}=0.00012). OEV contained 76.3% oleic acid and very little linolenic acid; but this oil oxidized at faster rates than did oils with a higher content of PUFA. This oil was very stable during storage without light (see 4.2.6.1, group #7). CAO contained a lower amount of linolenic acid than did FCO and BOR oils, but still oxidized at a significantly faster rate than did these oils (p_{k2-FCO}=0.00012; p_{k2-BOR}=0.00001).

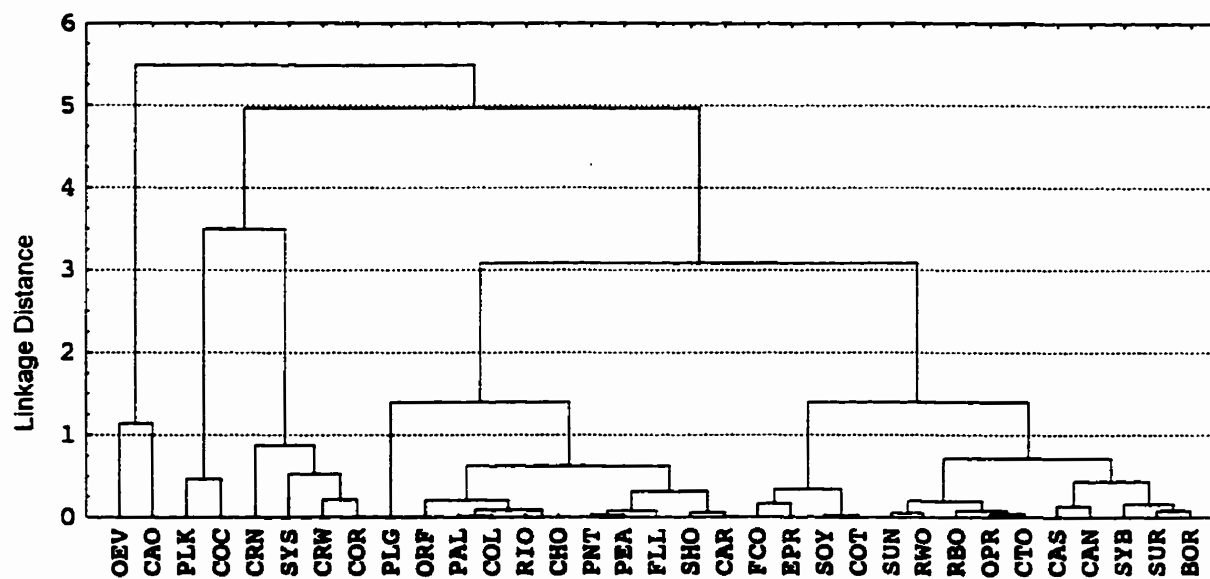

Fig. 4.2.6.2. Tree diagram for oils stored with light at 35°C, with complete linkage and Euclidean distances (for abbreviations see Table 3.1.1).

Table 4.2.6.2. Induction period (ip) and slopes of initiation (k_1) and propagation (k_2) lines during storage with light exposure (35°C).

Groups #	Oils[1]	K_1 (±STDx10^{-3})	K_2 (±STDx10^{-3})	ip(hrs)(±STD)
1	OEV	-	-0.173 (0.2)	-
	CAO	-	-0.138 (0.6)	-
2	FCO	-	-0.101 (0.7)	-
	COT	-	-0.096 (0.8)	-
	SOY	-	-0.099 (0.3)	-
	EPR	-	-0.103 (0.9)	-
3	RBO	-	-0.079 (0.2)	-
	SUR	-	-0.079 (0.6)	-
	SUN	-	-0.083 (0.8)	-
	OPR	-	-0.080 (0.7)	-
	SYB	-	-0.072 (0.5)	-
	CTO	-	-0.082 (0.5)	-
	CAS	-	-0.068 (0.3)	-
	CAN	-	-0.063 (0.6)	-
	BOR	-	-0.077 (0.8)	-
	RWO	-	-0.072 (0.7)	-
4	ORF	-	-0.036 (0.2)	-
	PAL	-	-0.039 (0.3)	-
	COL	-	-0.040 (0.8)	-
	RIO	-	-0.042 (0.2)	-
	CHO	-	-0.042 (0.6)	-
	PNT	-	-0.054 (0.4)	-
	PEA	-	-0.055 (0.7)	-
	FLL	-	-0.052 (0.4)	-
	SHO	-	-0.047 (0.3)	-
	CAR	-	-0.045 (0.6)	-
5	CRN	-0.029 (0.3)	-0.063 (0.3)	57.3 (2.3)
	SYS	-0.028 (0.2)	-0.070 (0.4)	31.4 (1.8)
	CRW	-0.030 (0.1)	-0.059 (0.7)	44.8 (1.3)
	COR	-0.030 (0.1)	-0.061 (0.5)	43.5 (0.7)
6	PLK	-0.018 (0.4)	-0.081 (0.6)	159.2 (3.2)
	COC	-0.018 (0.6)	-0.077 (0.3)	162.6 (4.1)
7	PLG	-	-0.012 (0.1)	-

STD= standard deviation; [1]abbreviations see Table 3.1.1.

Both oils, OEV and CAO, contained metals (Fig. 4.1.8) and chlorophyll (Fig. 4.1.6.1). However OEV contained about 58 times more chlorophyll, and 3 times more metals than did CAO. SOY and COT oils contained chlorophyll (Fig. 4.1.6.1) even at a higher level than did CAO, but oxidized at lower rates. Both SOY and COT contained more tocopherols (Fig. 4.1.4.1) and metals (Fig. 4.1.8) than did CAO, while CAO contained more sterols (Fig. 4.1.7).

Flax (FCO), soybean (SOY), cottonseed (COT) and evening primrose (EPR) oils formed group #2. These oils showed better stability than oils from cluster #1, which can be seen by the significantly lower oxidation rate of EPR compared to CAO (p_{k2}=0.00006). EPR showed no significant differences in the oxidation rate compared to FCO (p_{k2}=0.8838), but it had significantly faster oxidation than did SOY (p_{k2}=0.0013) and COT (p_{k2}=0.00006). FCO showed no significant differences in stability compared to SOY (p_{k2}=0.0506) and a significantly faster oxidation rate than did COT (p_{k2}=0.00005). SOY oxidized at a significantly faster rate than did COT (p_{k2}=0.0028). Oils from this group showed a high variability in fatty acid composition. FCO contained the highest amount of linolenic acid (54.2%), while EPR, SOY and COT contained 9.8, 7.2 and 0.3%, respectively. Again, EPR and SOY contained a lower amount of linolenic acid than did FCO, but these oils oxidized at similar rates. All oils from this group contained metals (Fig. 4.1.8), while SOY and COT also contained chlorophyll

(Fig. 4.1.6.1). Also, the amount of chlorophyll in SOY was 4.7 times higher than in COT. But the amount of tocopherols in SOY was twice as high as in COT (Fig. 4.1.4.1). These two oils (SOY and COT) had better stability than did regular sunflower and RBO oils during storage without light (see 4.2.6.1, group #3 and #4), but lower stability during storage with light. SOY and COT contained a lower content of PUFA than did regular sunflower oils, but neither RBO, SUN nor SUR contained chlorophyll.

Ten oils formed another cluster (group #3), RBO, SUR, SUN, OPR, BOR, SYB, CTO, RWO, CAN and CAS. These oils showed better stability than the oils in group #2. RBO, SUR, SUN, OPR, BOR and CTO showed a higher amount of oxygen depletion (about 38%) than did SYB and RWO (about 36%), and than did CAN and CAS (about 34%) oils, during the first 108 hours of storage. The BOR oil contained the highest amount of linolenic acid (22.8%) among oils from this group, and had oxidation similar to oils with higher amounts of linoleic and oleic acids. Both BOR and OPR contained chlorophyll, but at lower levels than did SOY, COT, CAO and OEV (Fig. 4.1.6.1). Also, the BOR contained a higher amount of tocopherols than did any other oil, while OPR contained the highest amount of phenolic acids when compared with other oils in this group (Fig. 4.1.5).

Another ten oils, ORF, PAL, COL, RIO, CHO, PNT, PEA, FLL, SHO and CAR formed group #4. These oils showed higher

stability than did oils from the previous group (#3). During 108 hours of storage PEA, PNT and FLL depleted a higher amount of oxygen (about 27.5%) than did CAR and SHO (about 23.0%), which was more than COL, CHO, RIO and PAL (about 22.1%), and finally more than ORF (17.8%). Low linolenic flax (FLL) contained a higher amount of linoleic acid (70.3%) than did peanut oils (about 30.0%), but similar oxidation rate. Also, FLL contained a higher amount of total tocopherols (Fig. 4.1.4.1) and phospholipids (Table 4.1.3.2) than did peanut oils. COL, CHO and SHO had a lower stability than did ORF, but all had similar amount of oleic acid (Table 4.1.1). These oils (CHO, SHO and COL) contained higher amounts of tocopherols (Fig. 4.1.4.1) and sterols (Fig. 4.1.7) than did ORF, while ORF contained a higher amount of phenolic acids (Fig. 4.1.5).

Soybean (SYS) and corn (COR, CRN and CRW) oils formed group #5. These oils showed an initiation period, which was absent for several other oils stored with light exposure. SYS showed no significant differences in relation to the initiation period (k_1) of COR (p_{k1}=0.0562), CRN (p_{k1}=0.8667) and CRW (p_{k1}=0.05372) oils, but a significantly shorter induction period than did corn oils (p_{ip-COR}=0.00017, p_{ip-CRN}=0.00006, p_{ip-CRW}=0.00010). The lower stability of SYS was also demonstrated by a significantly higher rate of oxygen depletion during the propagation period than that of COR (p_{k2}=0.00003), CRN (p_{k2}=0.00005) and CRW (p_{k2}=0.00003) oils. SYS contained a

higher amount of linolenic acid (Table 4.1.1) and tocopherols (Fig. 4.1.4.1) than did corn oils, while corn oils contained a higher amount of sterols (Fig. 4.1.7). Oils from this group showed better stability during the initial stages of storage (30-50 hours) than some oils from group #4 (PEA, PNT, FLL, CAR and RIO), but they showed faster oxidation during the remaining period of storage than did oils from the previous group.

Palm kernel (PLK) and coconut (COC) oils formed group #6. These oils showed high stability during storage without light (Table 4.6.2.2), but the same was not observed during storage with light exposure. In this storage COC and PLK oils showed an initiation period of about 160 hours, when about 17.8% of the oxygen was depleted. During this period (160 hours) these oils showed better stability than did other oils, except for PLG. After 160 hours of storage, COC and PLK started to deplete oxygen at faster rates ($k_{2-COC} = -0.077$; $k_{2-PLK} = -0.081$), with a consequent reduction of their stability. After 160 hours of storage, these oils depleted oxygen at faster rates than did oils with a higher content of unsaturated fatty acids, such as COL, CHO, SHO and ORF. Both oils, PLK and COC, contained metals (0.075 to 0.10ppm) and a low amount of phospholipids (Table 4.1.3.2). They also contained the lowest level of tocopherols and tocotrienols (Fig. 4.1.4.1 and 2) among all of the oils.

Palm golden (PLG) oil formed the last cluster (group #7).

This oil was the most stable during storage with light exposure among all the oils. PLG depleted oxygen at the lowest rates (k_2= -0.012) during all storage periods when compared with any other oil analysed. During the whole storage period PLG depleted only 13.7% oxygen. PLG contained higher amounts of MUFA and PUFA than did PAL, PLK and COC, but a lower amount than the other oils. PLG contained the highest amount of carotenoids among the oils analysed (Fig. 4.1.6.2).

4.3. Conventional storage of oils

The conventional Schaal Oven test applied in this study was used:

1) To evaluate selected oil storage stability using standard tests;
2) To verify the validity of oxygen consumption as a measurement of oil stability;
3) To assess the effect of minor components on the formation of oxidation products.

Due to the large total number of oils, 13 oils were selected on which to perform the Schaal Oven test in the absence and presence of light. Basically five main criteria were used to select the oils:

1) These oils represented the groups of oils with specific oxidation characteristics; they were selected on the basis of oxygen consumption;
2) Selected oils were representative of a wide range of major and minor components;
3) Olive (OEV and ORF); canola (CAO, CAS and CHO); soybean (SOY and SYS); rice bran (RIO and RWO) oils were selected because oils within these separate groups were from the same genetic background and presented different stability;
4) Low linolenic flax (FLL) and sunflower (SUR) were selected because they exhibited similar fatty acid composition, but these oils had different genetic make-ups, and also different stability. The same was applied to PEA, when

compared to RIO and RWO;

5) Finally, coconut (COC) oil was selected to represent an oil with a high content of saturated fatty acids.

4.3.1. Measurement of oil stability during accelerated oxidation

4.3.1.1. Peroxide value (PV)

The changes in peroxide value for oils stored without light (Fig. 4.3.1.1.1) followed different patterns during the storage period. In general, oils that showed faster rates of oxidation also exhibited a faster rate of PV accumulation.

Similarly to results for oxygen uptake (see 4.2.6.1), oils with an elevated content of saturated (COC) and monounsaturated fatty acids (OEV and ORF) showed the lowest PV accumulation. Oils with a higher content of polyunsaturated fatty acids (PUFA) exhibited higher PV values.

Virgin olive (OEV) oil showed a significantly higher initial PV (2.70) than RWO (PV= 1.35; $p=0.0018$), and both oils showed significantly higher PV values than all other stored oils, while no significant differences were found between the other oils at the initial time ($P<0.05$).

Among these oils, CAO demonstrated the fastest rate of oxidation (compare section 4.2.6.1), and also showed (Fig.4.3.1.1.1) a significantly faster PV accumulation up to the second day of storage than did other oils ($p<0.05$). Some oils started to accumulate peroxides at faster rates after the

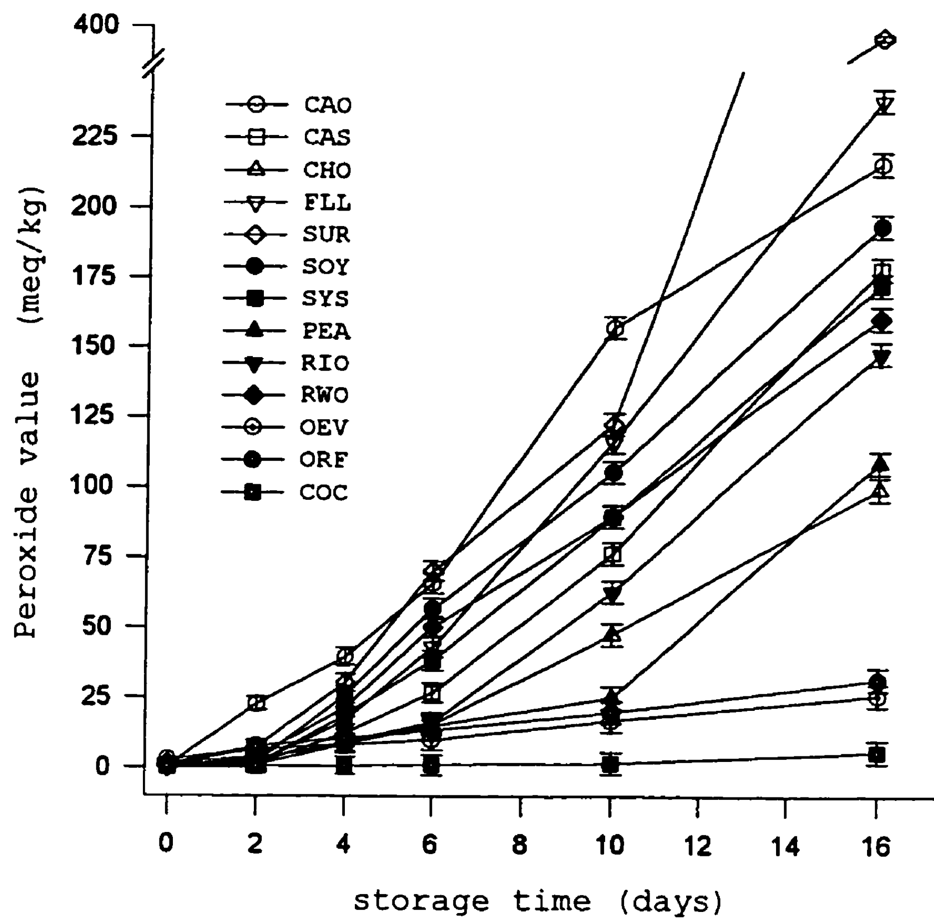

Fig. 4.3.1.1.1. Peroxide accumulation during storage without light at 65°C (error bars represent standard errors, and for abbreviations see Table 3.1.1).

second and fourth days of storage. Olive (OEV and ORF) and coconut (COC) oils maintained low PV values during the whole storage period. CAO exhibited the highest PV values until the 11th day of storage; then SUR and FLL surpassed it at the end of storage. SUR accumulated the highest amount of peroxides by the final day of storage, and that value was significantly higher than that for FLL (p=0.0001), but both oils had similar fatty acid composition. These two oils exhibited the highest content of polyunsaturated fatty acids (Table 4.1.1).

Coconut (COC), the most stable oil when measured by oxygen uptake, also had the lowest PV accumulation during storage compared to the other stored oils. Olive oil (OEV) showed significantly higher PV accumulation on the second day of storage (p=0.0017), while ORF had a significantly higher value on the fourth day (p=0.0001); however, the PV accumulation for both oils continued at low levels to the end of the storage period (PV< 30).

Oils with a high content of PUFA presented an initial resistance to oxidation (induction period) between 25 and 78.1 hours during the oxygen consumption measurement (Table 4.3.1.1.1). During the same period in storage using the Schaal Oven test, PV values lower than 5.93 were observed. An exception was for CAO where the PV value accumulated quickly to 22.26 at 25 hours of storage.

Table 4.3.1.1.1. PV values at the end of induction period During storage without light (65°C) measured by oxygen consumption.

Oils[1]	Induction period (hrs)[2]	PV (meq/kg)
OEV	79.1	6.89
ORF	236.4	19.56
CAO	25.0	22.26
CAS	46.9	1.11
CHO	72.7	4.70
FLL	55.7	1.52
SUR	28.4	4.26
SOY	49.2	1.87
SYS	51.5	1.19
PEA	78.1	5.93
RIO	68.1	5.81
RWO	56.7	4.54
COC	28.8	1.91

[1]Abbreviations see Table 3.1.1; [2]measured by oxygen consumption.

During storage in the presence of light the PV value started to increase at a faster rate from the first day of storage (Fig. 4.3.1.1.2). In the first day all oils showed significantly higher PV values than the PV at time-0 ($P<0.05$).

Coconut (COC) oil showed the lowest rate of PV accumulation through the whole storage time. But, on the last day of storage the PV value of COC was significantly higher ($p=0.0012$) than that of ORF. During storage with light exposure COC showed an induction period of 162.6 hours when measured by oxygen consumption (Table 4.2.6.1). The PV value for the same period of storage under Schaal Oven test conditions was 12.81.

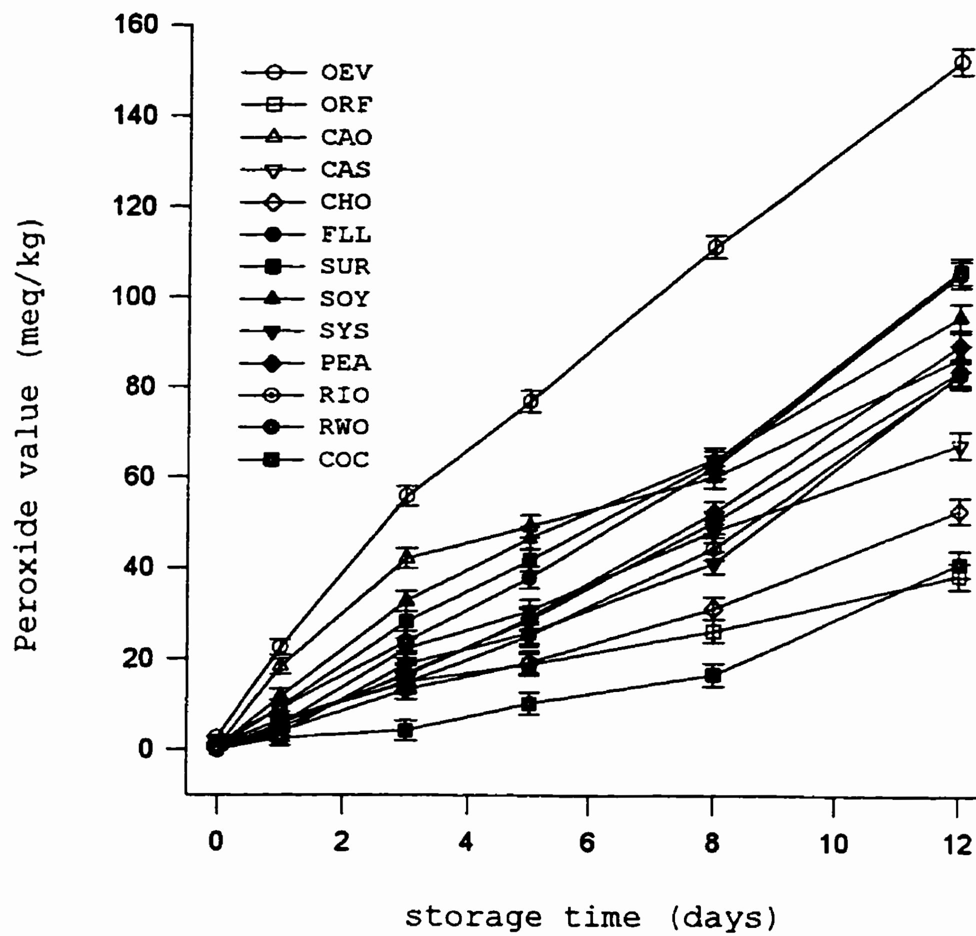

Fig. 4.3.1.1.2. Peroxide accumulation during storage with light at 35°C (error bars represent standard errors, and for abbreviations see Table 3.1.1).

After the third day, the rate of PV accumulation during this storage was lower than during storage without light. After the 12th day of storage, the PV values were similar to and/or lower than those observed for storage without light. Again exceptions were olive and COC oils.

The same results as were observed for peroxide formation during Schaal Oven storage without light were found during storage with light exposure, where oils that showed faster rates of oxidation also exhibited a faster rate of PV accumulation.

Olive oil (OEV) had a significantly higher accumulation of PV than did other oils during the whole storage period ($P<0.05$). At the end of storage OEV showed the highest PV, compared to oils composed of higher amounts of PUFA.

4.3.1.2. Conjugated dienoic acids (CLA)

During Schaal Oven storage without light at 65°C, CLA formation followed a similar pattern to PV accumulation.

