THE EFFECT OF MINOR COMPONENTS ON THE FRYING STABILITY OF REGULAR AND MODIFIED VEGETABLE OILS

BY

LAURA NORMAND

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

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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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Abstract

A study was conducted to compare the relationship between frying stability and levels and degradation rates of selected minor components in regular and modified canola, soybean and sunflower oils. Oils evaluated included regular (RCO), high oleic (HOCO), high oleic low linolenic (HOLLCO) and low linolenic (LLCO) canola oils; regular (RSY) and low linolenic (LLSY) soybean oils; and regular (RSO) and high oleic (HOSO) sunflower oils.

Oils were heated at 175 ± 2°C for a total of 72 hours, with french fries fried in the oil intermittently. Frying stability was compared based on rates of formation of free fatty acids (FFAs) and total polar components (TPCs). Significant differences ($p<0.05$) between oils were identified using analysis of covariance (ANCOVA) and t-tests for multiple comparisons. HOSO displayed a significantly faster rate of FFAs formation than did RSO. Among the canola oils, LLCO and HOCO displayed significantly faster rates of TPCs formation than HOLLCO and RCO. Among the soybean oils, LLSY had a significantly faster rate of TPCs formation than did RSY. Despite reductions in polyunsaturated fatty acid (PUFA) content, the modified oils showed no significant improvement in stability and, with the exception of HOLLCO, were significantly less stable than the corresponding regular oil.

Tocopherols may be important in frying stability as they can act as antioxidants to slow down oxidative deterioration. Of the canola oils, HOLLCO with the highest level of tocopherols exhibited a slower rate of degradation. RCO, with much lower levels of tocopherols, however, had the slowest tocopherol degradation rate. This could explain the greater frying stability of RCO and HOLLCO compared with HOCO and LLCO. RSY

and RSO were both higher in tocopherols than the corresponding modified oils (LLSY and HOSO) and exhibited slower rates of tocopherol degradation.

Certain phytosterols have been reported to act as antioxidants in oil systems heated to frying temperatures. Although phytosterol contents and degradation rates differed among oils, they could not explain the variability in frying stability.

The fresh oils were analyzed for metal content. Levels of Cu and Fe were near or below the detection limit, while Ni was not detected in any of the oils. While these metals are known to act as prooxidants, an effect of metals on frying stability was not determined in the current study.

The results of the current study showed that fatty acid composition was not the sole determinant of frying stability in these oils. Variations in tocopherol levels and degradation rates among the oils appeared to explain some of the differences in frying stability. In some cases, higher levels and/or slower rates of tocopherol degradation resulted in greater frying stability, despite higher levels of PUFAs. For example, HOSO and RSO both showed similar frying stability performance based on TPCs in spite of marked differences in PUFA content. The beneficial effect of high oleic acid levels in HOSO was lost due, in part, to much lower levels and faster degradation rates of tocopherols. Consequently, predicting frying stability on the basis of fatty acid composition alone is inadequate when other minor components such as tocopherols are not taken into consideration. However, differences in frying stability could not be totally explained based on the components evaluated in the current study. Future studies should focus on other factors which may contribute to frying stability.

Acknowledgments

I would like to express my deepest appreciation to my advisor, Dr. N.A.M. Eskin, for his excellent guidance, encouragement, enthusiasm and financial support during the completion of this project. I would also like to thank Dr. L. Malcolmson for her very active interest and support, as well as numerous contributions as a committee member. Appreciation is also extended to Dr. J. Daun for agreeing to serve on the committee, and for his contributions and interest in the project.

I would also like to thank Dr. R. Przybylski for his assistance and contributions, particularly during laboratory analysis and interpretations. Sincere thanks are extended to Marilyn Latta for her support and assistance in the lab. Appreciation is also expressed to Donna Ryland for her interest and assistance during the project. Thanks are also extended to Eugene Gawalko for performing metals analysis; and to Sherilyn Andres for providing statistical advice.

The friendship and support of my fellow students; Wei Li, Rui Zambiazi, Armando Conca Torres, Joanne McKinstry, Elaine Sopiwnyk, Ann Kiunga and many others; is deeply appreciated.

The financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

I would like to express my deepest appreciation to my parents for their support and for the sacrifices they have made to allow me the opportunity to pursue my goals.

This thesis is dedicated to my husband, Joe, in appreciation of the unwavering love, encouragement, support, patience and self-sacrifice shown to me throughout this project.

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Chapter 1. Introduction

The foodservice industry, which includes restaurants, hotels and various institutions, consumes a large proportion of edible vegetable oils. Figure 1.1 provides a breakdown of the uses of edible fats and oils in the foodservice industry (O'Brien, 1993). with the primary use as a frying medium (O'Brien, 1993). There has been a trend toward using liquid vegetable oils, which are high in mono- and polyunsaturated fatty acids, in place of solid, saturated animal fats. High levels of polyunsaturated fatty acids (PUFAs) make vegetable oils more susceptible to oxidative degradation. Oilseeds have been modified by changing fatty acid composition to improve stability and frying performance. In particular, oils have been modified to reduce their linolenic acid content and/or increase their oleic acid content (Erickson and Frey, 1994).

A number of studies have reported that modified vegetable oils show some improvement in frying stability over corresponding regular oils (Miller and White, 1988; Eskin et al., 1989; Liu and White, 1992; Dobarganes et al., 1993; Warner and Mounts, 1993; Warner et al., 1994; Romero et al., 1995b). However, no significant improvements in frying stability have also been reported by a number of researchers studying modified vegetable oils (Liu and White, 1992; Warner and Mounts, 1993; Mounts et al., 1994a; Mounts et al., 1994b). These findings suggest that fatty acid composition alone may not adequately explain the stability of frying oils.

Breeding of oilseeds to improve stability has tended to ignore minor components, focusing solely on levels of various fatty acids present in the resulting oil. However, as cited previously, the relative frying stability of an oil cannot always be accurately predicted based on its fatty acid composition alone. Therefore, it appears that minor

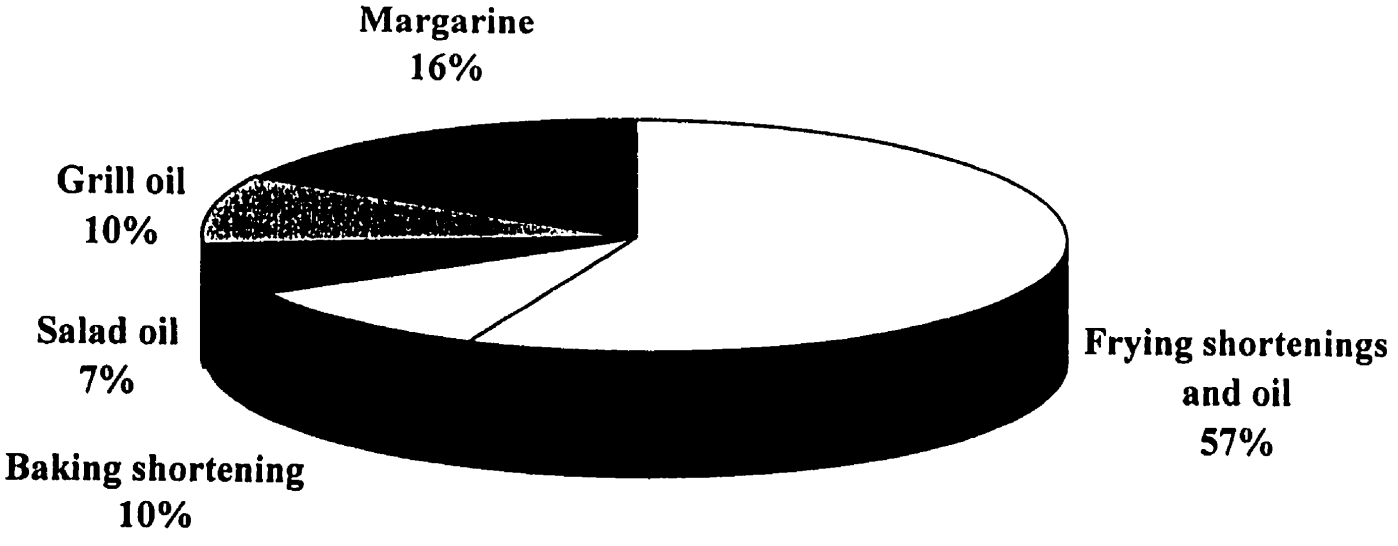


Figure 1.1. Foodservice uses for edible fats and oils (as % of total weight)(adapted from O'Brien, 1993)

components must play a significant role in oil stability, particularly if levels of these components are changed substantially during modification. Therefore, the current study was designed to compare the composition and frying stability of regular and modified canola, soybean and sunflower oils. In addition, an attempt was made to relate differences in composition, particularly of the minor components, to frying stability. The specific objectives of the study were:

1. to characterize regular and modified oils in terms of fatty acid composition, tocopherols, phytosterols and metals;
2. to determine the frying stability of these oils;
3. to relate differences in frying stability to one or more of the following factors: fatty acid composition; initial tocopherol level; rate of tocopherol degradation; initial phytosterol level; rate of phytosterol degradation; and metal content.

Chapter 2. Review of Literature

2.1 The Reactions of Frying

Frying (or specifically deep fat frying) is defined as submerging a food in a heated oil or fat which is exposed to air (Gutierrez et al., 1988). Typically, temperatures between 175-185°C are employed during frying (Berger, 1988). In addition to being the medium for heat transfer, the oil also influences the flavor and quality of the food product being fried (Perkins, 1992).