Coconut (COC) and olive oils (OEV and ORF) showed the lowest accumulation of conjugated dienes during storage (Fig. 4.3.1.2.1), the same as were observed for PV (Fig. 4.3.1.1.1) and oxygen uptake (see 4.2.6.1).

Coconut oil (COC) showed practically no changes in conjugated dienes formation during the whole storage period ($p=0.7512$ to 1.0000). OEV accumulated slightly higher amounts of CLA at the fourth ($p=0.0001$) and 16th ($p=0.0019$) days of

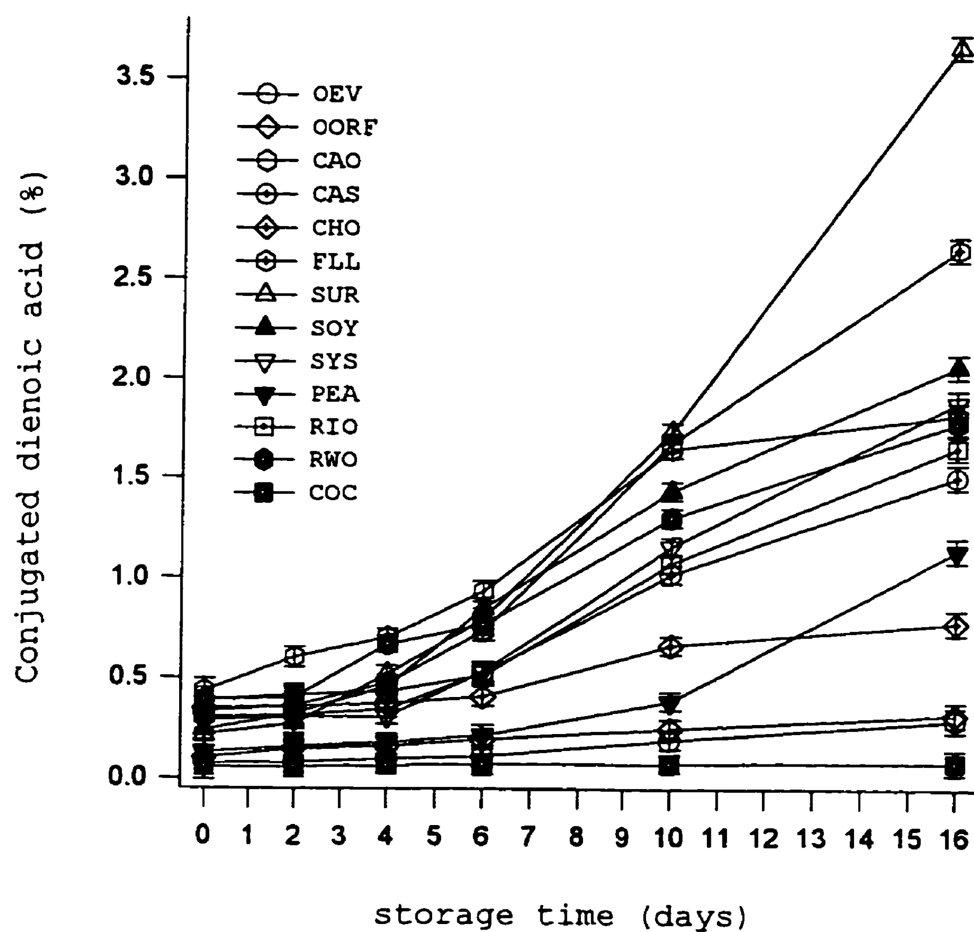

Fig. 4.3.1.2.1. Changes in conjugated dienes during storage without light at 65°C (error bars represent standard error, and for abbreviations see Table 3.1.1).

storage, while ORF showed higher values at the 10th ($p=0.00328$) and 16th ($p=0.00002$) days of storage. The CLA accumulation for both olive oils was then also significantly higher than for corresponding fresh oils ($P<0.05$).

For several other oils, CAS, CHO, SYS, PEA, RIO and RWO, no significant differences for the content of conjugated dienes before the second and fourth days of storage ($P<0.05$) were found, but after that day of storage the values increased at higher rates. CAO showed the highest content of conjugated dienes among all oils until the 10th day of storage, in which the content of these compounds in CAO showed no significant difference from SUR ($p=0.1139$) and FLL ($p=0.0569$).

As found for PV accumulation, SUR and FLL, which had the highest amount of PUFA, exhibited the fastest formation of CLA in the final day of storage.

During Schaal Oven storage with light exposure at 35°C, conjugated diene formation did not always follow the same trends as observed for peroxide accumulation (Fig. 4.3.1.2.2).

Different trends were observed for OEV and PEA in the rate of formation of these components. During storage with light exposure OEV showed the fastest oxygen uptake (see 4.2.6.2) and PV accumulation (Fig. 4.3.1.1.2), whereas the content of CLA formation remained low. Similar trends for ORF and COC were observed for this measurement. These oils exhibited higher stability than did OEV during storage with light exposure (see 4.2.6.2). On the 12th day of storage OEV

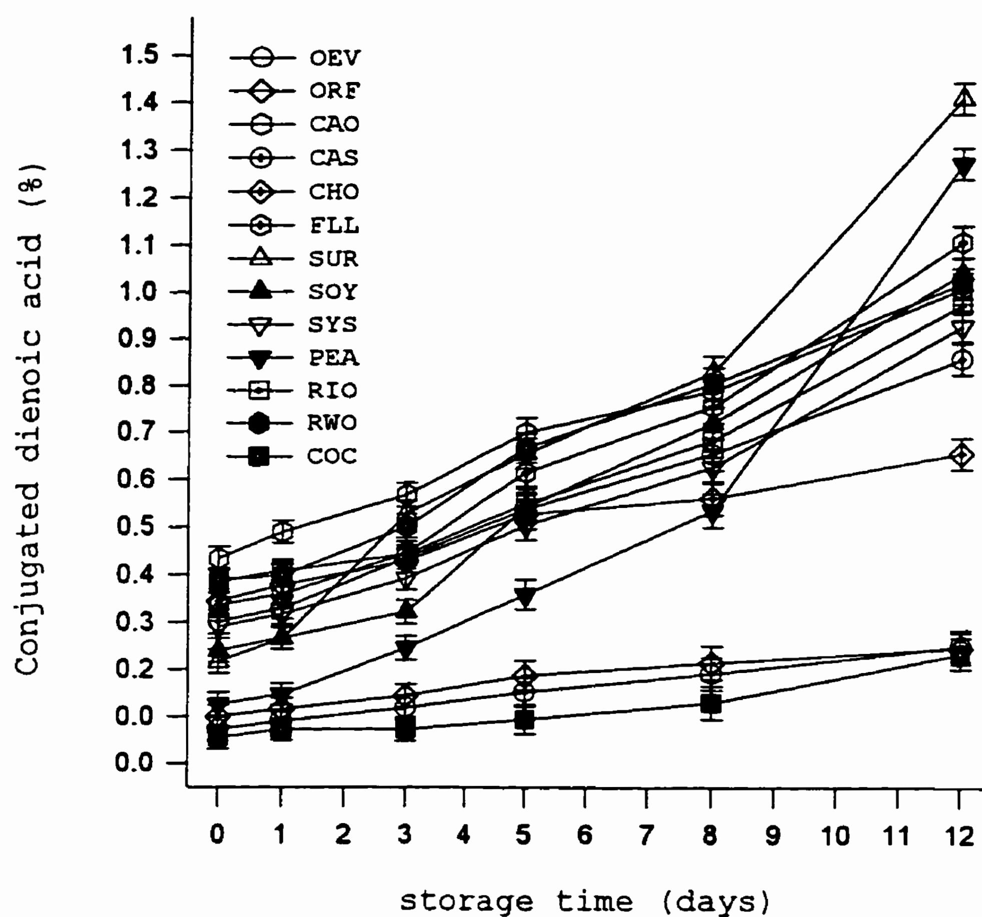

Fig. 4.3.1.2.2. Changes in conjugated dienes during storage with light at 35°C (error bars represent standard error, and for abbreviations see Table 3.1.1).

showed no significant differences in the content of conjugated dienes compared to ORF (p=0.7512) and COC (p=0.1173).

Conjugated dienoic acids are formed by a shift in the position of the double bond(s) during oxidation of PUFA (White, 1995). Both OEV and ORF exhibited very high content of MUFA (about 75%), but a low contribution of linoleic and only traces of linolenic acid (Table 4.1.1). COC showed a similar content of CLA at the 12th day of storage with light exposure compared to olive oils, while the former oil contained only 7.5% of MUFA and traces of PUFA (Table 4.1.1).

At the end of the storage period the amount of CLA in PEA reached significantly higher values (p=0.00001) than in FLL, but there were lower amounts of these compounds than in SUR.

4.3.1.3. Volatiles

Twenty-seven compounds were identified in the stored oils. These compounds were the major volatiles present in all oils, and the sum of them was presented as the total amount of volatiles (TAV).

The changes in the total amount of volatiles in oils stored without light followed trends similar to those observed for PV and conjugated dienes, which was expressed by the good correlation between these measurements (Table 4.3.1.3.1). An exception was with coconut (COC) oil, which showed poor correlation between these measurements.

Table 4.3.1.3.1. Coefficient correlation (r^2) between measurements of peroxide value (PV), conjugated dienoic acids (CLA) and total amount of volatiles (TAV) during storage without light (65°C).

OILS[1]	PV x CLA	PV x TAV	CLA x TAV
OEV	0.98	0.97	0.92
ORF	0.99	0.82	0.83
CAO	0.98	0.99	0.96
CAS	0.97	0.96	0.97
CHO	0.94	0.99	0.92
FLL	0.99	0.98	0.96
SUR	0.98	0.98	0.99
SOY	0.99	0.97	0.96
SYS	0.99	0.99	0.99
PEA	0.99	0.99	0.99
RIO	0.98	0.99	0.98
RWO	0.98	0.96	0.95
COC	0.65	0.21	0.69

[1]Abbreviations see Table 3.1.1.

As was observed for PV and CLA, oils that exhibited lower stability also showed higher amounts of total volatiles formation. CAO presented the highest content of TAV until the 14th day of storage, while SUR produced the highest amounts at the end of storage (Fig. 4.3.1.3.1).

The most stable oils, COC, OEV and ORF showed a lower rate of volatiles formation, and COC exhibited the lowest content during the whole storage period.

The major volatiles detected at the end of storage without light are presented in Table 4.3.1.3.2. Pentane, hexanal and decadienal were the major compounds found in

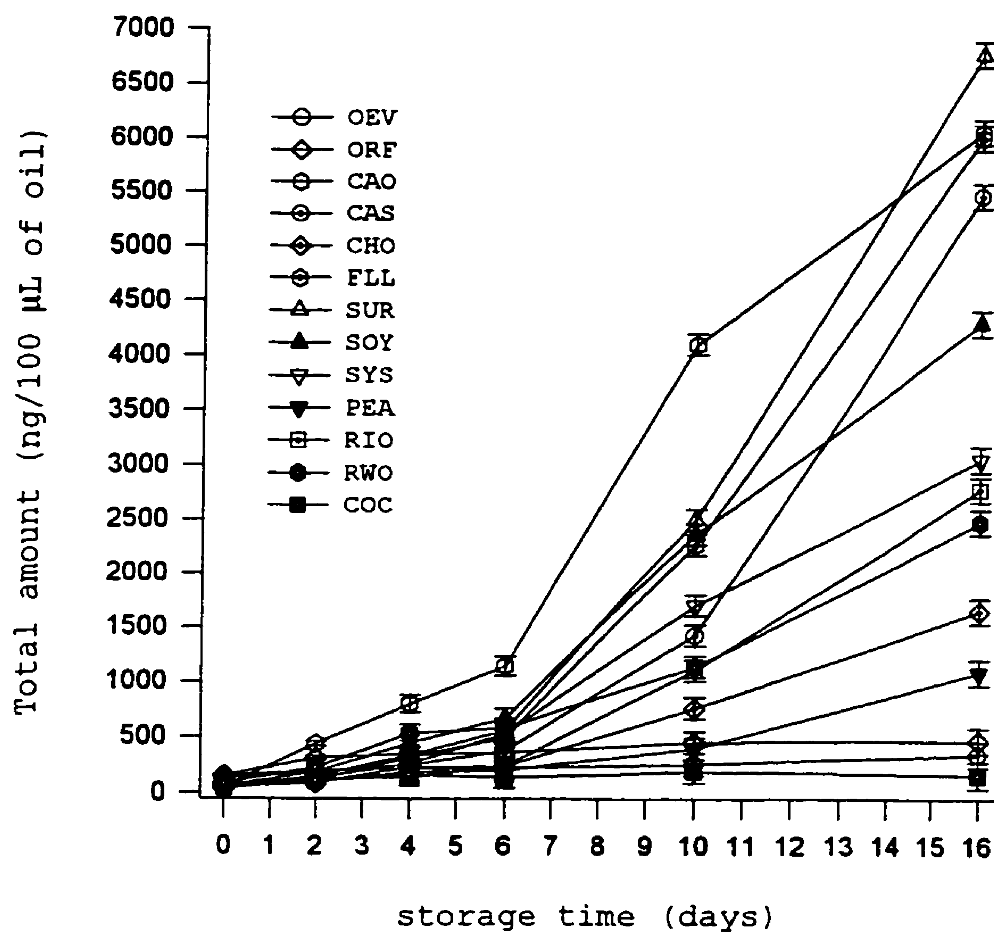

Fig. 4.3.1.3.1. Changes of total volatiles during storage without light at 65°C (error bars represent standard errors, and for abbreviations see Table 3.1.1).

Table 4.3.1.3.2. Volatiles[1] formed up to the 16th day of storage of oils[2] without light at 65°C (%).

Oils	Prop	Pent	Hexa	Hexl	Hept	Octe	Nona	Dece	Unde	Deca
OEV	1.65	16.71	8.70	12.54	1.47	7.92	18.72	3.69	2.33	5.72
ORF	1.55	17.64	8.08	13.46	1.41	6.12	17.93	3.50	2.09	6.51
CAO	7.18	8.31	8.30	14.87	7.01	4.32	5.90	4.58	4.66	17.14
CAS	7.91	11.01	9.17	15.73	5.93	3.41	5.38	4.09	4.44	16.33
CHO	12.04	9.11	9.22	12.24	9.05	3.73	11.68	4.46	2.25	7.22
FLL	1.45	22.58	2.07	23.43	0.98	3.30	1.69	0.63	7.06	20.80
SUR	1.42	21.01	1.88	22.46	0.92	3.18	1.69	0.48	7.91	23.63
SOY	4.54	23.23	4.41	14.74	3.21	2.97	1.82	0.62	8.46	23.09
SYS	5.41	18.53	6.20	15.26	4.02	3.19	2.58	0.91	7.13	20.57
PEA	0.99	12.36	3.27	23.39	1.33	2.71	10.53	2.62	6.10	13.86
RIO	1.33	19.11	3.56	21.68	1.42	4.54	4.16	1.86	5.04	16.09
RWO	1.66	11.62	2.75	21.30	1.28	5.53	5.00	2.44	5.85	18.16
COC	1.35	9.41	9.63	17.78	1.10	8.40	17.94	1.13	1.57	0.75

[1]Compounds which presented more than 5% in at least one oil; [2]abbreviations see Table 3.1.1; Prop= propenal; Pent= pentane; Hexa= hexane; Hexl= hexanal; Hept= heptadienal; Octe= octenal; Nona= nonanal; Dece= decenal; Unde= undecenal; Deca= decadienal.

almost all oils, while propenal, hexane and nonanal were present in relatively high proportion in some oils. These compounds are formed mainly from the decomposition of linoleic acid hydro-peroxides (hexanal, pentane, decadienal), oleic acid-hydro peroxides (nonanal) and linolenic acid hydro-peroxides (propenal, heptadienal) (Przybylski & Eskin, 1995).

Nonanal was the major compound detected in olive oils, and also one of the major volatiles found in CHO and COC oils. High oleic canola and olive oils exhibited the highest content of oleic acid among all stored oils (Table 4.1.1). Coconut oil contained only 7.5% oleic acid, but this acid was still

observed at a higher proportion than linoleic acid (1.8%).

Canola and soybean oils, which contained 4.9 to 10.1% linolenic acid, showed the highest proportion of propenal and heptadienal in volatiles. For oils where the major fatty acid was linoleic acid, a higher proportion of pentane, hexanal and decadienal was observed.

During storage with light exposure, the total amount of volatiles followed a pattern similar to that observed for conjugated dienes (Fig. 4.3.1.3.2). A high correlation was found between these two measurements, while in general a lower correlation was found with peroxide value, when compared to storage without light (Table 4.3.1.3.3). An exception again was with COC, which showed a better correlation between these measurements during this storage condition than during storage without light.

Table 4.3.1.3.3. Coefficient correlation (r^2) between measurements of peroxide value (PV), conjugated dienoic acids (CLA) and total amount of volatiles (TAV) during storage with light (35°C).

OILS[1]	PV x CLA	PV x TAV	CLA x TAV
OEV	0.59	0.74	0.95
ORF	0.96	0.91	0.92
CAO	0.94	0.89	0.98
CAS	0.99	0.97	0.96
CHO	0.95	0.75	0.91
FLL	0.99	0.98	0.99
SUR	0.99	0.97	0.98
SOY	0.95	0.95	0.93
SYS	0.99	0.76	0.79
PEA	0.95	0.99	0.94
RIO	0.99	0.90	0.88
RWO	0.98	0.88	0.90
COC	0.99	0.95	0.93

[1]Abbreviations see Table 3.1.1.

High oleic canola (CHO) showed the lowest while regular canola (CAO) exhibited the highest amount of total volatiles after the seventh day of storage (Fig. 4.3.1.3.2). At the end of storage the olive oils (OEV and ORF) exhibited higher amounts of total volatiles than did CHO, but were still lower than other oils, including coconut (COC) oil.

Similarly to what was found with conjugated dienes, olive oils (OEV and ORF) showed a small rate of TAV formation during storage compared to other oils, even though these oils showed the fastest oxidation under this storage condition as measured

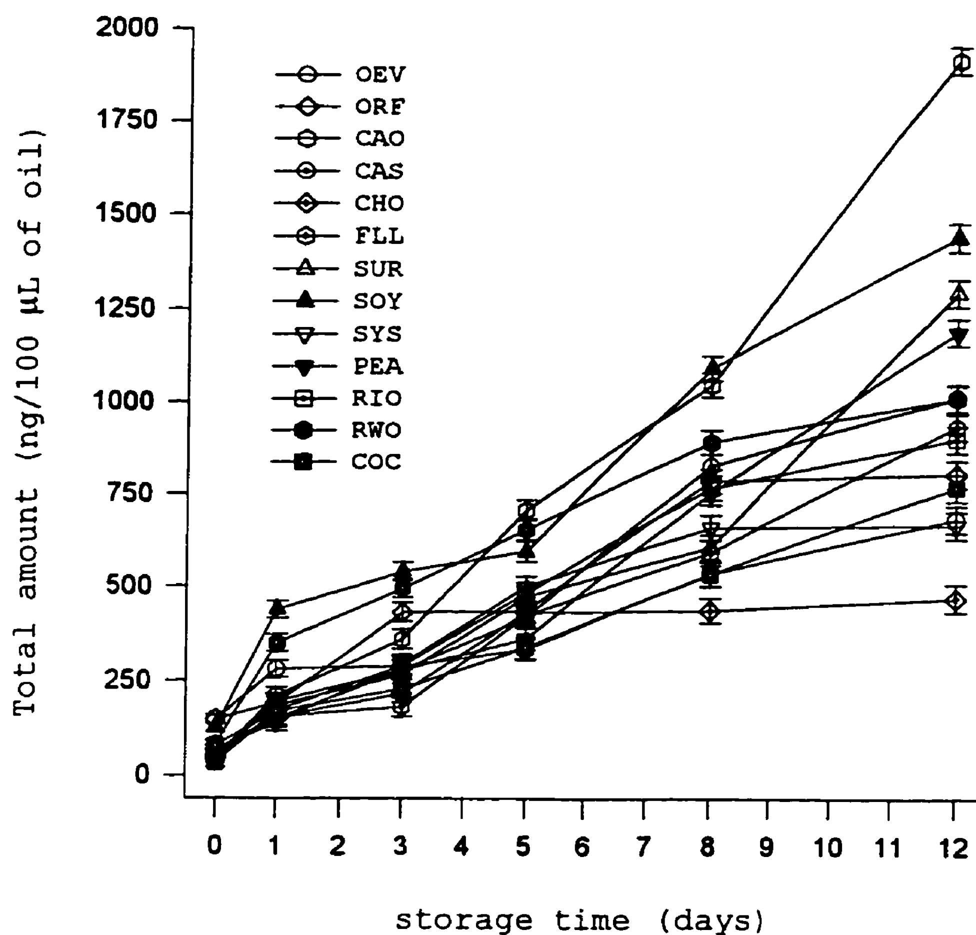

Fig. 4.3.1.3.2. Changes of total volatiles during storage with light at 35°C (error bars represent standard errors, and for abbreviations see Table 3.1.1).

by oxygen consumption (see 4.2.6.2). Coconut (COC) oil, with the lowest content of conjugated dienes and peroxide values, demonstrated a higher amount of total volatiles than did CHO and olive oils (OEV and ORF) at the end of storage, in spite of its low content of unsaturated fatty acids.

The major compounds formed after the 12th day of storage are presented in Table 4.3.1.3.4. Olive oils showed a higher proportion of hexanal, heptenal and decenal than during storage without light. The contribution of these compounds among total volatiles was higher in OEV than in ORF, together with a higher oxidation level of this oil. The higher proportion of these compounds in olive oils when stored with light exposure could be explained by the formation of heptenal from the photo-oxidative decomposition of linoleic acid hydro-peroxides, decenal from oleic acid hydro-peroxides, and finally hexanal from the decomposition of linoleic acid hydro-peroxides and also from cyclic-hydroperoxides formed from this acid (Przybylski & Eskin, 1995).

Propenal, heptenal, pentane and hexane were present in a higher proportion during storage with light while hexanal and decadienal had a lower contribution among canola oils volatiles. The higher proportion of propenal could be an effect of the oxidative and/or photo-oxidative decomposition of linolenic acid hydro-peroxides, whereas the decomposition of cyclic hydro-peroxides of linolenic acid can not be excluded. The same observations could be extended to pentane,

which is formed by a similar pathway (Przybylski & Eskin, 1995). In OEV and CAO the presence of sensitizers, which are usually responsible for photo-oxidative deterioration, were observed, while in ORF, CAS and CHO pigments were not detected.

Table 4.3.1.3.4. Volatiles[1] formed up to the 12th day of storage of oils[2] with light exposure at 35°C (%).