Frying oils are commonly reused for several frying cycles before being discarded. Therefore, it is particularly important to examine the changes which occur in the oil over the frying cycles. In very broad terms, the degradative products resulting from the frying process can be classified as volatile or nonvolatile decomposition products (Melton et al., 1994). Volatile decomposition products (VDPs) are responsible for the flavors and odors of deep fried foods and include aldehydes, acids, alcohols, ketones, esters, furans (Wu and Chen, 1992) and hydrocarbons (White, 1991). These components often evaporate from the oil, unlike nonvolatile components which tend to accumulate in the oil (Billek, 1988). Nonvolatile decomposition products (NVDPs), which include cyclic and noncyclic monomers, dimers and polymers (White, 1991), are more frequently measured to provide an indication of the amount of destruction the fat has experienced (Melton et al., 1994).

The process of frying is influenced by many variables; the type and amount of food being fried; the capacity and maintenance of the fryer; the type of frying oil used; the turnover rate of the oil (Stevenson et al., 1984); the surface to volume ratio of the oil; and whether heating is intermittent or continuous (Boskou, 1988). The oil is altered during frying due to its interactions with the food as well as with the atmosphere (Berger, 1988).

The major changes which occur in an oil during frying are shown in Figure 2.1 (Fritsch, 1981). These changes can be classified as hydrolytic, oxidative or thermolytic in nature (Gutierrez et al., 1988). Polymerization can occur in combination with these changes, resulting in the formation of high molecular weight compounds through condensation reactions (Fedeli, 1988). These changes are not mutually exclusive but interrelated. The rate of each type of reaction in a particular frying operation depends on the combination of all of the variables listed previously (Fritsch, 1981). Therefore, many different methods are used in combination to describe the extent of oil degradation which has taken place (Fritsch, 1981).

2.1.1 Hydrolytic Changes

Hydrolytic changes are produced by the destruction of ester bonds by water droplets, steam formed from water in the food, or fried material (Pokorny, 1989). The solubility of water in oil is much greater at frying temperatures compared to room temperature (Labuza, 1971; Pokorny, 1989) A typical hydrolysis reaction is depicted in Figure 2.2 (Perkins, 1996). Hydrolysis results in the release of free fatty acids (FFAs), monoglycerides, diglycerides and glycerol (Gutierrez et al., 1988). These products are lower in molecular weight but higher in polarity than triglycerides (Dobarganes and Marquez-Ruiz, 1996). FFAs are volatile and tend to be released from the oil in steam (Perkins, 1996). Water from food also adds oxygen to the oil, leading to oxidative as well as hydrolytic changes (Perkins, 1967).

2.1.2 Oxidative Changes

Oxidative changes are caused by interaction of the frying oil with oxygen (Gutierrez et al., 1988). When water from the food is converted to steam, its movement