Oils	Prop	Pent	Hexa	Hexl	Hepte	Nona	Dece	Deca
OEV	2.34	13.69	2.29	19.90	9.55	10.23	14.03	2.42
ORF	2.76	19.71	5.00	15.80	5.34	15.13	10.96	2.70
CAO	14.20	15.47	20.41	9.83	6.43	5.15	3.15	4.77
CAS	10.48	12.64	25.93	10.62	5.75	8.08	2.60	4.41
CHO	15.17	8.34	22.51	10.08	2.78	12.49	2.29	2.16
FLL	2.63	16.63	5.44	17.83	11.90	3.58	0.54	8.03
SUR	1.94	26.15	2.19	23.11	10.76	2.77	0.82	10.54
SOY	10.77	14.40	5.47	11.68	19.65	3.06	0.77	6.07
SYS	5.59	18.54	12.02	18.67	5.57	5.67	1.31	5.53
PEA	1.38	18.18	2.68	19.76	16.51	5.66	2.73	6.59
RIO	3.49	21.62	4.00	15.81	13.76	6.21	3.82	7.05
RWO	2.99	23.17	7.79	18.18	10.59	5.60	1.48	5.82
COC	0.92	7.63	4.92	28.40	1.51	26.96	4.29	1.22

[1]Compounds which presented more than 5% in at least one oil; [2]abbreviations see Table 3.1.1; Prop= propenal; Pent= pentane; Hexa= hexane; Hexl= hexanal; Hepte= heptenal; Nona= nonanal; Dece= decenal; Deca= decadienal.

Similarly, SOY showed higher contributions of propenal and heptenal than did SYS. Both oils contained higher amounts of these compounds when stored with light exposure than in oils stored without light.

In coconut (COC), nonanal and hexanal were the major volatile compounds in light and both compounds were observed at higher amounts than during storage without light. FLL, SUR, RIO and RWO also showed a higher contribution of heptenal and less decadienal when stored with light compared to storage without light.

4.3.2. Changes in oil composition

4.3.2.1. Fatty acids

Small changes in fatty acid composition for all stored oils and in both storage conditions were observed. The relative contribution of oleic acid increased, while the relative contribution of linoleic and linolenic acid decreased at the same time. An exception was COC where a reduction of oleic acid contribution (Table 4.3.2.1) was also found. When comparing oils from the same genetic background, the reduction in the contribution of linoleic and/or linolenic acids was higher for oils with a faster oxidation rate for both storage conditions.

The reduction in the contribution of linoleic and linolenic acids followed different patterns in oils with different genetic make-ups. In olive oils, the reduction in the contribution of linoleic acid was higher than for linolenic acid. The initial contribution of linolenic acid in these oils was only about 0.7% and showed practically no changes during storage. The contribution of linoleic acid

went down at a higher proportion in OEV (rate of disappearance of 12.8×10^{-2}%/day) than in ORF (rate of disappearance 9.8×10^{-2}%/day) when stored with light, while the opposite was observed during storage in the absence of light (Table 4.3.2.1). These results were related to the faster oxidation rate of OEV compared to ORF under light exposure, and the faster oxidation rate of ORF compared to OEV during storage without light (see 4.2.6.1 and 2). But both oils oxidized faster during storage with light than during storage without light, and they also showed a higher rate of disappearance during storage with light.

Table 4.3.2.1. Rate of disappearance [(%/day)$\times 10^{-2}$] of unsaturated fatty acids during storage.

OILS[1]	Absence of light[2]			Light exposure[3]		
	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
OEV	-	3.2	-	-	12.8	-
ORF	-	4.8	-	-	9.8	-
CAO	-	7.1	13.43	-	6.5	11.3
CAS	-	6.7	9.5	-	4.9	7.6
CHO	-	3.3	5.3	-	3.3	6.3
FLL	-	9.1	2.8	-	10.3	3.2
SUR	-	13.6	-	-	16.0	-
SOY	-	5.1	10.3	-	4.2	10.2
SYS	-	4.4	9.6	-	3.2	7.8
PEA	-	9.8	-	-	10.0	-
RIO	-	9.1	0.7	-	8.2	2.1
RWO	-	9.5	0.9	-	10.3	1.4
COC	0.3	0.5	-	2.5	4.6	-

[1]Abbreviations see Table 3.1.1; [2]storage for 16 days at 65°C; [3]storage for 12 days at 35°C.

Even though OEV showed the fastest rate of oxidation among all oils during storage with light exposure (see 4.2.6.2), the rate of disappearance of PUFA was lower than in CAO, FLL, SOY and SUR. But, in the same storage condition, oleic acid increased at a lower rate in OEV ($7.3x10^{-2}$%/day) than for these other oils ($8.7x10^{-2}$ to $17.9x10^{-2}$%/day).

Low linolenic flax (FLL) and rice bran oils (RIO and RWO), which contained 1.0 to 1.9% linolenic acid, showed trends in fatty acid changes similar to those observed in olive oils, where linoleic acid showed a faster rate of oxidation than did linolenic acid.

Canola (CHO, CAS and CAO) and soybean oils (SOY and SYS), with 4.9 to 10.1% linolenic acid, showed that this acid disappeared at a faster rate than did linoleic acid, in both storage conditions. The fastest rate of oxidation for CAO was observed during storage without light, and was also related to the highest changes in PUFA among oils stored at Schaal Oven test conditions. Among other canola oils, CAS showed higher changes than did CHO. A similar pattern of changes was observed for SOY and SYS.

Coconut oil (COC) with 1.8% linoleic acid, exhibited a higher rate of disappearance of linoleic than oleic acids. This oil was composed of 7.5% oleic acid and 1.8% linoleic acid.

These results demonstrated that the higher unsaturated fatty acids oxidized at a lower rate than did acids with lower

unsaturation when the contribution was below 2.0%. An exception was COC oil, where the contribution of linoleic acid was at lower levels, but the content of oleic acid was also at lower level in this oil.

4.3.2.2. Triacylglycerol species

The triacylglycerols showed practically no changes during both storage conditions. The variation in the relative percentages of the different triacylglycerol species were within the method error. The error in the determination for different molecular species was different because it was dependent on their relative concentration, but the standard deviation was between 0.04 and 2.35 for all molecular species.

The same peaks observed in the fresh oils were also present after the end of storage. These results were observed even from those molecular species present in low amounts, and formed mainly by polyunsaturated fatty acids, such as LnLnL in soybean oils, LnLL and LnLnO in canola oils, LLL and LnLO in rice bran oils, and LnOO in olive oils.

For several oils a small reduction in the polyunsaturated fatty acids occurred during storage (Table 4.3.2.1). As stated by Labuza (1971), small changes in fatty acids are sufficient to impart an objectionable degree of oxidation in oils and fats. However, these small changes in fatty acids were apparently not observed in any specific molecular specie. Therefore, these small changes in the total fatty acids were

probably distributed in the different molecular species that contained these specific fatty acids. As an example, linoleic acid decreased 2.18% (relative amount) in SUR stored without light, but it was not observed at the same reduction in the molecular specie LLL under the same conditions. Therefore, this reduction in linoleic acid probably occurred in the different triglycerides species that contained linoleic acid, such as LLO, LLP, LOO, LLS, POL, PPL, SOL, PLS and SLS, and a small decrease in this acid was difficult to observe. The relative increase in oleic acid was also not observed in relative increments of OOO. But oleic acid was present in many triglycerides species and its relative changes were probably spread among them. Also, in many molecular species there was polyunsaturated and oleic acid present, which could counterbalance the reduction in linoleic acids and/or linolenic with increments in oleic acid. The same could be expected for the different oils.

Therefore, these results suggested that deeper oxidation probably would be required to see differences in the triacylglycerol species, and/or maybe measurements of oxidized triacylglycerols could be more efficient to identify changes instead of measurements of normal triacylglycerol species.

4.3.2.3. Tocopherols and tocotrienols

The disappearance of tocopherols and tocotrienols during storage without light is presented in Table 4.3.2.3.1. The

rate of disappearance (ppm/hr) was calculated for the first 96 hours of storage, because after that time α-tocopherol was completely depleted in several stored oils.

Table 4.3.2.3.1. Depletion rate of tocopherols and tocotrienols during the first 96 hours of storage without light at 65°C (ppm/hr).

OILS[1]	α-tph	α-ttr	γ-tph	β-ttr	δ-tph	Total
OEV	1.11	-	-	-	-	1.11
ORF	0.75	-	-	-	-	0.75
CAO	1.18	-	0.60	-	-	1.77
CAS	1.00	-	0.53	-	-	1.53
CHO	0.88	-	0.13	-	-	1.01
FLL	-	0.40	0.18	-	-	0.58
SUR	1.27	-	-	-	-	1.27
SOY	0.71	-	1.04	-	0.50	2.24
SYS	0.42	-	0.79	-	0.55	1.75
PEA	0.44	-	0.06	-	-	0.49
RIO	0.62	-	0.07	0.11	-	0.80
RWO	0.87	-	0.09	0.14	-	1.09
COC	-	0.16	-	-	-	0.16

[1]Abbreviations see Table 3.1.1; tph= tocopherol; ttr= tocotrienol.

When comparing oils with the same genetic make-up, the total amount of tocopherols and tocotrienols decreased faster in the oils with higher oxidation rates, such as CAO and RWO oils. Among olive oils, in the first 96 hours OEV showed better stability than did ORF (see 4.2.6.1). However, depletion of α-tocopherol in OEV was almost twice as fast as in ORF. SOY and SYS exhibited a similar oxidation rate during

storage without light (see 4.2.6.1), but the rate of total tocopherols depletion was higher in SOY than in SYS.

When comparing oils from different genetic backgrounds, different trends in these changes were observed. SOY exhibited a higher depletion rate of total tocopherols at 4.5ppm/hr, than did CAO, at 3.5 ppm/hr and SUR, at 2.5 ppm/hr, but both CAO and SUR oxidized at a faster rate than did SOY (see 4.2.6.1). The total content of tocopherols in SOY was 3.6 and 4.9 times higher than CAO and SUR, respectively. SUR oxidized faster than did FLL (see 4.2.6.1) and also the depletion rate of tocopherols and tocotrienols was higher in this oil than in FLL. Similar trends were observed when comparing SUR to rice bran oils. COC was the most stable oil and also had the lowest depletion rates of α-tocotrienol, even when the initial amount of tocotrienols was only 33.6ppm.

The different isomers of tocopherols and tocotrienols showed different depletion rates, and this was related to their amounts in the oils. In general, when α-tocopherol was present in concentration over 100ppm, it was depleted at faster rates than were the other isomers of tocopherols and tocotrienols. This tocopherol was depleted at faster rates than was γ-tocopherol in rice bran, canola and peanut oils. In fresh rice bran oils, α-tocopherol was present at about 150ppm while γ-tocopherol was present at about 70ppm. In fresh canola and peanut oils, the concentration of γ-tocopherol was 1.3 to 2.6 times higher than was the

concentration of α-tocopherol, and the amounts of α-tocopherol in these oils were also higher than 100ppm.

In soybean oils γ-tocopherol was depleted at faster rates than was α-tocopherol. In those oils the initial amount of γ-tocopherol was 8 to 10 times higher than was the amount of α-tocopherol (Fig. 4.1.6.1). Soybean oils also contained δ-tocopherols at a concentration of 336 to 390ppm. The depletion rate for α-tocopherol was higher than that of δ-tocopherol in SOY, while in SYS the depletion rate was the reverse. But the initial content of α-tocopherol was 116ppm in SOY and only 67ppm in SYS.

Rice bran oils contained similar amounts of β-tocotrienol, about 145ppm, and α-tocopherol, about 150ppm. Again, α-tocopherol was depleted at a faster rate than was β-tocotrienol, which was similar to what was observed for its depletion when compared to other isomers in the other oils.

When the amount of α-tocopherol was below about 50ppm other isomers of tocopherols and tocotrienols started to deplete at faster rates. In order to compare the depletion rate among isomers of tocopherols and tocotrienols a rate of depletion was calculated based on the first 10 days of storage (240 hours). This depletion rate covered the storage time when α-tocopherol was present, and also when α-tocopherol was completely depleted and/or present at low concentration (Table 4.3.2.3.2).

Table 4.3.2.3.2. Depletion rate of tocopherols and tocotrienols during the first 10 days of storage of oils[1] without light at 65°C (ppm/hr).

Isomers	FLL	SOY	SYS	RIO	RWO
α-tocotrienol	0.87	-	-	-	-
β-tocotrienol	-	-	-	0.29	0.21
γ-tocopherol	0.35	2.19	1.36	0.15	0.19
δ-tocopherol	-	0.61	0.89	-	-

[1]Abbreviations see Table 3.1.1.

Soybean oils contained 2.0 to 2.5 times more γ-tocopherol than δ-tocopherol, and this tocopherol was depleted at faster rates than was δ-isomer.

Low linolenic flax (FLL) contained α-tocotrienol, 222ppm, and 1.6 times less γ-tocopherol, at 135ppm. In this oil, α-tocotrienol was depleted at a faster rate than was γ-tocopherol.

In rice bran oils, β-tocotrienol was depleted at faster rates than was γ-tocopherol, where the former was detected at about 150ppm while the γ-isomer of tocopherol was present at 1.9 times a lower amount, about 80ppm.

Similar trends were observed in oils stored with light. The depletion rates were calculated for the first 72 hours of storage (Table 4.3.2.3.3). Tocopherols and tocotrienols were

depleted at faster rates when oils were stored with light exposure than without light. During this storage experiment oils showed almost no initiation period, and this also caused tocopherols and tocotrienols to be depleted at faster rates.

Table 4.3.2.3.3. Depletion rate of tocopherols and tocotrienols during the first 72 hours of storage of oils with light at 35°C(ppm/hr).

OILS[1]	α-tph	α-ttr	γ-tph	β-ttr	δ-tph	Total
OEV	2.23	-	-	-	-	2.23
ORF	1.48	-	-	-	-	1.48
CAO	1.47	-	0.74	-	-	2.21
CAS	1.38	-	0.68	-	-	2.06
CHO	1.04	-	0.31	-	-	1.35
FLL	-	0.62	0.19	-	-	0.81
SUR	1.53	-	-	-	-	1.53
SOY	1.19	-	1.48	-	1.00	3.66
SYS	0.51	-	0.92	-	0.80	2.22
PEA	0.80	-	0.13	-	-	0.93
RIO	0.10	-	-	0.05	-	1.00
RWO	0.99	-	0.06	0.11	-	1.10
COC	-	0.38	-	-	-	0.38

[1]Abbreviations see Table 3.1.1; tph= tocopherol; ttr= tocotrienol.

Virgin olive (OEV) oil oxidized at the fastest rate during storage with light exposure, and tocopherols were depleted twice as fast compared to storage without light. Similar patterns were found for other oils.

In parallel to what was observed during storage without light, α-tocopherol was depleted at a higher rate than was γ-

tocopherol in canola oils, and at a higher rate than was δ-tocopherol in SOY oil, and finally at a higher rate than was β-tocotrienol in rice bran oils.

In table 4.3.2.3.4 the depletion rates of some isomers of tocopherols and tocotrienols, with the exception of α-tocopherol, are presented. Similarly to what was observed during storage without light, α-tocotrienol was depleted at a faster rate than was γ-tocopherol in FLL, γ-tocopherol depleted at a faster rate than did δ-tocopherol in soybean oils, and β-tocotrienol was depleted at a faster rate than was γ-tocopherol in rice bran oils.

Table 4.3.2.3.4. Depletion rate of tocopherols and tocotrienols during the first 8 days of storage of oils[1] with light at 35°C(ppm/hr).

Isomers	FLL	SOY	SYS	RIO	RWO
α-tocotrienol	0.64	-	-	-	-
β-tocotrienol	-	-	-	0.21	0.41
γ-tocopherol	0.32	2.54	1.22	0.19	0.23
δ-tocopherol	-	0.72	0.72	-	-

[1]Abbreviations see Table 3.1.1.

4.3.2.4. Phenolic compounds

Phenolic compounds were determined only for olive oils, because these oils contained measurable amounts of these compounds.

The eight major phenolic compounds present in olive oils were identified, and the sum was expressed as the total phenolics content (TPC). The changes in the total content of phenolics for OEV and ORF under both storage conditions are presented in Fig. 4.3.2.4.

During storage without light, the depletion of total phenolics was higher for OEV than ORF, with losses of 37.90% and 18.55%, respectively, for the whole storage period. OEV showed better stability than did ORF in this condition when measured by oxygen consumption (see 4.2.3), but depleted a higher amount of TPC than did ORF.

In the first 96 hours of storage, a period where α-tocopherol was still present at concentrations over 50ppm in these oils, the rate of depletion of total phenolics in ORF was lower than in subsequent periods. In this period, the losses of total phenolics were 79.79ppb/hr and 1.46ppb/hr for OEV and ORF, respectively, while after 96 hours of storage these losses were 36.70ppb/hr and 3.57ppb/hr, respectively.

During storage with light exposure, OEV also showed higher losses in TPC than did ORF, 40.73% vs 31.03%. But, in this storage condition the higher losses of OEV phenolics were also related to faster oxidation of this oil (see 4.2.3).

During both storage conditions, the losses in total phenolics were higher in OEV than in ORF, but the differences in losses between the two storage conditions were opposite.

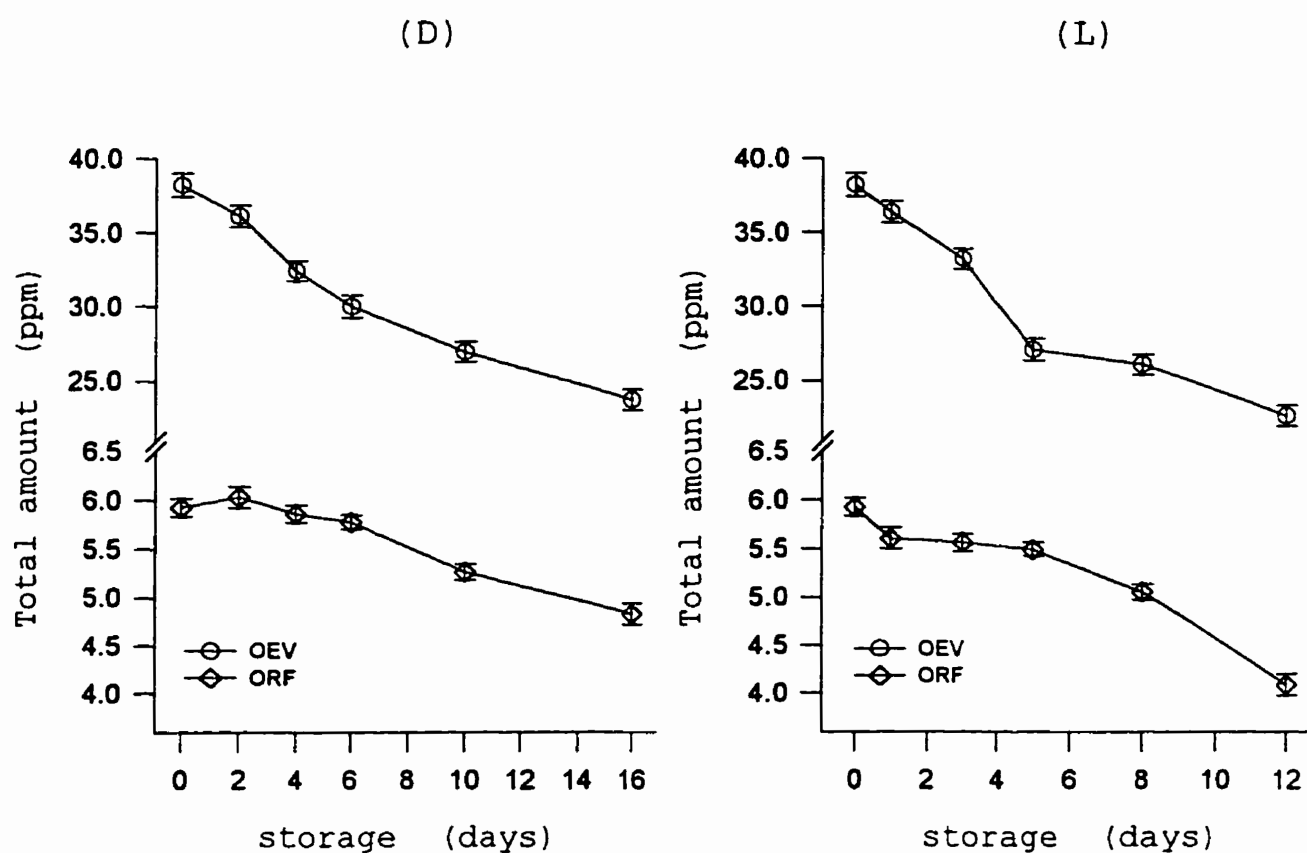

Fig. 4.3.2.4. Changes in total content of phenolic compounds in olive oils during storage without(65°C)(D) and with light(35°C)(L)(error bars represent standard errors, and for abbreviations see Table 3.1.1).

During the initial period of storage both olive oils showed higher losses of phenolics when stored with light exposure. The depletion rates for the first 72 hours of storage with light exposure were 139.7ppb/hr and 10.0ppb/hr for OEV and ORF, respectively.

Among the individual phenolics detected in OEV, the rate of disappearance (ppb/hr) when stored without light decreased in the following order: gentisic (19.9), *o*-coumaric (8.1), protocatechuic (6.7), ferulic (1.2), vanillic (0.9) and *p*-coumaric acids (0.9). Similar trends were observed during storage with light, where the depletion rate (ppb/hr) decreased from gentisic (24.3), o-coumaric (15.4), protocatechuic (10.1), ferulic (1.8), vanillic (1.2) to *p*-coumaric (1.2) acids. The depletion rates for *p*-hydroxybenzoic and ferulic acids were not calculated because they were present at low concentrations and they experienced practically no changes during storage. In ORF low concentrations of practically all phenolic compounds were observed, therefore the depletion rate was not considered.

The rate of depletion of the individual phenolic compounds in OEV was related to the concentration of the specific compound. Gentisic acid was detected as having the highest content; also a higher depletion rate of this compound was observed than for other phenolics.

4.3.2.5. Pigments

The changes in chlorophyll content were measured by the spectrophotometric method (AOCS) in OEV, CAO and SOY, which were the stored oils where this pigment was detected.

The content of chlorophyll decreased during both storage conditions in the above mentioned oils (Fig. 4.3.2.5.1). During the first 240 hours of storage without light, the depletion rate for chlorophyll was 7.0, 0.5 and 0.3 ppb/hr for OEV, SOY and CAO oils, respectively. Among these three oils, OEV showed the highest stability (see 4.2.6.1), while the content of chlorophyll was depleted at a faster rate than in CAO and SOY. Soybean oil (SOY) exhibited a higher stability and a higher depletion rate of chlorophyll than did CAO. When comparing the initial amounts of the chlorophyll in these oils it should be recalled that OEV contained 10 times more than SOY and 60 times more than CAO, while SOY contained 6 times more than CAO.