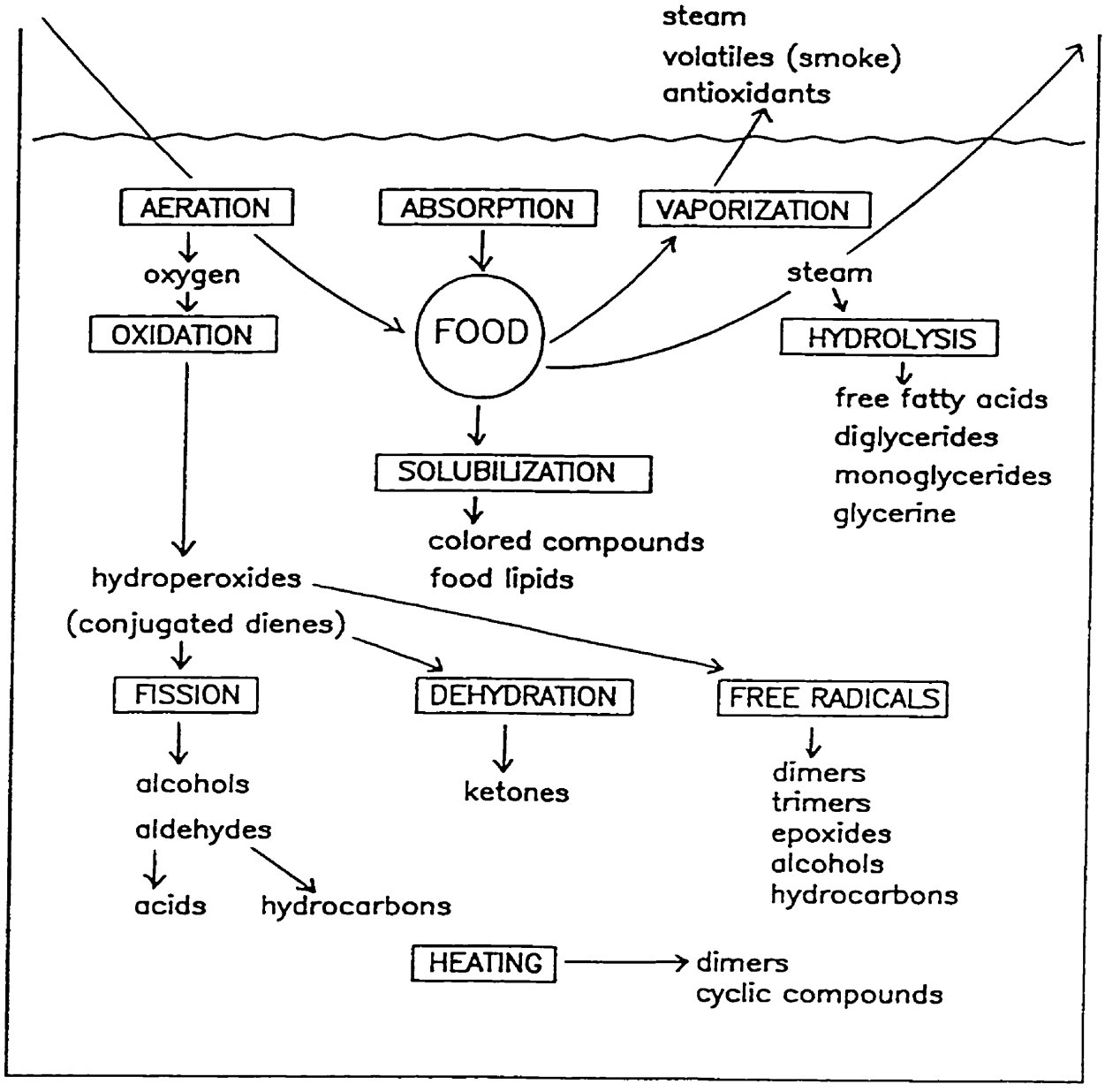


Figure 2.1. Major changes occurring during frying (Fritsch, 1981)

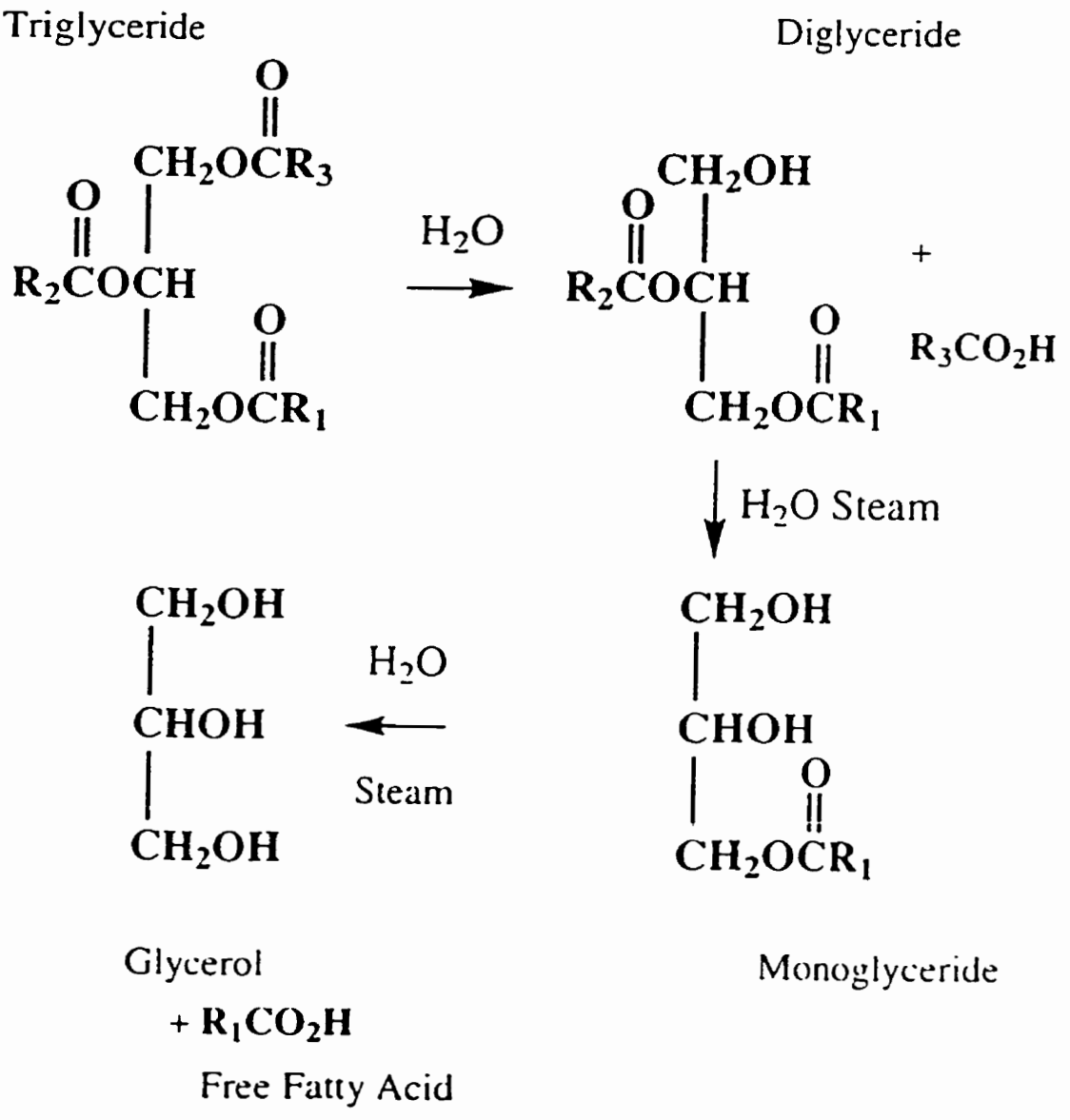


Figure 2.2. Hydrolysis of a triglyceride (Perkins, 1996)

through the oil increases aeration (Melton et al., 1994). Overall, however, the solubility of oxygen in oil at frying temperature is low (Pokorny, 1989). Therefore the amount of surface area available for air to oil contact is an important determinant of the extent of oxidation (Pokorny, 1989).

The oxidative process can be viewed as a chain reaction initiated by the formation of free radicals (Figure 2.3). A free radical is formed when a hydrogen atom located on the carbon next to a double bond is removed from an unsaturated fatty acid (Billek, 1988). A free radical contains an odd number of electrons and is extremely reactive (Labuza, 1971). The formation of a free radical may be catalyzed by factors such as temperature, light and metals (Artman, 1969; St. Angelo, 1996). This first stage of oxidation is referred to as the initiation phase (Frankel, 1980).

In the propagation stage of oxidation, free radicals react with oxygen to produce peroxy radicals. These radicals then react with other fatty acids to form hydroperoxides and more free radicals (Billek, 1988). Thus a chain reaction occurs as free radicals are continually produced and then react to produce more free radicals. Hydroperoxides are the primary products of the oxidative process. They are very unstable and rapidly break down at the high temperatures of frying, forming secondary oxidation products (Billek, 1988; Pokorny, 1989; Perkins, 1996). As indicated in Figure 2.1, hydroperoxides can undergo fission reactions to produce alcohols, aldehydes, acids and hydrocarbons; or dehydration reactions to form ketones (Fritsch, 1981). In addition, free radicals react to produce dimers, trimers, epoxides, alcohols and hydrocarbons (Fritsch, 1981).

Termination is the final stage of oxidation which occurs when free radicals react to form nonradical products (Frankel, 1980). Many of the secondary oxidation products

Initiation

$$RH \xrightarrow{\text{initiator}} R^{\cdot} + H^{\cdot}$$

Propagation

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

Termination

$$R^{\cdot} + R^{\cdot} \longrightarrow RR$$

$$R^{\cdot} + ROO^{\cdot} \longrightarrow ROOR$$

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow ROOR + O_2$$

Figure 2.3. The free radical mechanism of oxidation (Eskin et al., 1996); where RH is the unsaturated lipid, $R^{\bullet}$ is the lipid radical, $H^{\bullet}$ is hydrogen, O_2 is oxygen, $ROO^{\bullet}$ is the peroxy radical, ROOH is hydroperoxide, and RR and ROOR are nonradical products

are volatile and evaporate from the oil, while others accumulate in the oil (Billek, 1988) and in the fried food (Boskou, 1988; Warner et al., 1994). The formation of these compounds is the end result of the oxidative process (Billek, 1988).

The susceptibility of an oil to oxidation is dependent mainly on the amounts of linolenic (C18:3) and linoleic (C18:2) fatty acids (Warner and Mounts, 1993; Erickson and Frey, 1994). In general, the rate of oxidation of a fatty acid increases as the number of double bonds it contains increases (White and Miller, 1988). Linolenic acid oxidizes 20 to 30 times faster than oleic acid, and linoleic acid oxidizes 10 times faster than oleic acid (Labuza, 1971). The rationale behind this is that a methylene group located next to a single double bond (as occurs in oleic acid) is less likely to be oxidized than the same group located between two double bonds (as occurs in PUFAs such as linolenic acid) (Artman, 1969).

Figure 2.4 provides an example of how hydroperoxides can be produced from an unsaturated fatty acid, linoleic acid (Labuza, 1971). As Figure 2.4 indicates, hydroperoxides can then decompose to form a wide range of volatile and nonvolatile secondary oxidation products. These can include ketones, aldehydes, hydrocarbons, acids, alcohols, esters, lactones and epoxides (Artman, 1969; Labuza, 1971). Mechanisms of formation of hydroperoxides from oleate and linolenate are provided in Figure 2.5 (Frankel, 1980). As with linoleate, these hydroperoxides then decompose to form similar secondary oxidation products.