During the initial 24 hours of storage with light exposure, chlorophyll was depleted at rates of 47.5 and 8.5 ppb/hr for OEV and SOY, respectively. The rate of depletion for CAO could not be calculated because at the first day of storage with light chlorophyll was already undetectable in this oil. Again, under this condition OEV showed the highest rate of chlorophyll depletion, which was directly related to the faster oxidation of this oil (see 4.2.6.2).

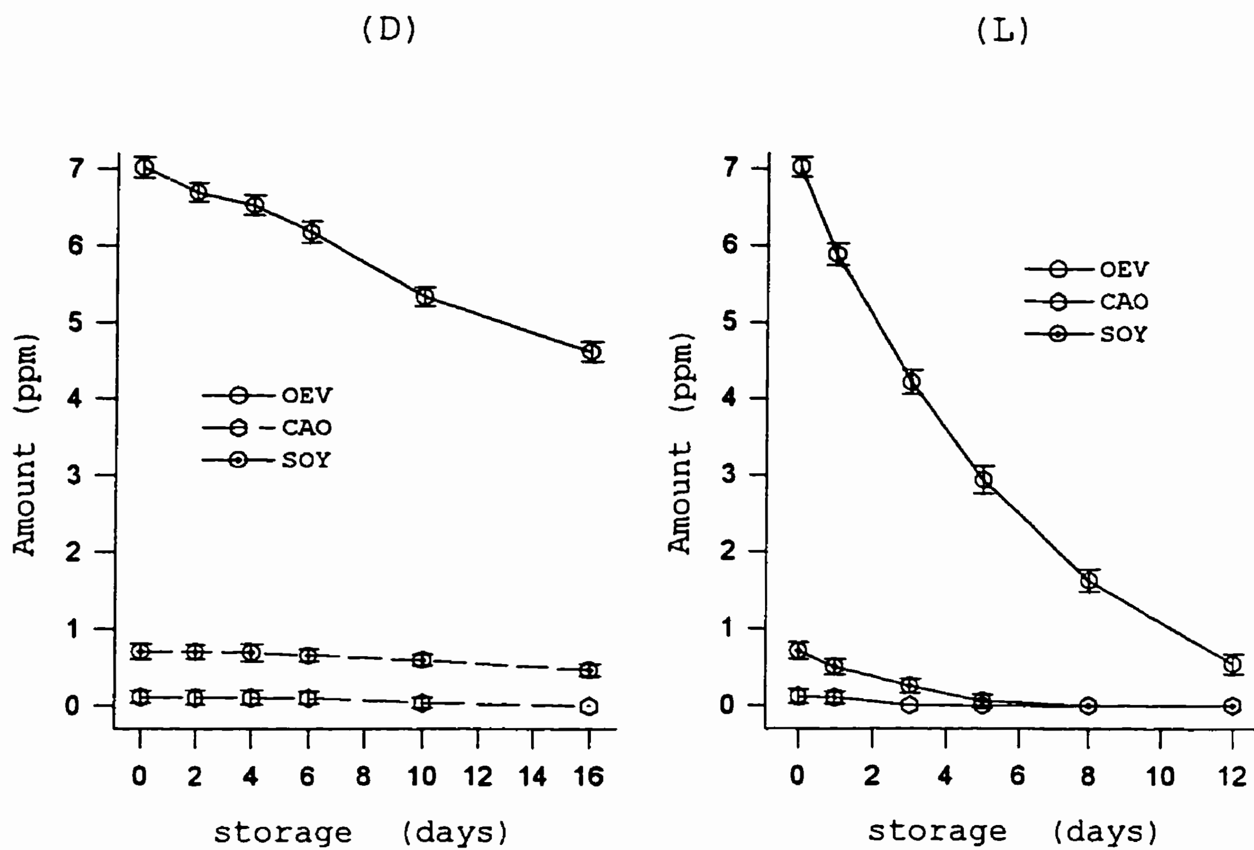

Fig. 4.3.2.5.1. Changes of chlorophyll during storage without(D) (65°C) and with light(L)(35°C)(error bars represent standard errors, and for abbreviations see Table 3.1.1).

When comparing the losses in chlorophyll between the two storage conditions, it was observed that OEV lost 92.37% and 34.15% at the end of storage with and without light exposure, respectively. Chlorophyll disappeared completely in SOY after the eighth day of storage with light exposure, while in storage without light only 34.09% of this compound vanished. No chlorophyll presence was observed in CAO after the first and 12th days of storage with and without light exposure, respectively.

The content of chlorophyll in these oils was depleted at faster rates in the first 72 hours of storage with light than in 96 hours of storage without light. These three oils also showed faster oxidation rates when stored with light than without light (see 4.2.6.1 and 2).

Among stored oils, carotenoids were found only in OEV. The changes in carotenoids during both storage conditions in Fig. 4.3.2.5.2 are presented.

During the first 10 days of storage without light, lutein was depleted at a faster rate than were β- and α-carotene, 8.3, 3.6 and 2.5 ppb/hr, respectively. During the first 8 days of storage with light exposure, the depletion rates were 17.5, 11.3 and 5.4 ppb/hr for these pigments, respectively.

As was observed for chlorophyll, carotenoids were depleted at faster rates during the initial period of storage (72 hrs) with light exposure than without light (48 hrs).

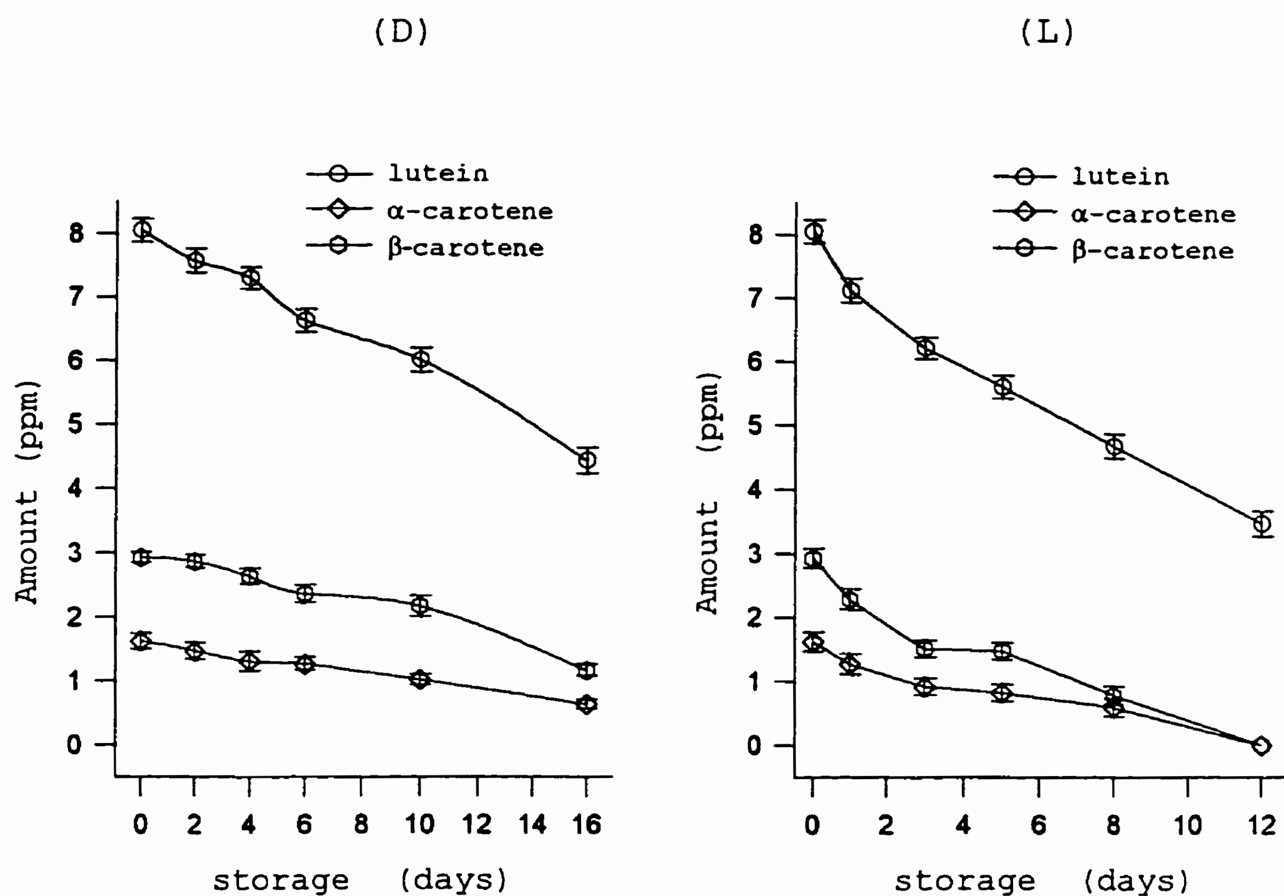

Fig. 4.3.2.5.2. Changes of carotenoids in olive oil (OEV) during storage without (D) (65°C) and with light (L) (35°C) (error bars represent standard errors).

During storage without light the losses were 44.8, 59.9 and 60.4% for lutein, α-carotene and β-carotene, respectively. In the presence of light 56.9% and 100% of lutein and both carotenes were lost, respectively.

4.3.2.6. Sterols

Table 4.3.2.6.1 presents the losses of sterols during storage without light. When comparing oils from the same genetic origin, the higher losses of sterols were associated with faster oxidation of oils. However, OEV showed a higher stability than did ORF, but also showed higher losses of sterols than did ORF. When comparing losses of sterols among oils from different genetic sources, this trend was not always observed. Soybean oils oxidized at faster rates than did rice bran oils (see 4.2.6.1), but sterols changed at a lower rate in soybean oils.

Sitosterol was the major sterol present in all stored oils (Fig. 4.1.7), and also the sterol that showed higher losses during storage, with the exception in canola oils. SUR oil showed the highest losses in this sterol (29.9%), while the losses for other oils were between 2.9 and 10.7%.

Campesterol was the second major sterol present in all stored oils (Fig. 4.1.7). This sterol presented the lowest changes, less than 4.2%, during storage when compared with other phytosterols. Only in olive oil (ORF) was campesterol lost at higher amounts than was stigmasterol.

Table 4.3.2.6.1. Losses of sterols[1] after 16 days of storage without light at 65°C (%).

Oils[2]	Brassicasterol	Stigmasterol	Campesterol	Sitosterol	Total
CAO	18.51	-	1.87	8.74	29.12
CAS	17.23	-	2.23	8.89	28.35
CHO	7.44	-	2.15	6.38	15.07
OEV	-	4.41	4.19	6.10	14.70
ORF	-	2.89	3.75	7.47	14.11
SOY	-	6.33	1.98	7.83	16.14
SYS	-	6.21	2.95	6.44	15.60
RIO	3.98	7.47	2.10	9.26	22.81
RWO	4.21	7.98	1.89	10.69	24.77
SUR	4.99	4.31	2.23	29.94	31.94
FLL	6.23	4.98	3.12	10.09	25.23
PEA	-	5.12	2.15	9.57	16.84
COC	-	-	1.15	2.92	4.07

[1]Calculated for sterols present at amounts higher than 10mg/100g; [2]abbreviations see Table 3.1.1.

Stigmasterol was not present in canola oils and there were only traces in COC oil, while this was the third major phytosterol found in the other oils (Fig. 4.1.7). In general, the losses of stigmasterol were higher than losses of brassicasterol and campesterol, but lower than losses of sitosterol. The loss of stigmasterol was between 2.9 to 8.0%, with soybean and rice bran oils showing the highest losses.

Brassicasterol disappeared in higher amounts than did other sterols in canola oils, 7.4 to 18.5%, while its losses were lower than 6.2% in other oils. Fresh canola oils also showed the highest content of brassicasterol, while the other

stored oils contained low amounts of this sterol (Fig. 4.1.7).

Similar results were observed during storage with light (Table 4.3.2.6.2). CAO, OEV, COC, CHO, ORF, SOY, SYS and PEA exhibited higher losses of total sterols when stored with light exposure than without light. The higher losses of total sterols in CAO was due to higher losses of brassicasterol under this condition. The higher losses of sterols for OEV was mainly due to higher losses of sitosterol during storage under light exposure.

Table 4.3.2.6.2. Losses of sterols[1] after 12 days of storage with light at 35°C (%).

Oils[2]	Brassicasterol	Stigmasterol	Campesterol	Sitosterol	Total
CAO	20.31	-	2.14	8.31	30.76
CAS	15.02	-	2.49	7.46	24.97
CHO	9.87	-	1.58	4.27	15.72
OEV	-	4.49	4.31	10.23	22.01
ORF	-	3.75	3.56	6.34	17.02
SOY	-	5.21	2.19	9.34	19.77
SYS	-	4.47	1.73	7.75	17.07
RIO	3.13	10.35	1.13	6.35	20.96
RWO	2.12	15.21	2.01	5.82	22.16
SUR	5.12	3.01	2.83	14.25	25.25
FLL	5.21	4.37	2.23	9.31	21.12
PEA	-	4.72	2.85	7.27	17.63
COC	-	-	1.13	4.08	8.63

[1]Calculated for sterols present at amounts higher than 10mg/100g; [2]abbreviations see Table 3.1.1.

Coconut oil showed lower stability during light exposure than during storage without light, and also higher

losses of sterols were observed. But, for CAS, RIO, RWO, FLL and SUR the losses during storage without light were higher than during storage with light exposure.

Among individual sterols, trends similar as to those during storage without light were observed. An exception was with rice bran oils. In these oils the losses of stigmasterol during storage with light were higher than the losses observed during storage without light.

For some individual sterols different rates of losses in both storage conditions were also observed. Brassicasterol in CAO and CAS, stigmasterol in RIO and RWO, and sitosterol in OEV and SUR, were decomposed at faster rates during the initial period of storage with light exposure compared to without light exposure (Table 4.3.2.6.3). When comparing the depletion rates of these sterols between oils from the same genetic make-up, the oils that showed faster initial oxidation (see 4.2.6.1 and 2) also showed higher rates of depletion during the beginning of storage; these were CAO among canola and RWO among soybean oils.

However, SUR showed the highest rates of sitosterol depletion, but both CAO and OEV were oxidized at faster rates than was SUR. CAO and CAS showed higher depletion rates for brassicasterol than did sitosterol in OEV, but this oil oxidized at a faster rate than did CAO in the initial period of storage with light.

Table 4.3.2.6.3. Depletion rate of sterols during initial periods of storage of the oils[1] (ppm/h).

Phytosterols		OEV	CAO	CAS	SUR	RIO	RWO
Brassicasterol	(D)	-	0.64	0.60	-	-	-
	(L)	-	1.58	1.17	-	-	-
Stigmasterol	(D)	-	-	-	-	0.29	0.35
	(L)	-	-	-	-	0.31	0.38
Sitosterol	(D)	0.16	-	-	1.52	-	-
	(L)	0.95	-	-	1.93	-	-

[1]Abbreviations see Table 3.1.1; (D)storage without light- first 96 hours; (L)storage with light- first 72 hours.

4.4. Application of Neural Networks to predict oil stability

About half of the 33 analysed oils were randomly selected to train the program (training set), while the other oils were used to predict the values related to their stability (testing set). The oil composition was used as input data, while the values from slopes of initiation (k_1) and propagation (k_2), and induction periods (ip) obtained from oxygen uptake measurements were used as output or pattern.

The Artificial Neural Networks (ANNW) program was trained using the standard back-propagation algorithm, and automatically tested each 20 runs while training. The training was stopped after the testing reached a squared error ≤ 0.05. The number of hidden neurons was initially determined by the relation: (inputs + outputs)/2 (Lawrence & Petterson, 1993). During the training the software was set up to automatically input and/or prune neurons after each training section. The learning and smoothing rates were set between 0.08 to 0.1, while the training tolerance was 0.1.

Several combinations of oil components were used to train the program. For each individual group of components, the predictability of k_1, k_2 and ip was done using the oils grouped in the testing set, where each oil was individually fed into the program using the same composition that was previously used to train the ANNW by using the training set.

4.4.1. Oils stored with light

Among oils stored with light exposure, CRN, COR, CRW, PLK AND COC oils had an induction period (ip), while all other oils had only slopes of propagation periods (k_2). In order to uniformize the data, a linear regression line was used for these six oils that had induction period to obtain only one value (k_2) to express their rate of oxidation (Table 4.4.1.1).

Table 4.4.1.1. Slopes of initiation (k_1) and propagation (k_2) lines, and induction period (ip) obtained by oxygen uptake measurements during storage with light at 35°C.

Oils[1]	Previous values			Calculated (k_2) for ANNW
	k_1	k_2	ip(hr)	
COR	-0.030	-0.061	43.5	-0.056
CRN	-0.029	-0.063	57.3	-0.051
CRW	-0.030	-0.059	44.8	-0.054
COC	-0.018	-0.077	162.6	-0.037
PLK	-0.018	-0.081	159.2	-0.040

[1]Abbreviations see Table 3.1.1.

Eighteen oils were initially selected to train the ANNW program (FCO, EPR, CAO, SUR, RBO, SOY, FLL, CTO, CAS, SHO, CAR, ORF, OEV, PLG, COR, PAL, PNT and COC), while the other fifteen oils were used to predict the values of slope of propagation (k_2). The selection was done by randomly taking oils from the different groups formed by cluster analysis,

which were related to different behaviours during stability test. Several groups of components were tested as inputs to verify their ability to predict values of k_2 (Table 4.4.1.2).

Initially all individual components measured in the oils, group L_1 (Table 4.4.1.2), were used as inputs and the values of slopes of propagation (k_2) (Table 4.2.6.2 and 4.4.1.1) were used as outputs (patterns). After that the program was trained; one by one the 15 oils were used to predict the values of k_2. The correlation coefficient (r^2) obtained was 0.70 (Table 4.4.1.3).

Due to the low value of the correlation coefficient found between the predicted value by ANNW and the experimental value obtained from slope of propagation measured by oxygen consumption, another set of variables was selected to retrain the program. The second set used the total amount for each group of components (L_2) (Table 4.4.1.2). The predictability using the second set (L_2) was slightly better (r^2=0.75) than using the first set (L_1) of oil components (Table 4.4.1.3). However, the correlation coefficient still was at unsatisfactory levels. Horimoto *et al.* (1995) stated that for useful prediction by ANNW, r^2 should be over 0.90. Therefore, other sets of components (L_3, L_4 and L_5, Table 4.4.1.2) were individually used to train the program. Based on previous results, these subsequent sets of components was chosen by using the total amount of component classes instead the individual oil components. For each set the values of k_2 were

again predicted by ANNW for each of the 15 oils.

Table 4.4.1.2. Groups of oil components used to train the ANNW.

Groups	Oil components
L_1	All individual FA, FFA, NL, PL, GL, tocopherols, tocotrienols, sterols, metals, pigments and phenolic compounds (see 4.1);
L_2	Total amount of NL, PL, GL, tocopherols and tocotrienols, sterols, chlorophylls, carotenoids, metals, phenolics, SFA, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$;
L_3	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, chlorophyll, metals, sterols and phospholipids;
L_4	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, metals, sterols and phospholipids;
L_5	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, chlorophyll and metals;
L_6	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, chlorophyll and metals, by excluding OEV and PLG and including CHO and OPR oils in the training set;
L_7	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, chlorophylls and metals, using 22 oils in the training set;
L_8	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols and chlorophylls, using 22 oils;
L_9	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and SFA fatty acids, using 22 oils.

FA= fatty acids; FFA= free fatty acids; NL= neutral lipids; PL= phospholipids; GL= glycolipids; SFA= saturated fatty acids; $C_{18:1}$= oleic acid; $C_{18:2}$= linoleic acid; $C_{18:3}$ = linolenic acid.

As can be noticed (Table 4.4.1.3), a slight improvement of the predictability of k_2 was observed using these different sets, but still the best predictability was at r^2:0.81, using the L_4 group of components.

Table 4.4.1.3. Correlation coefficient (r^2) between predicted and experimental values of k_2 and standard error of the estimate (SEE).

Group of oil components	r^2	SEE ($x10^{-3}$)
L_1	0.70	4.9
L_2	0.75	3.3
L_3	0.68	8.5
L_4	0.81	6.9
L_5	0.80	3.0
L_6	0.87	2.9
L_7	0.95	3.1
L_8	0.59	5.6
L_9	0.44	11.7

As an effort to achieve better results the data used to train the program was altered. PLG and OEV were excluded from the training set, because these oils were outliers in total carotenoids and total phenolics compounds, respectively. CHO and OPR were used to replace these oils in the training set. The program was trained again using this new set of oils as inputs (L_6). The results obtained were more satisfactory and the correlation coefficient increased to 0.87 (Table 4.4.1.3).

Lawrence & Petterson (1993) proposed making small changes

in the facts used to train ANNW in order to obtain better predictability. In this study the facts corresponded to the number of oils and the group of components used to train the program. As a new effort to increase accuracy in the predictability, the number of oils in the training set was increased to 22, by the inclusion of RWO, SYB, BOR and CRW oils. With this new set (L_7) better results were obtained, and the predictability of stability based on k_2 was very close to experimental data obtained from oxygen consumption (Fig. 4.4.1, Table 4.4.1.4).

Table 4.4.1.4. Values of k_2 predicted by ANNW and experimental values obtained by oxygen consumption with standard error of the estimate (SEE), using group L_7 as the training set.

Oils[1]	K_2 (O_2 consumption)	K_2 (ANNW)	SEE ($x10^{-3}$)
CAN	-0.0635	-0.0741	5.3
COL	-0.0450	-0.0491	2.1
CRN	-0.0511	-0.0499	0.6
COT	-0.0961	-0.0957	0.2
OEV	-0.1730	-0.1351	18.9
PLG	-0.0120	-0.0131	0.6
PLK	-0.0402	-0.0381	1.0
PEA	-0.0550	-0.0537	0.7
RIO	-0.0420	-0.0425	0.3
SUN	-0.0850	-0.0806	2.2
SYS	-0.0687	-0.0651	1.8

[1]Abbreviations see Table 3.1.1.