Pokorny et al. (1976) reported that, when heated to 210°C with exposure to air, oils with high levels of PUFAs (soybean oil: 44.2%; sunflower oil: 52.4%) resulted in higher levels of trimers and tetramers than oils with medium PUFA content (peanut oil:

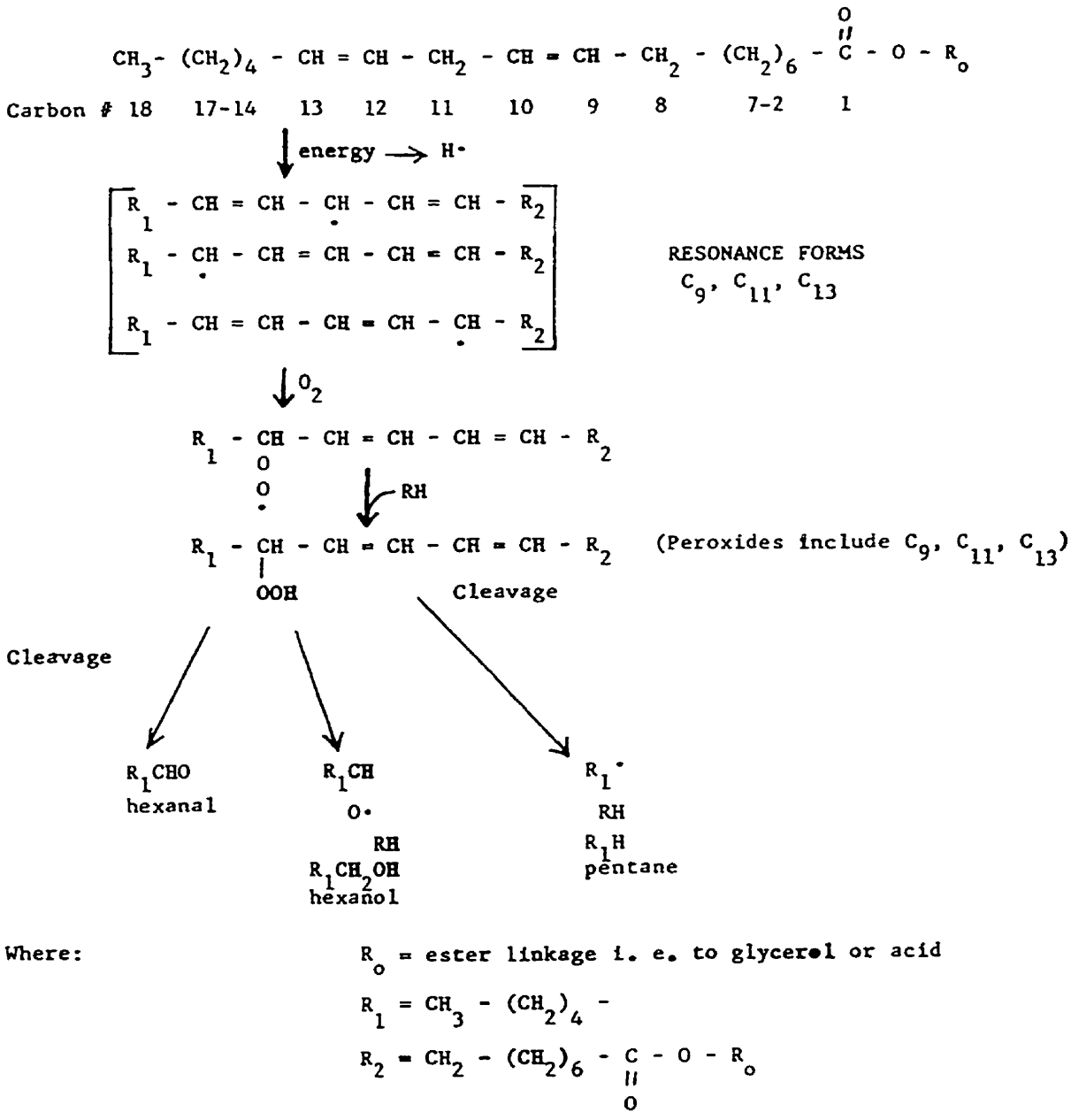


Figure 2.4. Formation of hydroperoxides and secondary oxidation products from the oxidation of linoleate (Labuza, 1971)

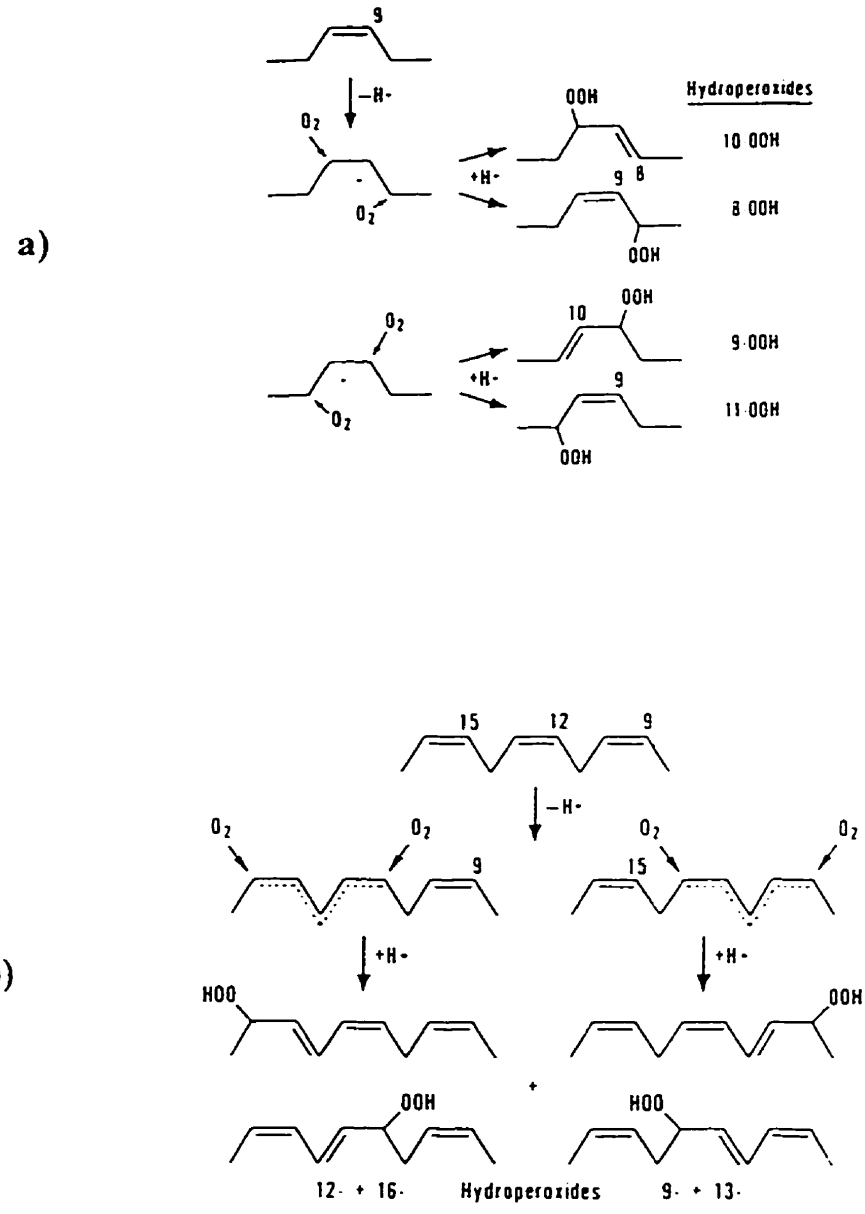


Figure 2.5. Formation of hydroperoxides from a) oleate and b) linolenate during oxidation (Frankel, 1980)

26.8%; rapeseed oil: 19.4%; mustard seed oil: 20.2%). Therefore, oils with higher PUFA content experienced more extensive polymerization.

2.1.3 Thermolytic Changes

Thermolytic changes occur in unsaturated fatty acids as the result of exposure to high temperature when no oxygen is present (Perkins, 1967). Artman (1969) identified three classes of products which can be formed from fats and oils when they are heated without exposure to oxygen. These three classes include:

1. volatile components (which include aldehydes, ketones, alcohols, acids, esters and hydrocarbons),
2. monomers (which consist of approximately the same amount of carbon atoms as the original fatty acid), and
3. dimers and polymers (which consist of at least two fatty acids joined together).

Monomers can occur in the form of cyclic monomers, which are formed by the reaction of the fatty acid with another section of itself to form a ring structure (Artman, 1969; White, 1991). A possible mechanism resulting in the production of a cyclic monomer is shown in Figure 2.6 (Paquette et al., 1985). Sebedio et al. (1989) heated two oils, one high in linolenic acid (linseed oil) and one high in linoleic acid (sunflower oil) to 240°C for 10 hours under both nitrogen and air. The researchers looked at the structures of the cyclic fatty acid monomers formed in each oil. Under either air or nitrogen, linseed oil contained both cyclopentane and cyclohexane monomers, while sunflower oil contained mainly cyclopentane monomers.

Thermal dimers are usually in the form of cyclic dimers produced by Diels-Alder reactions as shown in Figure 2.7 (Perkins, 1992; Dobarganes and Marquez-Ruiz, 1996).