Virgin olive (OEV) oil had the higher deviation from the experimental value (SEE= 18.9 x 10^{-3}), while SEE lower than 5.3 x 10^{-3} were found for the other oils. As another approach, the number of components used to predict oil stability was decreased, and two new sets of components were tested (L_8 and L_9), but no successful results were obtained (Table 4.4.1.3)

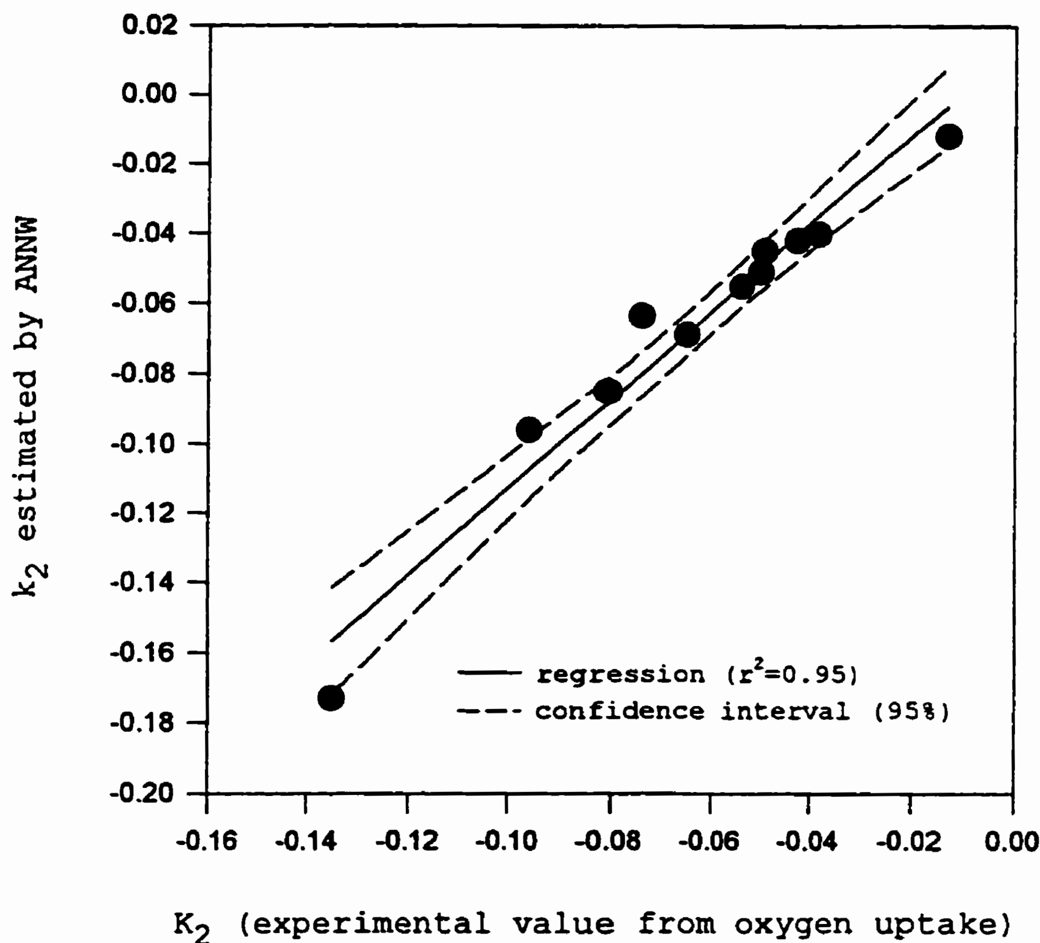

Fig. 4.4.1. Correlation between experimental values of propagation period (k_2) obtained by oxygen consumption during storage with light(35°C) and values of k_2 predicted by ANNW.

4.4.2. Oils stored without light

The last 22 oils used to train the ANNW program with data obtained during storage with light exposure was used as training set for data obtained during storage without light.

Similarly to what was done before, several groups of oil components were used to test the predictability by ANNW (Table 4.4.2.1).

Initially the composition of oils as the total amount of components in each class (D_1) (Table 4.4.2.1) was used as inputs and the values of slopes of initiation (k_1) and propagation (k_2), and values of the induction period (ip) were used at the same time as outputs (three outputs).

Table 4.4.2.1. Groups of oil components used to train the ANNW.

Groups	Oil components
D_1	Total amount of NL, PL, GL, tocopherols and tocotrienols, sterols, chlorophylls, carotenoids, metals, phenolics, SFA, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$;
D_2	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, metals, sterols and phospholipids;
D_3	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total amount of tocopherols and tocotrienols, metals and sterols;
D_4	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total tocopherols and tocotrienols, metals and sterols, using k_1, k_2 and ip, one each time as outputs;
D_5	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total tocopherols and tocotrienols, metals, sterols and phospholipids, using ip as output;
D_6	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$, SFA, total tocopherols and tocotrienols and metals, using ip as output;
D_7	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$,SFA and total tocopherols and tocotrienols, using ip as output;
D_8	$C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and SFA fatty acids, using ip as output;

FA= fatty acids; FFA= free fatty acids; NL= neutral lipids; PL= phospholipids; GL= glycolipids; SFA= saturated fatty acids; $C_{18:1}$= oleic acid; $C_{18:2}$= linoleic acid; $C_{18:3}$ = linolenic acid.

The correlation coefficients (r^2) obtained between the values predicted by ANNW and experimental values from oxygen consumption are presented in Table 4.4.2.2. Due to the low correlation coefficient found for the prediction of k_1, k_2 and

ip, two new data sets (D_2 and D_3) were used in order to find the variables that could give the best predictability, similarly to what was done with data from storage with light exposure. The correlation coefficients and standard error of the estimate (SEE) for the other data sets are also presented in Table 4.4.2.2.

Table 4.4.2.2. Correlation coefficient (r^2) between predicted and experimental values and standard error of the estimate (SEE).

Groups of oil	r^2			SEE ($x10^{-3}$)		SEE
components	k_1	k_2	ip	k_1	k_2	ip
D_1	0.71	0.65	0.70	7.5	9.2	6.7
D_2	0.68	0.57	0.54	6.4	7.3	11.3
D_3	0.63	0.68	0.67	5.1	9.7	8.4
D_4	0.69	0.63	0.91	6.1	5.9	1.5
D_5	-	-	0.95	-	-	3.1
D_6	-	-	0.88	-	-	2.8
D_7	-	-	0.91	-	-	1.4
D_8	-	-	0.53	-	-	14.3

As can be noticed, poor correlation coefficients were obtained between predicted values using ANNW and values obtained from oxygen consumption measurements when the three parameters (k_1, k_2 and ip) were used as outputs for each training set. Even though the ANNW can predict more than one value (output) at the same time, usually this program is used to predict a single value as output (Lawrence, 1994). Based

on the unsatisfactory results obtained when 3 outputs were used simultaneously, it was decided to use only one value each time as an output for each training set. Firstly the program was trained using k_1 as output, then k_2 and finally ip, using the same set of oil components (D_4). The correlation coefficient of predicted and experimental values for the induction period (ip) was markedly improved (0.91), but for k_1 and k_2 a poor correlation coefficient was still observed (Table 4.4.2.2).

As better predictability was obtained for induction period values, other sets of components were tested (D_5, D_6, D_7 and D_8) trying to find the oil components which could give better predictability of the induction period. The best predictability was reached with the group of components- D_5, which are presented in more detail in Table 4.4.2.3 and Fig. 4.4.2.1. As can be noticed, only PLG had a SEE of 21.1, while for other oils the SEE was below 9.8, with an r^2=0.95.

Table 4.4.2.3. Values of induction period obtained experimentally by oxygen uptake measurements during storage without light (65°C) and values predicted by ANNW.

Oils[1]	ip(hr)		SEE
	Oxygen uptake	ANNW	
CAN	38.6	44.3	2.9
COL	87.1	85.2	1.0
CRW	79.9	67.4	6.3
COT	37.5	38.9	0.7
OEV	79.1	98.8	9.8
PLG	0.0	42.1	21.1
PLK	288.0	283.5	2.3
PEA	78.1	71.6	3.3
RIO	68.1	61.2	3.4
SUN	27.6	26.1	0.8
SYS	51.5	41.4	5.1

[1]Abbreviations see Table 3.1.1.

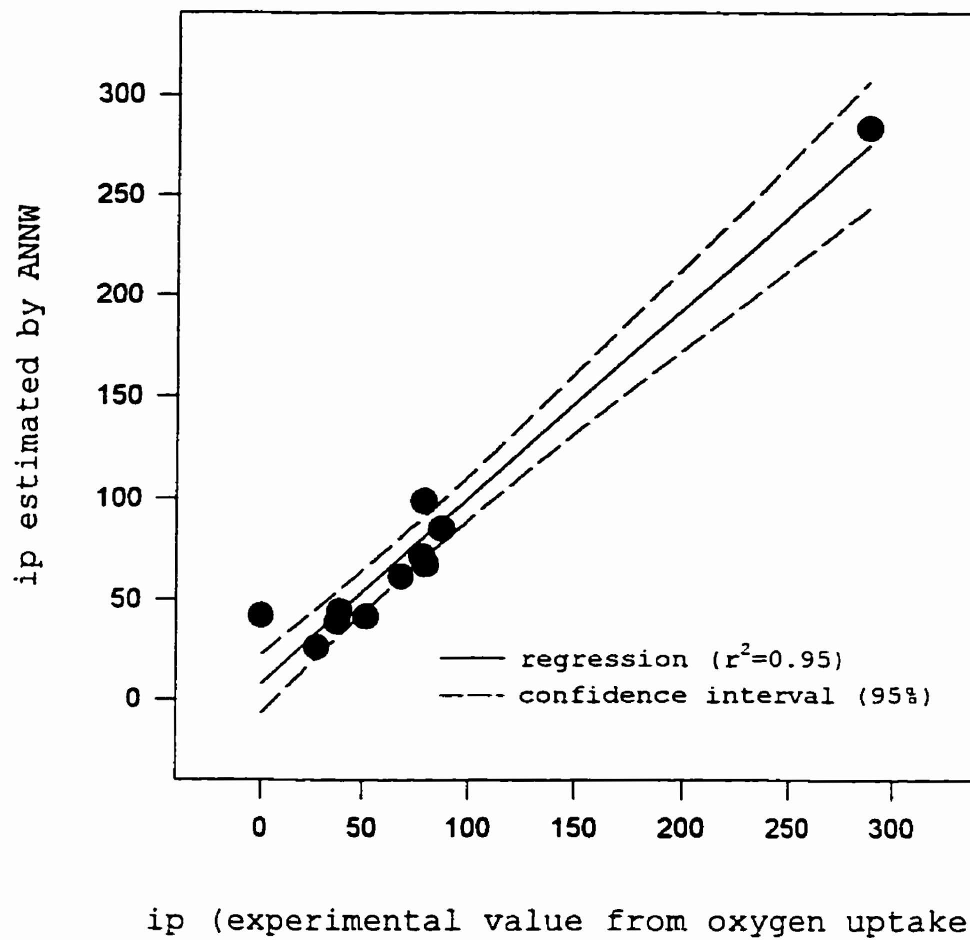

Fig. 4.4.2.1. Correlation between experimental value of induction period (ip) obtained by oxygen consumption during storage without light (65°C) and values of ip predicted by ANNW.

Based on the low predictability of k_1 and k_2, a single value was calculated from oxygen consumption data using linear regression for each individual oil (Table 4.4.2.5).

Table 4.4.2.5. Previous values of the slopes of initiation (k_1) and propagation (k_2) lines and induction period (ip), and the single value calculated (k_3) to run ANNW.

Oils[1]	Previous values			k_3
	k_1	k_2	ip(hr)	
CAN	-0.0151	-0.0953	38.6	-0.0845
COL	-0.0083	-0.0844	87.1	-0.0541
CRW	-0.0101	-0.1090	79.9	-0.0615
COT	-0.0153	-0.1258	37.5	-0.0910
OEV	-0.0140	-0.0234	79.1	-0.0203
PLG	-0.0000	-0.0540	0.0	-0.0540
PEA	-0.0123	-0.0483	78.1	-0.0370
RIO	-0.0163	-0.0748	68.1	-0.0550
SUN	-0.0240	-0.1160	27.6	-0.1123
SYS	-0.0083	-0.1300	51.5	-0.0990

[1]Abbreviations see Table 3.1.1.

These values of k_3 were used as output to train the ANNW. The same set of components described before were used as inputs (D_2, D_3, D_6, D_7 and D_8, Table 4.4.2.1), one each time, trying to find the set that could give the best predictability. The results obtained using k_3 as outputs were more satisfactory than when k_1 and k_2 were used (Table 4.4.2.6).

Table 4.4.2.6. Correlation coefficient (r^2) between experimental and predicted values of k_3 and standard error of the estimate (SEE).

Factors	D_2	D_3	D_6	D_7	D_8
r^2	0.92	0.85	0.80	0.88	0.56
SEE ($\times 10^{-3}$)	1.9	3.3	6.1	4.0	9.2

The set of components that gave the best results of predictability was D_2 (Table 4.4.2.7, Fig. 4.4.2.2) which was the same set that gave the best results on predictability of the induction period, which included fatty acids, the total amount of tocopherols and tocotrienols, metals, sterols and phospholipids. The set of components containing only fatty acids and the total amount of tocopherols and tocotrienols (D_7) also gave a reasonable correlation coefficient (0.88), which was also observed when the induction period was predicted using this same set.

Table 4.4.2.7. Values of k_3 obtained by oxygen uptake measurements during storage without light (65°C) and values predicted by ANNW.

Oils[1]	k_3		SEE ($\times 10^{-3}$)
	Oxygen uptake	ANNW	
CAN	-0.0845	-0.0924	3.9
COL	-0.0541	-0.0578	1.9
CRW	-0.0615	-0.0770	7.8
COT	-0.0910	-0.0785	6.3
OEV	-0.0203	-0.0387	9.2
PLG	-0.0540	-0.0487	2.7
PLK	-0.0040	-0.0110	3.5
PEA	-0.0370	-0.0464	4.7
RIO	-0.0550	-0.0654	5.2
SUN	-0.1123	-0.1096	1.4
SYS	-0.0990	-0.0886	5.2

[1]Abbreviations see Table 3.1.1.

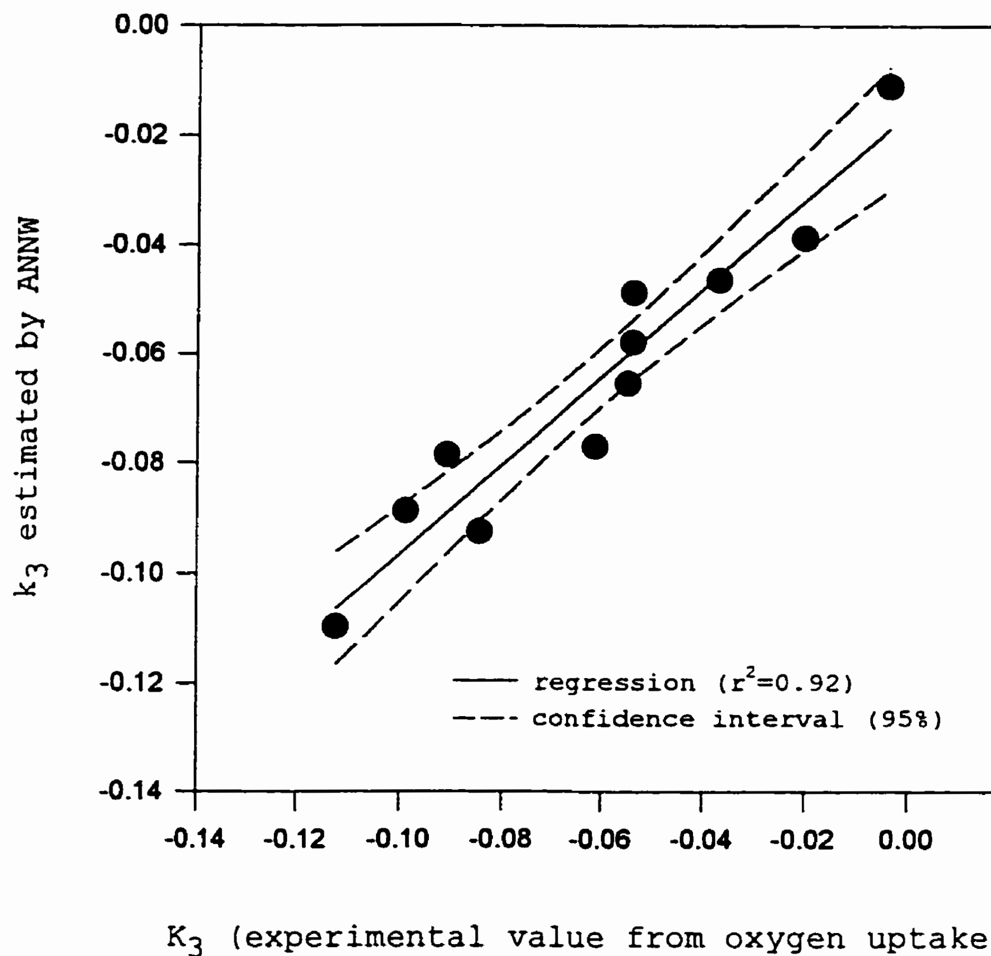

Fig. 4.4.2.2. Correlation between experimental values of propagation period (k_3) obtained by oxygen consumption during storage without light (65°C) and values of k_3 predicted by ANNW.

5. DISCUSSION

5.1. Effect of oil components on oil stability

5.1.1. Fatty acid composition

The type of fatty acid played an important role in the oil stability. This was clearly observed when the stability of regular and genetically modified oils as measured by oxygen consumption were compared. High oleic (CHO), low linolenic (CAR) and high oleic low linolenic (COL) canola oils were more stable than regular canola oils (CAN, CAS and CAO), high oleic sunflower (SHO) oil was more stable than regular sunflower oils (SUN and SUR), and low linolenic flax (FLL) oil was more stable than conventional flax oil (FCO) at both storage conditions (Tables 4.2.6.1 and 2). The stability of these oils increased when the content of polyunsaturated fatty acids decreased, mainly due to the reduction in the content of linolenic acid in canola and flax oils, and linoleic acid in sunflower oils.

Similar results were observed when the stability was measured by other chemical methods (Table 5.1.1.1). Again, CHO showed better stability than CAO and CAS , due to lower values in PV, CLA and total volatiles observed at the end of the storage period.

Table 5.1.1.1. Chemical measurements at the end of storage.

Measurements	Absence of light[4]			Light exposure[5]		
	CAO	CAS	CHO	CAO	CAS	CHO
PV[1]	216.07	178.36	99.60	86.84	67.55	53.00
CLA[2]	1.82	1.51	0.78	1.01	0.82	0.66
Volatiles[3]	6066.44	5473.15	1641.76	2180.50	1014.11	470.96

[1]meq peroxide/kg; [2]conjugated dienes (%); [3]total volatiles (ng/100µl of oil); for oil abbreviations see Table 3.1.1; [4]in 16 days of storage at 65°C; [5]in 12 days of storage at 35°C.

These results are in agreement with other reports. Purdy (1985) reported that high oleic sunflower oils were more stable than regular varieties, when their stability was measured by the Active Oxygen Method (AOM). The values of AOM were 11, 38, 60 and 100 hours for regular sunflower, 69% linoleic acid, and high oleic sunflower oils, 12, 7 and 1% linoleic acids, respectively. Przybylski *et al.* (1993) found that low linolenic canola oil (3.1% linolenic acid) exhibited marked improvement in stability when compared with regular canola oil (11.5% linolenic acid) during accelerated storage at 60°C. The stability was measured by PV, thiobarbituric acid value (TBA), carbonyls and dienals, and sensory evaluation for overall odour intensity and pleasantness.

Similar results were also found for other genetically modified oils. White & Miller (1988) found better stability of low linolenic soybean oils (2 to 4% linolenic acid) than

regular varieties (7 to 10% linolenic acid). These oils were stored at 65^{0}C for 8 days and the stability was measured using the AOCS flavour intensity scale. O'Keefe *et al.* (1993) found that high oleic peanut oil (75% oleic acid) had an AOM induction time of 69 hours compared with 7.3 hours of normal lines (56% oleic acid).

However, in the present study the stability of oils from the same genetic source, which were composed of similar fatty acids, was not always the same. Canola (CAO) was less stable than other regular canola (CAN and CAS) oils, and rice bran (RBO) was less stable than other rice bran (RIO and RWO) oils, at both storage conditions. Virgin olive (OEV) and olive pomace (OPR) were more stable than olive (ORF) when stored without light, but were less stable with light exposure. Corn (COR and CRW) oils were less stable than the other corn (CRN) oil, cottonseed (COT) was less stable than the other cottonseed (CTO) oil, and soybean (SOY) was less stable than the other soybean (SYB and SYS) oils during storage with light exposure (see 4.2.1 to 4.2.5). Therefore, these results implied that fatty acid composition was not the only factor responsible for stability of the oils.

When oils from different genetic backgrounds were compared, the differences in stability were markedly higher between oils, due to the bigger differences in the composition of saturated and polyunsaturated fatty acids. A better stability of oils with a high content of saturated fatty acids

(COC and PLK) than oils with a high content of oleic acid (ORF, CHO, CAR, COL and SHO) was observed. In addition, oils with a high content of linoleic acid (FLL, SUR and SUN), and linolenic acid (FCO and BOR) exhibited decreasing stability.

Several comparisons in stability between oils with different fatty acid composition are available in published literature. Snyder & Mounts (1990) compared the stability of corn, canola, soybean and sunflower oils stored without light at 60°C. After 8 days of storage higher values of PV and total volatiles in sunflower, followed by canola, soybean and corn oils were found. Higher values of PV and total volatiles were found in sunflower oil, while lower values in corn oils.

Sattar *et al.* (1976) found similar results when comparing Peroxide values and flavour scores of canola, soybean and corn oils stored with light exposure at 25°C. Ostrić-Matijašević *et al.* (1982) found that corn oil stored at 60°C and at ambient temperature showed lower PV accumulation than sunflower oil. Kaya *et al.* (1993) reported that olive oil had a higher stability than did sunflower oil, when they were stored in clear glass under fluorescent light at 10°C and 20°C. Warner *et al.* (1989) found that soybean oil was more stable than canola oil, and canola oil more stable than sunflower oil when stored without light at 60°C. The same authors found different trends when they used different methods to assess stability. Canola oil showed better stability than soybean oil when it was analysed by AOM and Rancimat. Also, canola

oil exhibited a lower amount of total volatile compounds formed than soybean oil when stored with light exposure.

In the present study, slightly different results of oil stabilities from the cited literature were found when compared by PV, CLA, TAV and oxygen uptake, during both storage conditions. After the sixth day of storage without light, ORF showed better stability than other oils, when measured by PV and total volatiles, and during all storage periods when measured by CLA. Regular canola (CAS) showed lower stability than SYS until the second day, and better stability after that day, when measured by PV and total volatiles, but CAS showed lower CLA amounts than SYS only after the 10th day of storage. Regular sunflower (SUR) showed higher PV values than CAS and SYS during the whole storage period, and higher CLA values and total volatiles after the fourth day of storage (Table 5.1.1.2).

Table 5.1.1.2. Chemical measurements during storage without light at 65°C.

Analyses	Oils[1]	Time (days)					
		0	2	4	6	10	16
PV[2]	CAS	0.10	1.14	5.26	26.92	76.70	178.36
	SYS	0.10	1.11	6.68	30.53	79.63	172.63
	SUR	0.44	7.20	30.52	70.40	122.50	326.63
	ORF	0.75	7.50	10.56	13.40	19.90	31.69
CLA[3]	CAS	0.30	0.31	0.35	0.53	1.02	1.51
	SYS	0.29	0.30	0.31	0.53	1.16	1.89
	SUR	0.22	0.27	0.52	0.79	1.74	3.67
	ORF	0.10	0.14	0.16	0.19	0.25	0.35
Volatiles[4]	CAS	53	189	243	376	1425	5473
	SYS	69	135	260	527	1699	3061
	SUR	54	145	332	559	2501	6783
	ORF	146	309	348	355	450	468

[1]Abbreviations see Table 3.1.1; [2]meq peroxide/kg; [3]conjugated dienes (%); [4]total volatiles (ng/100µl oil).

Similar results were obtained when oxygen uptake was measured (see 4.2.6.1 and 2). Corn (COR) was found to be the most stable oil during the first 100 hours of storage, when olive (ORF) showed better stability than the others during the whole storage period.

Small variations in stability between these oils were observed during storage with light exposure. Soybean (SYS) showed better stability than CAS until the eighth day of storage, when measured either by PV or CLA values. Higher amounts of volatiles were formed in SYS than in CAS after the first 3 days of storage. Olive (ORF) produced more volatiles than SYS during the whole storage period, while OPR exhibited

higher amounts of these compounds than did CAS during the first 5 days of storage (Table 5.1.1.3). Regular sunflower (SUR) exhibited the highest PV after the second day and the highest CLA after the third day of storage, while SUR produced the highest amount of total volatiles just in the final days of storage.

Table 5.1.1.3. Chemical measurements during storage with light at 35°C.

Analyses	Oils[1]	Time (days)					
		0	1	3	5	8	12
PV[2]	CAS	0.10	5.38	22.28	30.64	48.33	67.55
	SYS	0.10	3.46	18.86	25.79	41.26	83.34
	SUR	0.44	9.21	28.23	41.75	63.58	106.20
	ORF	0.75	6.54	14.88	18.82	26.21	38.46
CLA[3]	CAS	0.30	0.33	0.44	0.54	0.66	0.86
	SYS	0.29	0.32	0.39	0.50	0.63	0.93
	SUR	0.22	0.27	0.53	0.66	0.83	1.41
	ORF	0.10	0.12	0.14	0.19	0.22	0.30
Volatiles[4]	CAS	53	195	280	419	827	1014
	SYS	69	141	295	500	660	667
	SUR	54	185	267	474	607	1296
	ORF	147	192	432	435	786	806

[1]Abbreviations see Table 3.1.1; [2]meq peroxide/kg; [3]conjugated dienes (%); [4]total volatiles (ng/100µl oil).

When the stability was measured by oxygen uptake, SYS showed better stability than CAS, while both were more stable than SUR (see 4.2.6.1 and 2). Olive (ORF) was the most stable oil after the first 60 hours of storage (2.5 days), which agrees with PV and CLA values, but not with the TAV formed

during this period. Regular sunflower (SUR) also showed the fastest oxidation when measured by PV, CLA and oxygen uptake; however, the TAV was higher only in the final period of storage. Similar to by Warner *et al*. (1989), the stability of an oil is dependent on the storage condition and the method used to assess stability.

Comparing the stability of oils measured by oxygen uptake, it was observed that virgin olive (OEV) and olive (ORF) showed better stability than did sunflower oils (SUR and SUN) in storage without light, but they were less stable during storage with light (see 4.2.6.1 and 2). Olive oils contained about 75% oleic acid, and regular sunflower oils about 70% linoleic acid. Virgin olive (OEV) and canola (CAO) showed better stability than did conventional flax (FCO) when stored without light, but both exhibited poorer stability during storage with light. Conventional flax (FCO) contained 54% linolenic acid, while canola (CAO) only 10%. These results also implied that different fatty acid composition was not the only factor determining stability of the oils.

The combined results showed that valid comparisons of the stability between oils was dependent on the fatty acid composition, the method used to evaluate the stability, the storage conditions, and the presence of other minor constituents in the oils.

5.1.2. Chlorophylls

Chlorophylls were present at detectable levels in OEV, OPR, COT, CAO, SOY and BOR oils. Among olive oils, OEV and OPR showed better stability than ORF during storage without light, and lower stability with light exposure. SOY showed a stability similar to SYB and SYS during storage without light, but lower when stored with light. Similar results were found for cottonseed oils, where COT showed the same stability as did CTO without light, while lower with light. CAO showed a lower stability than did other regular canola oils in both storage conditions.

These results demonstrated that during storage with light exposure, oils that contained chlorophyll had a lower stability than did oils without it. Therefore, the presence of chlorophyll accelerated the photo-oxidative reactions in these oils.

Several studies have reported the pro-oxidant activity of chlorophylls and their derivatives in oils stored with light exposure. Endo *et al.* (1984a,b) found that added chlorophylls catalysed oxidation of methyl linoleate at concentrations as low as 2.2×10^{-9}mol/g (2ppm). The same authors found that the pro-oxidant activity of chlorophyll *b* was twice as high as chlorophyll *a*, while pheophytins and pheophorbides exhibited even stronger pro-oxidant activities than chlorophylls. Similar results were found by Usuki *et al.* (1984b) when canola oil was used as a substrate, which was previously purified

with activated carbon-celite column chromatography to remove minor components.

Kiritsakis & Dugan (1985) also demonstrated that the addition of 4ppm of chlorophyll greatly reduced the stability of purified olive oil stored with light exposure for 6-8 hours, as expressed by higher values of PV in stored oil. The pro-oxidant effect of chlorophylls and their derivatives was also confirmed by Tautorus & Low (1994). These authors added pheophytin to crude canola oils, and then they subjected the oils to processing conditions (degumming, refining and deodorization). The stability of the oils significantly decreased with increasing content of pheophytin before processing at concentrations higher than 30ppm.

In the present study, the individual chlorophylls were determined only for fresh OEV oil, in which pheophytin *b* was the major chlorophyll pigment present. However, the total amount of chlorophylls and their derivatives determined by the HPLC method in OEV was 1.85 times higher than the amount determined by the AOCS method. Ward *et al.* (1994a) also reported that the HPLC method detected higher levels of total chlorophyll *per* sample than did the AOCS spectrophotometric method. These authors found on average 1.4 times more chlorophyll when using the HPLC procedure than the AOCS method. They suggested that this difference could be due to an underestimation of chlorophyll derivatives because the spectrophotometric method (AOCS) is calibrated with only

chlorophyll *a*, which has a much higher absorption at 665nm than their derivatives. However, in the present study, several other factors could be responsible for these differences. The total content of chlorophyll was determined by the sum of individual pigments, more specifically chlorophyll *a* and *b* and pheophytin *a* and *b*. A calibration curve for each pigment was made using both chlorophyll *a* and *b*, which were not 100% pure. Also, pheophytin *a* and *b* were synthesised in the laboratory from the corresponding counterpart chlorophylls. Therefore, it is reasonable to assume that some errors may have been made during the preparation of these compounds.

During accelerated storage, the changes of specific pigments were not monitored, and only the total content of chlorophyll was measured by spectrophotometer method. The depletion rate of chlorophyll was higher during storage with light exposure than without light. However, the higher depletion rate of chlorophyll was related to its higher concentration, but not always to faster oxidation of the oil. Usuki *et al*. (1984a) reported that pheophytins *a* and *b* were stable in methyl linoleate and showed no decomposition during exposure to light for 24 hours at 500 μW. Chlorophylls *a* and *b* showed degradation at first-order reaction, and chlorophyll *a* showed better stability than chlorophyll *b*.

When the stability of oils containing chlorophylls was compared, the results showed that chlorophyll content alone

was not responsible for the reduction in stability of oils stored with light. OEV contained the highest amount of chlorophyll, and also showed the fastest oxidation among oils. However, SOY had 6 times more chlorophyll than CAO, but CAO oxidized faster than SOY. SOY and COT exhibited similar oxidation rates, but SOY contained 4.6 times more chlorophyll than COT (Table 5.1.2). Therefore, when comparing SOY to CAO and COT, the higher content of chlorophyll in SOY was not directly related to faster oxidation.

When the stability of oils containing chlorophylls was associated with their fatty acid composition, it could also be observed that these two components were not the only factors responsible for the oxidation rate of the oils exposed to light. BOR and OPR showed similar stability during storage with light, as well as similar content of chlorophyll. However, the content of PUFA in BOR was much higher than in OPR. Therefore, there were other factors accelerating the oxidation in OPR and/or retarding it in BOR.

Table 5.1.2. Chlorophyll, fatty acid content and oxygen uptake during oil storage.

Oils[3]	Chlorophyll (ppm)	%O_2 depleted per hour[1]	Fatty acid composition[2]		
			SFA	MUFA	PUFA
OEV	7.02	0.126	13.0	76.3	10.7
OPR	0.10	0.075	13.5	74.0	12.5
CAO	0.12	0.099	6.7	59.0	34.3
SOY	0.70	0.089	15.1	21.4	63.5
COT	0.15	0.087	25.7	16.6	57.7
BOR	0.08	0.074	13.0	16.5	70.5

[1]During first 108 hours of storage with light at 35°C; [2]relative amount %; [3]abbreviations see Table 3.1.1; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.

During storage without light, the role of chlorophyll was not as clear as it was for storage with light exposure. Other studies have related the different behaviour of chlorophylls during storage without light. Endo *et al.* (1985a,b) found that chlorophyll and pheophytin retarded the oxidative deterioration of methyl linoleate and triacylglycerides from canola and soybean oils when stored under absence of light at 30°C. They also reported that the antioxidant activity of these compounds decreased when the temperature increased to 50°C, and that this activity was dependent upon substrate. Gutiérrez-Rosales *et al.* (1992) reported that adding chlorophyll (9.38ppm) to olive oil increased its stability when compared to the control without chlorophyll. These oils were stored at 36°C without light.

In the conditions of the present study, chlorophyll showed

no apparent antioxidant activity during storage without light in CAO, SOY and COT oils. During this storage condition, CAO showed faster oxidation than other regular canola oils. COT showed the same rate of oxidation than CTO, and SOY showed the same rate of oxidation than SYS and SYB oils. Therefore, in these oils chlorophyll apparently did not exert any antioxidant activity in storage without light, and could even be acting as slight pro-oxidants in CAO.

In olive oils, OPR showed slightly better stability until 180 hours of storage than OEV, and the amount of chlorophyll in OEV was 70 times higher than in OPR. Therefore, comparing the stability of OEV with OPR, there was no evidence that chlorophyll acted as an antioxidant. Both OEV and OPR were more stable than ORF, which did not contain chlorophyll. Again, taking into account the behaviour of chlorophyll in the other oils, and the fact that the highest content of chlorophyll in OEV (7.02 ppm) was not effective in assuring better stability in OEV than in OPR (0.10 ppm), it is not likely that the absence of chlorophyll would be the only factor that can be attributed to the faster oxidation of ORF in relation to OEV and OPR.

When the stability of the oils between both storage conditions was compared, it was observed in all oils, except PLG had, that there was a faster initial oxidation during storage with light than without light, even though several oils showed no presence of chlorophyll. However, in oils that

contained chlorophyll, the initial oxidation during storage with light exposure was even higher than oils without chlorophyll. Malcolmson *et al.* (1994) found that samples stored under fluorescent light (2690 lux) showed a higher development of rancid odour intensity than did the same samples stored under the absence of fluorescence light, when both were stored at 40°C. Oštrić-Matijašević *et al.* (1982) found that corn and sunflower oils stored at room temperature without light were more stable than when stored with daylight exposure.

Therefore, factors other than chlorophyll are affecting the oxidation of the oils. The presence of sensitizers that were not detectable with the methodology used in this study cannot be disregarded. Also, a strong effect of light with shorter wavelengths on micro components has been reported (Bekbölet, 1990).

5.1.3. Carotenoids

The presence of carotenoids was detected only in two oils, OEV and PLG.

Palm golden oil (PLG) showed better resistance to oxidation when stored with light exposure than without light. The amount of oxygen depleted after 168 hours of storage was 47.1 and 9.9% without and with light, respectively. This behaviour was the opposite from that found for all other oils, which showed faster oxidation in the initial period of storage

with light exposure. Palm (PAL) showed similar fatty acid composition to palm golden (PLG), but in the same period of time PAL depleted 24.2 and 35.72% of oxygen during storage without and with light, respectively. As can be seen, PAL showed better stability without light and poorer stability with light than did PLG.

These results suggest that the high content of carotenoids in PLG, 711.9ppm, likely acted as pro-oxidants during storage without light, but could act as antioxidants when this oil was stored with light exposure.

The pro-oxidant effect of carotenes during storage without light was reported by Warner & Frankel (1987). When they added β-carotene (1 to 20ppm) to a commercial soybean oil, and stored the oil without light at 60°C the oil showed significantly higher PV values than samples without β-carotene. Based on these results they suggested that free radical oxidation was promoted by β-carotene during storage without light.

Lee & Kim (1992) also found that β-carotene acted as pro-oxidant in soybean oils stored without light. These authors reported that the pro-oxidant activity of β-carotene increased as its concentration increased from 50 to 200ppm.

The antioxidant effect of carotenes under light exposure of oils that contained no chlorophyll was also related by Haila & Heinonen (1994). These authors found that β-carotene alone acted as a pro-oxidant at a level of 20ppm in purified

canola oil stored with light exposure. However when β-carotene was used with γ-tocopherol (20 to 50μg/g), the stability of the oil increased. The effect of both components was more pronounced than with γ-tocopherol alone. Similar results were reported by Warner & Frankel (1987). They reported that β-carotene at levels of 5 to 20ppm with tocopherols protected purified soybean oil against photo-oxidative deterioration.

In the present study, PLG contained 137ppm of tocopherols and 789ppm of tocotrienols. The high content of tocopherols, tocotrienols and carotenes could have acted synergistically to increase the stability of PLG oil during storage with light.

The role of carotenoids in olive oil (OEV), which also contained chlorophyll, was not as clear as observed for PLG. However, the content of carotenoids in OEV was only 12.6 ppm, which was 56.5 times lower than the content in PLG.

When the stability of olive oils was compared, it was observed that OEV and OPR, both with chlorophylls, showed better stability than ORF in storage under absence of light, while OPR showed slightly better stability than OEV. As in PLG, the presence of carotenes present in OEV could in part explain the slightly lower stability of OEV than OPR oil. Therefore, carotenoids could have acted as pro-oxidants under the absence of light in OEV, and their lower effects on the stability in OEV could be due to their lower concentrations than in PLG, and/or due to interaction between other

components present in OEV.

During the storage with light exposure, even containing carotenes, OEV showed faster oxidation than OPR. However, as discussed previously, the content of chlorophyll in OEV was 70 times higher than in OPR. All carotenoids present in OEV showed decomposition during both storage conditions, whereas the amount of carotenoids depleted was higher during storage with light (72.5%) than without light (50.4%). The highest disappearance of carotenoids was also accompanied by higher changes of chlorophyll during storage with light exposure (92.4%) than without light (34.2%). These results could indicate the participation of carotenoids in the photo-sensitized oxidation, where they could be acting as singlet oxygen quenchers.

Other workers have also found that carotenes acted as antioxidants in chlorophyll sensitized photo-oxidation of vegetable oils. Lee & Min (1988, 1990) reported that lutein and β-carotene at levels of 5 to 20ppm acted as effective antioxidants in soybean oils, which contained 4ppm of chlorophyll, when stored with light. The same authors reported that as the level of carotenoids increased, the oxygen uptake decreased. Kiritsakis & Dugan (1985) found that β-carotene added to olive oils, which contained 6ppm of chlorophyll, increased their stability when the oil was stored with light. These authors stated that β-carotene substantially inhibited oxidation in the first hours of

illumination thus supporting the concept that chlorophyll causes formation of singlet oxygen, which is quenched or its formation inhibited by β-carotene. Lee & Kim (1992) found that the photo-sensitized oxidation of soybean oil was effectively retarded by the addition of 50 to 200ppm of β-carotene.

Some authors also reported that the antioxidant effect of carotenes is complicated by its susceptibility to autoxidation, which is dependent on temperature, wavelength of irradiation and type of substrate (Warner & Frankel, 1987; Haila & Heinonen, 1994).

In this study, faster decomposition of carotenoids during storage with light exposure was related to the time when chlorophyll was depleted at faster rates. The α-tocopherol was initially present in OEV at level of 188ppm, and after 72 hours (the period that carotenes showed faster depletion rates) the content of α-tocopherol dropped to 28ppm. Therefore, it seems probable that carotenoids were depleted at faster rates during the initial stages of storage with light exposure mainly due to their participation as antioxidants instead of their autoxidation.

5.1.4. Metals

Several oils contained metals, but the content in many of them was lower than 0.05ppm. However, it is well known that traces of heavy metals in edible oils have serious and

deleterious effects on their stability. Ringkasan (1982) reported that the presence of metals such as iron, copper and nickel at levels of 1.5 to 15.0ppm, greatly reduced the stability of crude palm oil when measured by AOM and Totox value (PV + 2 Aniside value). Benjelloun *et al.* (1991) found an increase in the stability of canola oils when traces of metals were removed.

In the present study, metals were found in several oils, and the oil stabilities were clearly affected. CAO had 0.024ppm of copper, while metals were not detected in CAS and CAN oils. CAO was less stable than CAS and CAN at both storage conditions. During storage with light, the lower stability of CAO could be explained by the presence of chlorophyll. The presence of copper in CAO could explain, at least in part, its lower stability than CAS and CAN during storage without light.

The same trends were observed for cottonseed oils, where COT contained higher amounts of metal, and also showed slightly lower stability than CTO when stored without light.

In rice bran oils, the presence of a higher amount of iron in RBO (0.12ppm) was accompanied by significantly lower stability of this oil than RIO (0.030ppm) and RWO (0.027ppm), at both storage conditions.

Virgin olive (OEV) oil contained a slightly higher amount of metals (0.074ppm) than OPR (0.061ppm) and accordingly its stability was slightly lower. A higher content of

carotenoids, as discussed before, and a higher amount of metal could be responsible for the lower stability of OEV when compared to OPR during storage without light.

Palm (PAL) oil showed a very high content of metals (1.29ppm) compared to PLG (0.10ppm), and also a lower stability in storage with light. PAL was more stable than PLG when stored without light, but at this condition the strong pro-oxidant effect of carotenoids in PLG could overcome the effect of metals in PAL oil. PAL showed the highest content of metals among all oils, and also showed lower stability than several oils with higher content of PUFA under both storage conditions (see 4.2.6.1 and 2). Therefore, the higher content of metals in PAL oil greatly affected its stability.

Coconut (COC) and palm kernel (PLK) oils had a similar composition, and both contained metals at levels of 0.08 to 0.10ppm. These oils showed higher stability during storage without light, but both oils showed a poorer stability when stored with light. The COC oil also showed a higher PV values (41.1) than did ORF (38.4) oil, and higher amount of total volatiles than did SYS, CHO and OEV oils during storage with light. The content of metals in these oils apparently played a role as pro-oxidants during storage with light, but with no apparent effect when stored without light. Sattar *et al.* (1976) also found that coconut stored under fluorescent light with 550 ft-c intensity at 25^0C showed significantly lower flavour scores and higher PV values when compared with samples

stored without light.

Low linolenic flax (FLL) oil showed a fatty acid composition similar to regular sunflower oils, but had higher stability under both storage conditions. Both SUR and SUN contained iron (about 0.030ppm), while metals were not detected in FLL oil. Again, the presence of metals could be responsible for low stability of regular sunflower oils when compared with low linolenic flax oil.

Borage (BOR) oil showed similar stability when stored without light and better stability when stored with light than EPR. As discussed before, some minor components other than chlorophylls, carotenes and fatty acid composition could be responsible for these differences. EPR contained a higher amount of metals (0.076ppm) than did BOR (0.036ppm), which could explain the faster oxidation of EPR during storage with light.

For other oils, the presence of metals did not directly affect stability. RIO contained a slightly higher amount of iron (0.030ppm) than did RWO (0.027ppm), but showed better stability than did RWO in both storage conditions. Among soybean oils, SOY contained the highest amount of iron (0.147ppm), but showed the same stability as SYB and SYS during storage without light. SYB also contained 0.029ppm of iron, but its stability was the same as SYS, which metals were not detected, during both storage conditions. OEV and OPR contained slightly higher amounts of metals than did ORF

(0.053ppm). Both oils showed a better stability than did ORF during storage without light. In these oils other minor components could interfere in the pro-oxidant action of metals by the chelating effect.

Therefore, these results suggest that metals had a strong pro-oxidant activity when present at concentration higher than 1.0ppm. In storage with light exposure, even at concentrations lower than 1.0ppm, metals had a strong effect in oils that contained low amounts of other minor components. However, the effect of low concentrations of metals was not apparent in oils that contained higher amounts of other minor constituents.

5.1.5. Tocopherols and tocotrienols

Tocopherols are considered the major natural antioxidants present in vegetable oils (Pokorný, 1991). Tocotrienols, which possess a phenolic hydroxyl group similar to tocopherols, are also claimed to protect vegetable oils in a similar way as tocopherols (Clark *et al.*, 1990). The main form of antioxidant effect of these compounds is attributed to their capacity to donate a hydrogen atom to a hydroperoxyl radical, consequently stopping or retarding the chain reaction that continuously increases oxidation (Wong, 1989). Therefore, due to their participation in free radical reactions, tocopherols and tocotrienols are decomposed during the oxidation process, with the consequent formation of

tocopherol derived radicals (Yamauchi & Matsushita, 1977; Jacobsberg *et al.*, 1978).

The disappearance of these compounds during storage has been mainly associated with their antioxidant activity, even though these compounds may also be decomposed due to their autoxidation, which is associated with temperature, substrate and light intensity (Clark *et al.*, 1990; Blekas *et al.*, 1995).

In the present study, tocopherols and tocotrienols were depleted during both storage conditions. However, when oils from the same genetic background were compared, it was observed that the rate of depletion of tocopherols and tocotrienols was higher for oils that showed faster oxidation. A faster depletion of tocopherols and tocotrienols was observed in virgin olive (OEV) among olive oils during storage with light, in CAO among canola oils, in SOY among soybean oils, and in RWO among rice bran oils at both storage conditions (Table 5.1.5).

These results suggest that tocopherols and tocotrienols were mostly decomposed due to their action as free radical scavengers, because when their disappearance at the same conditions were compared, they decomposed at faster rates in oils where faster oxidation occurred, and consequently more free radicals were present.

The rate of depletion of these compounds also was faster during storage with light than without light, when oils from the same genetic background were compared (Table 5.1.5). Even

comparing the rate of depletion of tocopherols and tocotrienols between oils from different genetic background, it was found that oils which contained chlorophyll (OEV, CAO and SOY) showed the highest rates of depletion of these components during storage with light exposure.

These results indicate that tocopherols and tocotrienols could also be involved as singlet oxygen quenchers and/or free radical scavengers during photo-sensitized oxidation. Several authors reported the action of tocopherols as singlet oxygen quenchers in vegetable oils containing chlorophyll when exposed to light (Yamauchi & Matsushita, 1977; Kiritsakis & Dugan, 1985; Jung *et al*, 1991; Huang *et al.*, 1994).

Table 5.1.5. Depletion rate of total tocopherols and tocotrienols (ppm/hr).

Oils[1]	Absence of light[2]	Light exposure[3]
OEV	1.11	2.23
ORF	0.75	1.48
CAO	1.77	2.21
CAS	1.53	2.06
CHO	1.05	1.35
FLL	0.58	0.81
SUR	1.27	1.53
SOY	2.24	3.66
SYS	1.75	2.22
PEA	0.49	0.93
RIO	0.80	1.00
RWO	1.09	1.10
COC	0.16	0.38

[1]Abbreviations see Table 3.1.1; [2]during first 96 hours of storage at 65°C; [3]during first 72 hours of storage at 35°C.

The results in the present study also indicate that the rate of disappearance of the individual tocopherols and tocotrienols was dependent on their concentration in the oil. Compounds that were present at higher concentrations tended to oxidize at faster rates. This could be an indication of a competition between these compounds to react with free radical substrates- peroxyl radicals. Usually the compound that was present at a higher concentration had a greater effect on the oxidation process. When these compounds were present at similar concentrations, α-tocopherol showed a faster depletion rate than did other tocopherols, and γ-tocopherol also showed a faster depletion rate than did δ-tocopherol. These results suggest that among tocopherols, α-tocopherol showed a better antioxidant activity, followed by γ-tocopherol and δ-tocopherol.

The published data about the antioxidant activity of the individual isomers of tocopherols is controversial. Some workers reported that δ-tocopherol was the most active antioxidant, followed by γ- and α-tocopherols (Pokorný, 1991). In these studies the tocopherols were added as single compounds into purified oils, in which there was no competition between the different tocopherols. Neither were there other endogenous components present in the oils.

Huang *et al.* (1994) found that γ-tocopherol showed less antioxidant activity than α-tocopherol when present at 100ppm,

but higher antioxidant activity was observed at higher concentrations. These authors also added individual tocopherols into corn oil stripped of minor components.

Jung & Min (1990) found that the antioxidant activity of individual tocopherols was dependent on their concentrations, when they were individually added into the purified oils. Optimum concentration for α-, γ- and δ-tocopherols was 100, 250 and 500ppm, respectively.

In the present study, when these compounds were present in similar concentration, the rate of depletion of α-tocopherol was higher than α-tocotrienols, which depleted at faster rates than γ-tocopherol. These results suggest that tocotrienols exhibited antioxidant activity between the different tocopherols, which agrees with data published by Clark *et al.* (1990). Jacobsberg *et al.* (1978) proposed that tocotrienols present in palm oil could be more effective as antioxidants than the corresponding tocopherols.

In the present study, all soybean oils (SOY, SYS and SYB) showed the same rate of oxidation during storage without light, while SOY was oxidized at faster rates with light exposure. As previously discussed, faster oxidation would be expected for SOY and SYB compared to SYS during storage without light, due to the presence of metals in these oils. SOY, which contained a higher amount of iron also had a higher content of tocopherols. The same was observed when SYB and SYS were compared. SYB showed a higher amount of metal and

also a higher amount of tocopherols than did SYS. Therefore, a higher content of tocopherols could be suppressing or retarding the effect of metals on oxidation.

During storage with light, SOY oxidized at faster rates than SYB and SYS. These results suggest that even the higher amount of tocopherols in SOY was not sufficient to suppress the photo-oxidation stimulated due to the presence of chlorophyll.

Soybean oils showed a better stability at the beginning of storage without light than did CAS and CAN oils. Soybean oils contained a difference of about 1-2% less linolenic acid than regular canola oils, but there was a difference of more than 30% in respect to linoleic acid. Also soybean oils contained metals, which was not detected in regular canola oils. Based on these compositions, it could be expected that soybean oils have faster oxidation than regular canola oils. The content of tocopherols in soybean oils was between 1096 to 1458ppm, while in canola oils it was 470 to 518ppm. Again, it seemed that tocopherols acted more efficiently as antioxidants in soybean oils, at least during the beginning of storage, when they were present at higher concentrations.

These observations were supported by the comparison of the oxidation between CAO and SOY during storage with light exposure. In this condition CAO showed a faster oxidation than did SOY. However, SOY showed higher content of chlorophyll and metals than CAO. Based on what was observed

for other oils, a faster oxidation of SOY than CAO would be expected. The content of total tocopherols in SOY was 3.6 times higher than in CAO. Again, it seems that tocopherols played an important role as antioxidant in SOY exposure to light, proportionally retarding its oxidation when compared to CAO.

Similar results were obtained for other oils. FLL, SUR and SUN exhibited similar fatty acid composition, but both regular sunflower oils presented faster oxidation than FLL. As previously discussed, the metal content in sunflower oils could be partially responsible for their faster oxidation. But, the total amount of tocopherols and tocotrienols in FLL was also higher than in regular sunflower oils, which could also be partially responsible for better stability of this oil when compared to regular sunflower oils.

The same trends were observed for a few other oils. CAO was oxidized faster than CAS and CAN in both storage conditions. CAO contained chlorophyll and metal, and the content of total tocopherols was lower (404ppm) than in CAS (487ppm) and CAN (470ppm).

Corn (CRW) oil showed slightly lower stability than CRN oil during storage with light exposure, and also a lower content of total tocopherols was observed in this oil.

Palm (PAL and PLG) oils showed similar fatty acid composition, while PAL contained a higher amount of metals (1.29ppm) than did PLG (0.10ppm). During storage without

light, PAL showed better stability than PLG. This could be attributed to the pro-oxidant effect of carotenes in PLG. However, during storage with light PAL oxidized at a faster rate than did PLG. This could be due to the pro-oxidant activity of metals, as previously discussed. Also, PLG contained much higher amounts of tocopherols and tocotrienols (925ppm) than did PAL (210ppm). Again, the high content of these compounds could retard the oxidation in PLG during storage with light.

When BOR and EPR were compared, it was observed that BOR had a higher content of linolenic acid (22.8%) than did EPR (9.8%). Both oils showed similar stability during storage without light, but BOR had better stability than EPR during storage with light. As previously discussed, the higher amount of metals in EPR could be partially responsible for faster oxidation of this oil. However, BOR also contained more tocopherols (1631ppm) than EPR (394ppm). Again, the elevated content of tocopherols could be responsible for partially retarding the oxidation of BOR when compared to EPR, and also explain the similarity in stability between these oils during storage without light. However, BOR also contained chlorophyll (0.08ppm), which acted as a pro-oxidant in OPR (0.10ppm) when stored with light. The BOR had an even better stability than EPR during storage with light. In this case, the same as observed for COC, PLK and PAL oils, the pro-oxidant effect of metals seemed to be stronger in storage with

light than during storage without light.

Rice bran oil (RBO) contained an amount of iron similar to that in SOY, which showed a similar stability to other soybean oils during storage without light. The RBO had a faster oxidation than did RIO and RWO during both storage conditions. The amount of total tocopherols and tocotrienols in RBO was 406.6ppm; therefore, 3.6 times lower than in SOY. This amount of tocopherol present in rice bran oil seemed not to provide the same effectiveness in preventing oxidation when metals were present, the same result as observed for SOY oil.

These combined results indicate that tocopherols and tocotrienols can play an important role in protecting vegetable oils from oxidation.

The pro-oxidant activity of tocopherols present at high concentrations, as proposed by Jung & Min (1990), was not evident in any situation in this study. In the present study, there were indications that oils with higher amounts of tocopherols and tocotrienols exhibited even better stability, as previously discussed.

5.1.6. Phenolic compounds

Phenolics were found only in traces for almost all refined, bleached and deodorized oils. Only in olive (OEV, OPR and ORF) and BOR oils were the amount of phenolics higher than 1.0ppm.

The total content of phenolics was 38.2, 8.6 and 5.9ppm in

OEV, OPR and ORF, respectively. Similar to chlorophyll and carotenoids, the phenolic compounds in OEV and ORF were depleted at higher levels during storage with light than without light exposure. Also, during the initial period of storage with light exposure the depletion rate of phenolics in OEV was higher than during similar period of storage without light (see 4.3.2.4).

These results suggest that the decomposition of phenolics during storage, at least partially, was due to their participation as antioxidants in olive oils.

Chimi *et al.* (1991) reported that phenolic compounds were degraded as a consequence of their antioxidant activity, and their degradation rate was positively correlated to their antioxidant efficacy. The antioxidant efficacy was proposed as free radical scavenging activity, particularly in their ability to quench peroxyl radicals.

In this study OEV and OPR showed a higher amount of total phenolics than did ORF, which also was accompanied by better stability of these oils than ORF during storage without light. Therefore, these results suggest that phenolics could act as antioxidants in olive oils. However, the total content of phenolics in OPR was 2.7ppm higher than in ORF, but 29.6ppm lower than in OEV. Both OPR and OEV showed similar stability only in the initial 48 hours of storage, when OEV started to oxidize at slightly faster rates than OPR. Therefore, the total content of phenolics was not responsible for better

stability of OPR than OEV. The OEV also showed a higher amount of metals and a higher amount of carotenoids than did OPR, which could be the factors responsible for reducing the stability of OEV compared to OPR, as previously discussed.

Other studies also related the antioxidant effect of phenolics. Dziedzic & Hudson (1984a) found that added phenolics at a concentration of 0.025% to 0.10% increased the stability of lard. Marinova & Yanishlieva (1994) reported that *p*-hydroxybenzoic, vanillic, syringic and *p*-coumaric acids exercised no stabilizing effect, whereas syringic acid had a weak inhibitory effect on methyl esters of sunflower oil fatty acids. However, in lard these phenolics showed greater antioxidant activity.

Papadopoulos & Boskou (1991) reported that hydroxytirosol, caffeic, protocatechuic and syringic acids had a antioxidant activities in virgin olive oil. The same authors observed that natural olive oils had a longer shelf life due to their fatty acid composition and the presence of endogenous phenolic antioxidants.

Chimi *et al.* (1991) found that phenolics markedly slowed the rate of conjugated diene formation in a linoleic acid system when stored at room temperature.

In contrast, Tsimidou *et al.*(1992b) compared the stability of 24 samples of Greek virgin olive oils, and found that the total content of phenols determined by HPLC did not correlated with better stability. They also suggested that

other compounds present in the polar fraction, which were not eluted under the chromatographic conditions applied, might contribute to the antioxidant effect.

Papadopoulos & Boskou (1991) found that hydroxytirosol was the most effective phenolic in refined oil stored at 63^0C, followed by caffeic and protocatechuic acids. These authors added isolated phenolic at concentrations of 200ppm into the refined oil.

In the present study, it was observed that the disappearance of the individual phenolics was related to their concentration (see 4.3.2.4). Similar to tocopherols, these results suggest that the participation of phenolics in oxidative reactions was mainly related to their respective concentration. Therefore, these results suggested that in a complex system where several components were present, the higher concentration of a specific component determined its form of action.

The effect of phenolics in BOR was not evident, but their amount was only 1.5ppm. As previously discussed, some components could retard the oxidation in BOR when compared to EPR, which was attributed to the possible antioxidant effect of tocopherols. But as was the case in tocopherols, phenolics could be acting as antioxidants in BOR, because these compounds were present at higher amounts than in EPR.

5.1.7. Phospholipids

Some authors have related the antioxidant activity of phospholipids, mainly as synergists to tocopherols. Hudson & Ghavami (1984) found that added phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidyl serine (PS) at concentrations of 0.3%, slightly improved the stability of refined soybean oil. They found that their antioxidant activity was improved when α-tocopherol was present at 0.02%. These authors also stated that the chemical structure of the phospholipid has an important synergistic activity, with PE and phosphatidic acid (PA) as the most effective.

Hildebrand *et al.* (1984) also reported that the addition of tocopherols (1220 to 4000ppm) and phospholipids (0.25 to 0.50%) enhanced the stability of purified soybean oil. Phosphatidyl inositol (PI) and PA acid were more effective with tocopherols than was PC. The authors proposed that phospholipids were extending the effectiveness of the tocopherols by a free radical termination, rather than just as a matter of metal chelating. Kashima *et al.* (1991) added individual phospholipids and a combination of phospholipids with tocopherols (500ppm) into purified perilla oil. They found that phospholipids enhanced the stability of the oil when in combination with tocopherols.

Dziedzic & Hudson (1984b) reported that PE at a level of 0.5% increased stability of refined and deodorized canola oil when tocopherols were added. The effect was more pronounced

when the temperature increased from 80 to 140^{0}C. At lower temperatures the synergistic activity of this phospholipid was negligible.

In the present study, the content of phospholipids in the vegetable oils ranged from 0.17 to 0.68%, which was similar to the concentrations reported in the literature. Phosphatidyl choline was the major phospholipid present in all oils, which was usually cited in the literature as having the lowest synergistic activity among phospholipids.

As previously discussed, tocopherols could be acting as antioxidants by preventing faster oxidation of SOY and SYB compared to SYS during storage without light. However, the content of total phospholipids in SOY (0.66%) was higher than in SYB (0.40%) and SYS (0.37%). Therefore, a higher content of phospholipids in SOY and SYB could also be acting synergistically with tocopherols and decelerate oxidation of SOY when compared to SYB, and SYB when compared to SYS.

Similarly, FLL was more stable than SUR and SUN, and also contained higher amounts of phospholipids (Table 4.1.3.2) and tocopherols and tocotrienols (Fig. 4.1.4.1 and 2). Again, phospholipids could have acted as synergists to tocopherols, and explain the better stability of FLL when compared to regular sunflower oils.

Similar results were observed for other oils. In rice bran oils, RIO showed a better stability than did RBO and RWO, and also a higher content of phospholipids (Table 4.1.3.2).

Among corn oils, CRN showed a slightly better stability than did CRW and COR during storage with light, where content of phospholipids were 0.52, 0.46 and 0.26%, respectively.

However, for some oils the elevated amount of phospholipids could not be directly related to better stability. EPR showed a higher content of phospholipids (0.41%) than did BOR (0.23%), but a lower stability when stored with light exposure. In this case, if there was some synergism between tocopherols and phospholipids in EPR, they were less effective that the higher amount of tocopherols in BOR, and/or the more pronounced synergistic effect in BOR due to higher content of tocopherols and/or phenolic compounds.

A similar trend was observed in olive oils, where OEV showed the highest content of phospholipids (0.33%), but presented a lower stability than did OPR. However, the content of tocopherols in olive oils was lower than in other oils. Therefore, the lower level of tocopherols and/or a combination of other factors such as the content of phenolics, metals and carotenes, had a stronger effect than did phospholipids.

5.1.8. Sterols

In the present study the sterols were depleted at levels between 7.3% to 31.9% compared to the initial content.

Li (1996) found decomposition levels between 1.0 to 12.8% for phytosterols in fried potato chips stored for 16 days at

60°C without light. Yanishlieva & Schiller (1984) reported that 56% of sitosterol disappeared when oil was heated for 7 hours at 120°C. Ghavami & Morton (1984) reported losses between 20 to 45% for campesterol, stigmasterol and β-sitosterol when heated for 96 hours at 180°C.

In the present study, brassicasterol showed the highest losses (7.4 to 20.3%) in canola oils. In the other oils, in general sitosterol showed the highest changes, while campesterol showed the lowest losses during both storage conditions. Stigmasterol usually exhibited higher losses than brassicasterol and campesterol, with the exception of canola oils (see 4.3.2.6).

Li (1996) found the highest changes in brassicasterol, followed by sitosterol and campesterol, during storage of fried potato chips. This author also reported that during heating of individual phytosterols, stigmasterol was more stable than campesterol and β-sitosterol when heated for 12 hours at 120°C. However, Ghavami & Morton (1984) found higher losses in stigmasterol than campesterol in soybean oil heated for 96 hours at 180°C.

In the present study, the losses of specific sterol was not always related to its relative concentration, where brassicasterol seemed to be the most susceptible to oxidation, followed by sitosterol. Stigmasterol usually showed lower losses than did sitosterol, but the latter was present at a much higher concentration than stigmasterol in all oils. Both

brassicasterol and stigmasterol contain a double bond in the side chain, which could be, at least partially, responsible for their higher susceptibility to oxidation. Campesterol was the second major sterol present in almost all oils, and also was the most stable among phytosterols.

Studies have related the effect of sterols on oil stability, but mainly at frying temperatures. Fucosterol and Δ^5-avenasterol, both with ethylidene side group, were reported to be as efficient antioxidants at elevated temperatures (180 to 190°C) when added at concentrations of 0.1 to 0.2% (Sims *et al.*, 1972; Boskou & Morton, 1976; Gordon & Magos, 1983). However, the other sterols were reported to have no activity, or even acted as pro-oxidants. Yanishlieva & Schiller (1984) reported that the PV values increased at faster rates when 5% of sitosterol was added to tristearin at 120°C.

Boskou & Morton (1975b) found that Δ^5-avenasterol was the most susceptible to air oxidation at 180°C, which was directly related to its higher capacity to retard the oxidation of the oil. Results in the present study suggest however that the rate of disappearance of sterols was related to their autoxidation and/or pro-oxidant activity, rather than their participation in retarding oxidation reactions.

Corn oil (CRN) showed slightly better stability when stored with light than did CRW and COR. This oil had a lower content of sterols than did COR.

In rice bran oils, RBO showed faster oxidation due to

higher content of iron. Between RIO and RWO, the content of iron was similar, but RIO showed better stability. Again, this oil contained a lower amount of total sterols than did RWO.

When the stability of BOR was compared to EPR, it was deduced that the higher content of tocopherols and phenols in BOR could be responsible for the better performance of this oil when compared to EPR. Also, EPR contained much more total sterols than did BOR (Fig. 4.1.7); these could act as pro-oxidants in EPR.

Similarly, when compared to regular canola oils, the higher amount of tocopherols present in soybean oils was assumed to be partially responsible for their better performance. Again, canola oils showed a higher amount of total sterols than did soybean oils (Fig. 4.1.7).

Therefore, these results indicate that sterols could have some pro-oxidant activity, which was also proposed by Yanishlieva & Schiller (1984).

5.1.9. Triacylglycerol species

Some studies have suggested that the distribution of fatty acids in the triacylglycerol molecule could influence the stability of the oil (Neff *et al.*, 1992, 1993, 1994). These authors reported a negative correlation between peroxide formation and oleic acid contribution in the triacylglycerol molecule. The resistance of soybean and canola

triacylglycerols to oxidation decreased when an increased amount of linolenic acid was attached to carbons *sn*-1,3 and linoleic acid was present at the position *sn*-2. The increasing concentration of oleic acid in any position of the glycerol molecule was associated with increased resistance to oxidation.

In the present study, only small differences were observed in the triacylglycerides when oils from the same genetic background were compared. ORF showed a lower content in LOO and a higher content in POO than did OPR (Table 5.1.9), which could give better stability to ORF due to relatively higher content in species with saturated and monounsaturated fatty acids, but the opposite was found in terms of stability.

High oleic sunflower (SHO) oil showed a similar fatty acid composition to olive oils. During the first 80 hours of storage without light, SHO showed a better stability than did ORF. The content of OOO was also higher in SHO, which could be an indication that a higher content in OOO could improve stability, at least during the initial stages of oxidation.

Low linolenic flax (FLL) and regular sunflower oils showed similar fatty acid composition; however, FLL showed a higher content of trilinoleate (LLL) triacylglycerols and a lower content of LLO species than did regular sunflower oils. Taking into consideration only the glycerol distribution, it would be expected that sunflower oils would have better stability than FLL due to a higher content of triglycerides

with lower amounts of PUFA, but the opposite was observed.

Also, the major triacylglycerols in soybeans were LLL, LLO and LLP (about 60% of the total), while LOO and OOO were the major triacylglycerols in canola oils (about 68% of the total). Based on the composition of glycerides, it would be expected that canola oils would have a better stability than soybean oils, but again this was not observed in the initial stages of storage.

Table 5.1.9. Triacylglycerol species (area %).

Oils[1]	LLL	LLO	LOO	POO	OOO	LLP
FLL	48.20	21.23	-	-	-	-
SUN	42.32	27.76	-	-	-	-
SUR	45.10	28.65	-	-	-	-
OPR	-	-	12.11	21.78	53.25	-
ORF	-	-	8.44	25.98	53.26	-
SHO	-	-	13.17	5.51	67.74	-
PNT	-	-	-	9.05	25.73	-
RWO	-	-	-	11.04	13.39	-
SYB	21.73	21.59	-	-	-	15.60
CAN	-	-	29.82	-	38.81	-

[1]Abbreviations see Table 3.1.1; L=linoleic acid; O=oleic acid; P=palmitic acid.

Therefore, based on these observations, it seems that the distribution of fatty acids in the triacylglycerol species did not substantially affect the stability of the oils.

5.1.10. Other minor components

Other minor components determined in this study, neutral

lipids, glycolipids, free fatty acids and diacylglycerides, were present at low concentrations, and apparently had little or no effect on the stability of the oils.

The initial level of free fatty acids presented in the oils was lower than 0.05%, except for only a few oils. Among olive oils, OEV showed the highest content in FFA (0.15%), while OPR and ORF presented less than 0.05%. However, ORF showed faster oxidation during storage without light than OEV. No apparent influence was observed due to the higher content of free fatty acids in OEV. Mistry & Min (1987) observed the pro-oxidant effect of free fatty acids when added to purified soybean oil at a level of 0.5 to 1.0%. Miyashita & Takagi (1986) also showed a pro-oxidant effect of free fatty acids during oxidation of methyl esters of fatty acids at 50^{0}C only when they were present at a concentration over 0.5%. In the present study only BOR had levels of free fatty acids more than 0.5% (1.59%). However, as discussed before, it seems that the oxidation of BOR was retarded instead of accelerated, when the stability of oil was compared to EPR. Therefore, no apparent pro-oxidant activity of free fatty acids was observed.

Diacylglycerols (DAG) were also suggested by Mistry & Min (1988) to act as pro-oxidants in purified soybean oil at levels of 0.25 to 0.5%, when the stability was measured by oxygen uptake. In the present study FLL contained 1.56% of diacylglycerols, while sunflower oils contained about 0.64%

and this oil showed faster oxidation. In olive oils, OPR showed a higher content of diacylglycerides (1.83%) than did ORF (1.40%), and OPR also showed a better stability during storage without light. CTO contained 0.96% of DAG while COT 0.82%, but they showed no significant difference in stability during storage without light. RWO contained 1.85% and RIO 1.77% of DAG, and RIO was more stable than RWO. PEA showed similar stability to PNT, but PNT was composed of a higher amount of diacylglycerols (0.72%) than did PEA (0.64%). Therefore, there was no evident effect of diacylglycerols on oxidative stability of the oils.

5.2. Prediction of oil stability using Neural Networks

Neural Networks are processing techniques that have the ability to learn by experience. They are useful when no exact mathematical information is available, and also to solve linear and nonlinear prediction problems (Wythoff *et al.*, 1990; Borggaard & Thodberg, 1992; Katz *et al.*, 1992).

Artificial Neural Networks (ANNW) are usually composed of computer-simulated layers of processing elements or artificial neurons (Arteaga & Nakai, 1993). The artificial neurons receive input signals and pass them to the hidden layers through a set of weighted connections (Fig. 5.2). Each processing unit (neuron) can process a piece of information at the same time as other units do. The processing of information occurs in parallel and is distributed throughout

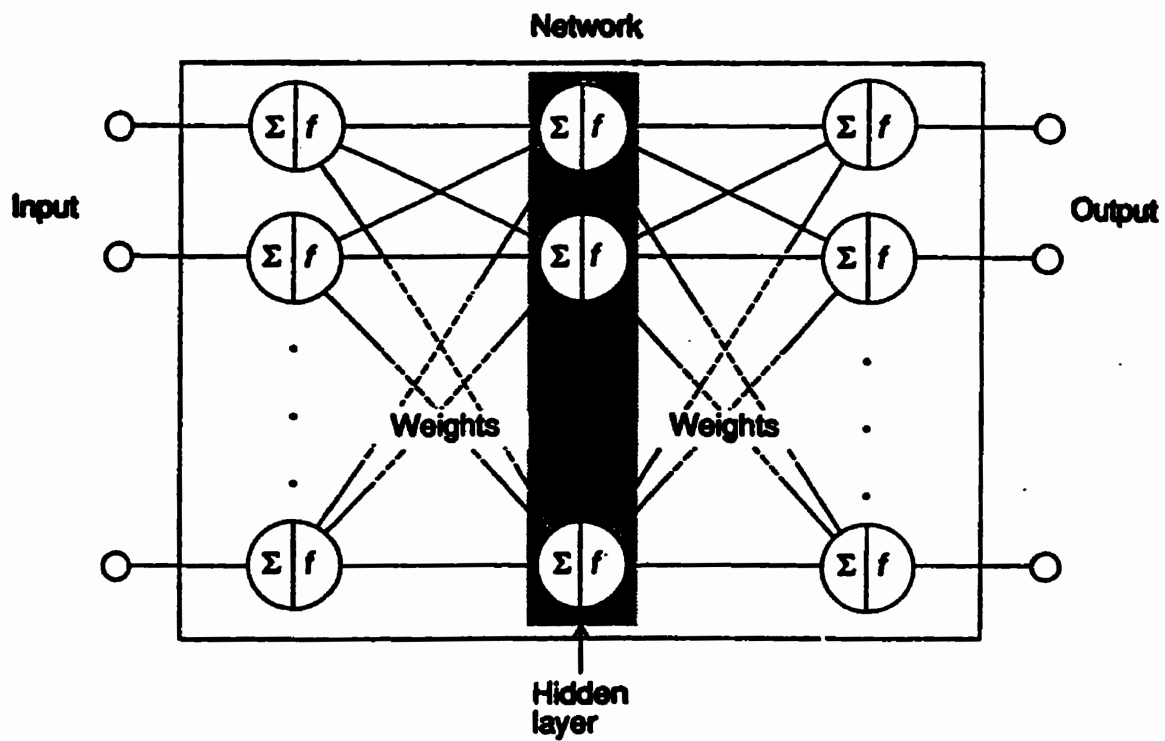

Fig. 5.2. Three layer feed-forward back-propagation network (Σ=sum of inputs; f=transfer function) (Jansson, 1991).

each unit composing the network. The processing units transfer the weighted sum through a sigmoidal function that compresses a wide domain of inputs to a limited range of outputs (Jansson, 1991; Katz *et al.*, 1992; Eerikäinen *et al.*, 1993).

During the training phase of ANNW, the predicted output is subsequently compared with the actual output, and the connection weights between the processing units are modified to minimize the deviation between the values. This is accomplished by a feed-forward layered structure, where each unit in a given layer is fully connected to every unit in the succeeding layer. This structure includes the forward propagation of neuron activation and the backward propagation of the error, with concomitant adjustments to the connections weights. In this back-propagation algorithm concept, the ANNW continues to make changes to internal connections by a trial and error process until a defined accuracy has been reached (Wythoff *et al.*, 1990; Katz *et al.*, 1992; Ruan *et al.*, 1995; Vallejo-Cordoba *et al.*, 1995).

Once trained by a set of training facts (inputs and outputs), a new set of inputs can be presented and the ANNW will predict the corresponding value of outputs (Jansson, 1991; Lawrence, 1994). Borggaard & Thodberg (1992) demonstrated that ANNW are able to predict well on new observations (inputs and outputs) that even fall outside the learning set range.

The proper functioning of a Neural Network is highly dependent on the way signals are propagated through the network. During the program training, several settings can be adjusted in order to improve the learning capacity and consequently improve the predictability of the program. In general, settings are not known beforehand and the initial choice of parameters is empirical and differs for varying topologies and pattern sets (Lawrence, 1994; Horimoto *et al.*, 1995).

The number of neurons and hidden layers is essential to obtain a well trained program. It is strongly recommended that one hidden layer be the first choice for any practical feed-forward network design. More hidden layers may cause over fitting, since the network focuses excessively on the idiosyncrasies of individual samples, but with too few hidden layers a network may not become trained. The smoothing factor, learning rate and noise are also reported as important parameters in a training process (Katz *et al.*, 1992; Lawrence & Petterson, 1993; Lawrence, 1994).

In the present study better results were found in the different training sets using hidden layers, smoothing factors, noise and learning rates between 1-2, 0.8-1.0, 0.00-0.05 and 0.8-1.0, respectively. These values were similar to published settings for different ANNW applied in the area of food science (Arteaga & Nakai, 1993; Horimoto *et al.*, 1995; Ruan *et al.*, 1995).

Also it was observed that the training tolerance, which specifies how close each output of the network must be to the empirical response to be considered correct during training, markedly affected the training time. As reported by Horimoto *et al.* (1995), smaller tolerances required longer time to train the program. It was found helpful for training sets that were taking over an hour, to increase the training tolerance, and then consecutively narrow it to desired levels. For all networks the final training tolerance used was 1.0, while for some networks it was started at tolerance 2.0 and gradually decreased to 1.0.

Recently, Artificial Neural Networks have been successfully applied in many areas of food science. Goodacre *et al.* (1992, 1993) assessed the adulteration of olive oils with other oils by an ANNW trained with Curie-point pyrolysis mass spectra of a virgin olive oil. Zhang *et al.* (1992) presented ANNW models for predicting the secondary structure of globular proteins. Arteaga & Nakai (1993) developed an ANNW using physicochemical properties of food-related proteins for predicting foam capacity and stability, and the emulsion activity index. These authors reported that ANNW had better prediction ability than did Principal Component Analysis (PCR). Horimoto *et al.* (1995) used several variables to predict the loaf volume of breads made from different wheat cultivars by ANNW. Vallejo-Cordoba *et al.* (1995) used ANNW for the prediction of milk shelf-life by multivariate

interpretation of gas chromatographic profiles and flavour-related shelf-life. These authors also reported that ANNW had better predictive ability than PCR. Ruan *et al.* (1995) predicted at levels over 94% the rheological properties of dough from the torque developed during mixing.

In the present study, a good prediction of oil stability, measured by slopes of linear regression and induction periods obtained from oxygen measurements, were obtained using ANNW with oil components as training factors. These results suggested that oil stability can be successfully predicted by ANNW using some of the oil components as variables for the training process.

For both storage conditions, the use of too many variables (oil components) could not be related to better predictability of the values of induction periods and slopes of propagation. These results suggested that many of the oil components could not be directly related to oil stability, and they probably cause interference during the program training.

The group of oil components found to be the best predictors of the propagation period (k_2) obtained from oxygen consumption with light exposure, was associated with the components which had an apparent influence on oil stability (fatty acids, chlorophylls, tocopherols and tocotrienols, and metals).

During storage without light, a more complete set of oil components from that used to predict data of storage with

light was found to be the best predictors of the induction period (ip) and slope of oxygen uptake (k_3), with the presence of fatty acids, tocopherols and tocotrienols, metals, sterols and phospholipids. These oil components present in this set were also found to affect the oils' stability during storage without light.

But only the fatty acid composition and the total content of tocopherols and tocotrienols also gave a reasonable correlation coefficient between predicted and experimental values (r^2=0.91). These results suggest that a good predictability of stability of oils stored under conditions without light could be obtained using only their fatty acid content and the total content of tocopherols and tocotrienols.

6. CONCLUSIONS

Based on the results from this study the following conclusions can be drawn:

1. The rate of oxidation of the oils was affected by a complex interaction among major and minor endogenous components;

2. The fatty acid composition was the major determinant of oil stability for most oils, but it was evident that the endogenous minor components also affected the stability of the oils;

3. Oils that had faster oxidation rates also presented higher depletion rates of PUFA. Linolenic acid with a relative contribution of less than 2.0% of the total fatty acid composition was depleted at lower rates than linoleic acid, but when present at a relative contribution higher than 2.0% the opposite was found;

4. Chlorophylls acted as strong pro-oxidants during storage with light even at concentrations lower than 1ppm in OEV, CAO, COT, OPR and SOY, but no effect of these components on oil

stability was observed during storage without light;

5. Chlorophylls were depleted at faster rates during storage with light than during storage without light in OEV, CAO and SOY oils. Among these oils OEV had a faster oxidation rate and also a faster rate of depletion of chlorophylls during storage with light exposure;

6. Carotenoids, when present at high concentration (789ppm), had a pro-oxidant effect in PLG oil during storage without light, while an apparent antioxidant effect was observed during storage with light exposure;

7. Carotenoids in OEV oil were depleted at faster rates during storage with light exposure, when OEV oxidized faster. In this condition, the depletion rate of these components was faster at the initial period of storage when the disappearance of chlorophylls was also faster;

8. Among the individual carotenoids presents in OEV, the depletion rate was related to their concentration, where lutein was present at a higher content was also depleted at a faster rate than α- and β-carotenes;

9. Metals, at concentration over 1ppm, markedly reduced the stability of PAL oil during both storage conditions. Even at

concentrations of 0.08 to 0.10ppm these components markedly reduced the stability of the highly saturated oils (PLK and COC) during storage with light exposure. These oils also contained very low amount of natural antioxidants such as tocopherols and tocotrienols;

10. Tocopherols and tocotrienols apparently acted as antioxidants by suppressing free radicals, and their effects were more pronounced at higher concentrations (up to 1000ppm);

11. The depletion rate of tocopherols and tocotrienols was higher in oils that oxidized at faster rates, when compared to oils from the same genetic background. The depletion rate was higher during storage with light exposure than during storage without light, mainly for oils that contained chlorophylls (OEV, CAO and SOY);

12. Among tocopherols, the rate of depletion was dependent on their concentration, where isomers present in higher amounts were depleted at faster rates. When these compounds were present in similar amounts, α-tocopherol depleted at faster rates, followed by γ- and δ-isomers, which started to deplete at faster rates when the amount of α-tocopherol decreased at low levels;

13. Among olive oils (OEV, OPR and ORF), which contained a

higher amount of phenolic compounds, OEV and OPR showed better resistance to oxidation than did ORF oil, which contained a lower amount of these compounds, during storage without light. Due to the low content of these compounds in the other oils, no apparent effect of phenolics was observed on their stability;

14. The rate of depletion of the total amount of phenolic compounds in OEV and ORF was higher when the content of α-tocopherol decreased to levels below 50ppm. The rate of depletion of the individual phenolic compounds in OEV was related to their concentration, where the phenolics present at a higher content were depleted at faster rates;

15. The higher content of phospholipids in several oils from the same genetic background was accompanied by better stability, which could be related to the antioxidant and/or synergistic effect of phospholipids with other antioxidants;

16. The higher content of sterols in several oils from the same genetic background was accompanied by poorer stability, which could be related to the pro-oxidant effect of sterols;

17. The sterols were depleted at faster rates in oils that had faster oxidation, when compared to oils from the same genetic background. Among the individual sterols, brassicasterol was

lost at a higher percentage in canola oils, while β-sitosterol was lost at a higher percentage in the other oils, which contained low amounts of brassicasterol. Stigmasterol was lost at a higher percentage than other sterols in RIO and RWO stored with light exposure. RIO and RWO also contained the highest content of stigmasterol among stored oils;

18. Other minor components, TAG, DAG, GL, SE and FFA had no marked effect on oil stability during both storage conditions;

19. ANNW was accurate in predicting the oil stability based on oil composition, with a correlation coefficient (r^2) over 0.92;

20. For predicting the stability of oils stored with light exposure, the best set of components used to train the ANNW program were related to the same components that had a marked effect on oil stability during storage at the same condition (fatty acids, tocopherols and tocotrienols, chlorophylls and metals)(r^2=0.95);

21. For predicting the induction period of oils by ANNW during storage without light, a set of compositions based only on fatty acids and total tocopherols and tocotrienols were very accurate (r^2= 0.91), and could be successfully used to predict the stability of oils stored at this condition. More accurate

predictability was achieved using a more complete set of components (fatty acids, tocopherols and tocotrienols, metals, sterols and phospholipids) (r^2=0.95).

7. SUGGESTIONS FOR FUTURE RESEARCH

The results obtained in this study suggest that the stability of the vegetable oils is determined by the fatty acid composition and by a complex interaction among the minor endogenous oil components. Further research is needed to evaluate with more details the effect of these components on oil stability:

1. Minor components such as tocopherols, tocotrienols, chlorophylls, sterols, carotenoids, metals, phenolic acids and sterols influenced the oil stability. Their effect based on individual concentrations need be defined;

2. To clarify the interaction among these minor components, several studies could be carried out by adding different concentrations of individual components, group of components and/or purified fractions isolated from vegetable oils, and analyse their effect on stability and oxidation products formation;

3. Minor components which were not determined in this study such as chlorophyll derivatives in oils containing

chlorophylls, oryzanols in rice bran oils, fatty alcohols in olive oils, and steryl-coumarates in corn oils could play an important role in some oils. Therefore, the evaluation of the changes of these components during storage could define their role during an oil oxidation;

4. A deep oxidation of oils to higher peroxide values could help to evaluate changes in fatty acids and triacylglycerol molecules;

5. The measurement of oxidation products such as oxidized tocopherols, tocotrienols, sterols and triacylglycerols could clarify the mechanism of oxidation of vegetable oils;

6. The application of Artificial Neural Networks proved to be very helpful in predicting oil stability when measured by oxygen consumption (output) and composition used as predictor. Future work could investigate the applicability of this program to predict PV values, volatiles and /or flavour scores using composition as predictor.

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APPENDIX

Appendix I. Fatty acid composition of oils (area %).

OILS[1]	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$
BOR	-	-	-	0.06	8.75	0.29	0.06
CAN	-	-	-	0.06	3.75	0.21	0.04
CAO	-	-	-	0.04	3.65	0.20	0.04
CAS	-	-	-	0.05	4.14	0.21	0.06
CAR	-	-	-	0.05	3.97	0.17	0.04
CHO	-	-	-	0.04	3.53	0.22	0.05
COL	-	-	-	0.07	3.87	0.21	0.04
COR	-	-	-	-	10.47	-	0.08
CRN	-	-	-	-	10.34	-	0.07
CRW	-	-	-	0.04	10.58	-	0.09
COC	6.38	5.56	45.46	18.82	10.08	-	-
COT	-	-	-	0.77	21.87	0.47	0.08
CTO	-	-	-	1.15	21.73	0.44	0.09
EPR	-	-	-	0.04	5.47	-	0.07
FCO	-	-	-	0.05	4.81	-	0.05
FLL	-	-	-	0.05	6.07	0.01	0.08
OEV	-	-	-	-	8.70	0.51	0.17
OPR	-	-	-	0.02	9.31	0.73	0.09
ORF	-	-	-	-	10.84	0.92	0.14
PAL	-	-	-	1.12	42.33	-	0.11
PLG	-	-	-	0.98	33.89	-	0.08
PLK	3.43	3.23	46.14	16.17	8.65	-	-
PEA	-	-	-	0.03	9.40	0.06	0.12
PNT	-	-	-	0.00	9.67	-	0.11
RBO	-	-	-	0.21	16.90	-	-
RIO	-	-	-	0.29	14.07	0.16	-
RWO	-	-	-	0.26	13.93	0.21	-
SHO	-	-	-	-	3.49	0.07	-
SUN	-	-	-	0.06	5.70	-	0.04
SUR	-	-	-	0.05	5.76	0.06	0.05
SOY	-	-	-	0.06	9.90	0.08	0.10
SYB	-	-	-	0.06	9.63	0.04	0.11
SYS	-	-	-	0.06	9.81	0.08	0.11

[1]Oils abbreviations see Table 3.1.1.

(Cont.) Appendix I. Fatty acid composition of oils (area %).

OILS[1]	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	α-$C_{18:3}$	γ-$C_{18:3}$	$C_{20:1}$
BOR	0.07	3.46	16.52	38.47	0.22	22.75	4.17
CAN	-	1.87	62.41	20.12	8.37	-	1.54
CAO	0.07	1.64	59.31	21.00	10.10	-	1.54
CAS	0.09	2.32	63.06	18.93	7.91	-	1.39
CAR	-	2.39	67.59	20.45	2.26	-	1.13
CHO	0.09	1.99	77.00	9.02	4.90	-	1.50
COL	0.09	2.68	74.44	12.17	3.24	-	1.18
COR	0.05	2.02	24.23	60.38	0.99	-	0.28
CRN	-	2.04	25.54	59.27	1.07	-	0.37
CRW	0.11	1.99	24.31	60.60	0.95	-	0.22
COC	-	4.31	7.45	1.80	-	-	0.06
COT	0.11	2.27	16.61	56.35	0.33	-	0.14
CTO	0.13	2.42	17.37	54.78	0.53	-	0.07
EPR	0.06	1.83	7.50	74.00	0.16	9.60	0.24
FCO	0.12	3.03	21.42	15.18	54.24	-	0.40
FLL	0.06	4.12	16.73	70.29	1.86	-	0.16
OEV	0.25	3.47	76.34	8.64	0.75	-	0.34
OPR	0.14	3.20	74.00	10.33	0.77	-	0.39
ORF	0.21	3.59	75.55	7.01	0.66	-	0.32
PAL	0.06	4.55	39.37	10.62	0.21	-	0.17
PLG	-	4.00	46.71	12.85	0.32	-	0.21
PLK	-	2.27	16.46	2.76	-	-	0.17
PEA	0.01	2.65	48.66	31.01	0.23	-	1.43
PNT	0.13	2.65	49.04	29.45	0.23	-	1.41
RBO	-	1.78	40.50	35.69	2.11	-	0.53
RIO	-	2.13	43.87	36.28	0.99	-	0.64
RWO	-	1.88	44.41	35.91	1.39	-	0.63
SHO	0.13	3.82	76.33	12.56	0.96	-	0.42
SUN	0.06	4.79	15.26	71.17	0.45	-	0.22
SUR	0.03	4.76	16.86	70.69	0.27	-	0.16
SOY	0.08	3.94	21.35	56.02	7.15	-	0.21
SYB	0.01	4.38	23.44	52.84	7.60	-	0.36
SYS	0.08	4.10	24.30	52.80	7.51	-	0.22

[1]Oils abbreviations see Table 3.1.1.

(Cont.) Appendix I. Fatty acid composition of oils (area %).

OILS[1]	$C_{20:0}$	$C_{20:2}$	$C_{22:0}$	$C_{22:1}$	$C_{22:2}$	$C_{24:0}$	$C_{24:1}$
BOR	0.22	0.35	0.30	2.34	-	0.11	2.09
CAN	0.64	0.11	0.35	-	-	0.27	0.26
CAO	0.63	0.10	0.35	0.61	-	0.17	0.34
CAS	0.75	0.08	0.38	0.23	-	0.23	0.18
CAR	0.68	0.07	0.52	0.28	-	0.30	0.10
CHO	0.70	0.05	0.37	0.16	-	0.16	0.21
COL	0.73	0.12	0.87	-	-	0.18	0.12
COR	0.39	-	0.76	-	-	0.15	0.21
CRN	0.44	0.09	0.31	-	-	0.26	0.18
CRW	0.39	0.13	0.21	-	-	0.20	0.19
COC	0.10	-	-	-	-	-	-
COT	0.26	0.10	0.36	-	-	0.12	0.17
CTO	0.25	0.07	0.36	0.17	-	0.11	0.16
EPR	0.30	0.14	0.31	-	-	0.09	0.18
FCO	0.20	0.39	-	-	-	0.01	0.08
FLL	0.12	0.09	0.15	-	-	0.12	-
OEV	0.46	-	0.13	-	0.20	0.05	-
OPR	0.55	-	0.25	-	-	0.11	0.10
ORF	0.50	-	0.15	-	0.07	0.06	-
PAL	0.39	-	0.58	-	-	0.06	0.06
PLG	0.36	0.07	0.29	-	-	-	0.09
PLK	0.15	-	-	-	-	0.30	0.28
PEA	1.38	-	3.14	0.12	-	1.66	-
PNT	1.37	0.12	3.40	0.06	-	1.98	0.38
RBO	0.61	0.11	0.77	-	-	0.41	0.38
RIO	0.75	-	0.33	-	-	0.48	-
RWO	0.73	-	0.26	-	-	0.37	-
SHO	0.41	0.12	1.14	0.05	-	0.31	0.18
SUN	0.30	0.09	1.16	-	-	0.31	0.40
SUR	0.30	-	0.78	-	-	0.22	-
SOY	0.41	-	0.48	-	-	0.21	-
SYB	0.35	0.12	0.67	-	-	0.24	0.07
SYS	0.40	-	0.42	-	-	0.11	-

[1]Oils abbreviations see Table 3.1.1.