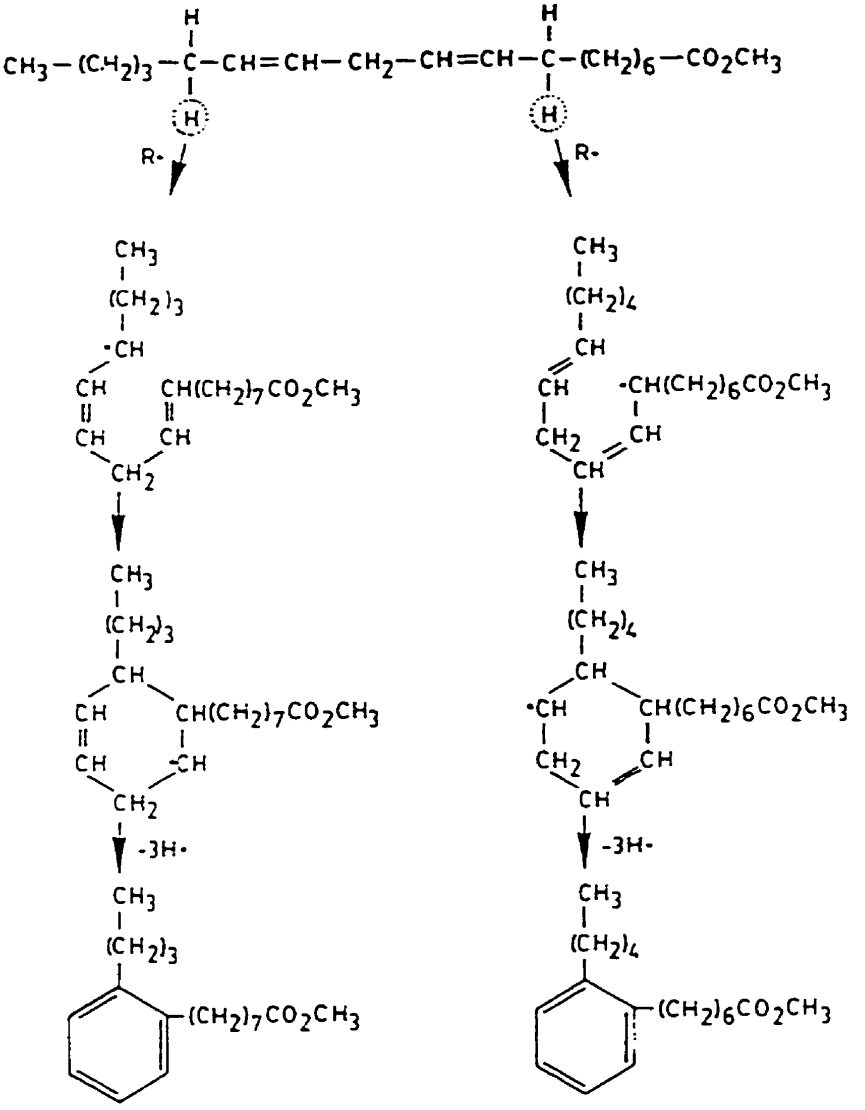


Figure 2.6. Possible mechanism for the formation of cyclic monomers from methyl linoleate (Paquette et al., 1985)

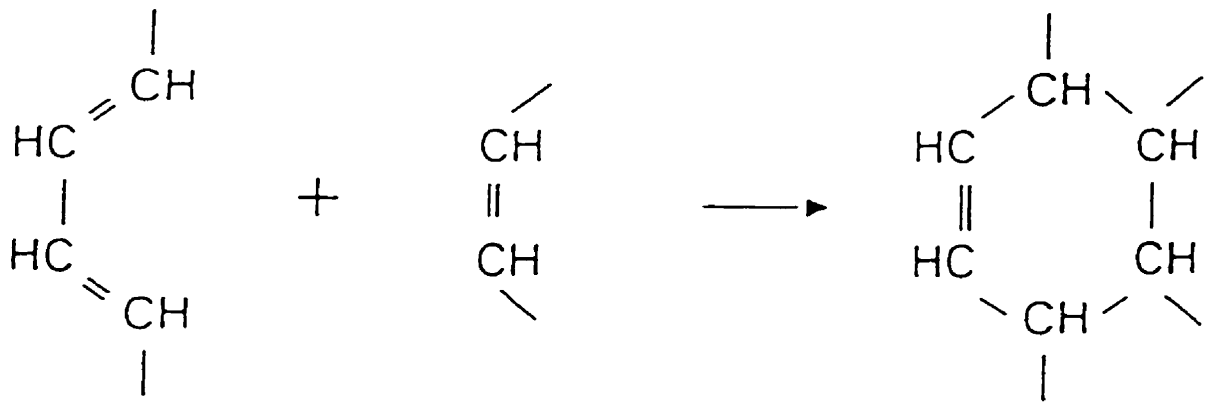


Figure 2.7. Formation of a cyclic dimer by the Diels-Alder reaction (Dobarganes and Marquez-Ruiz, 1996)

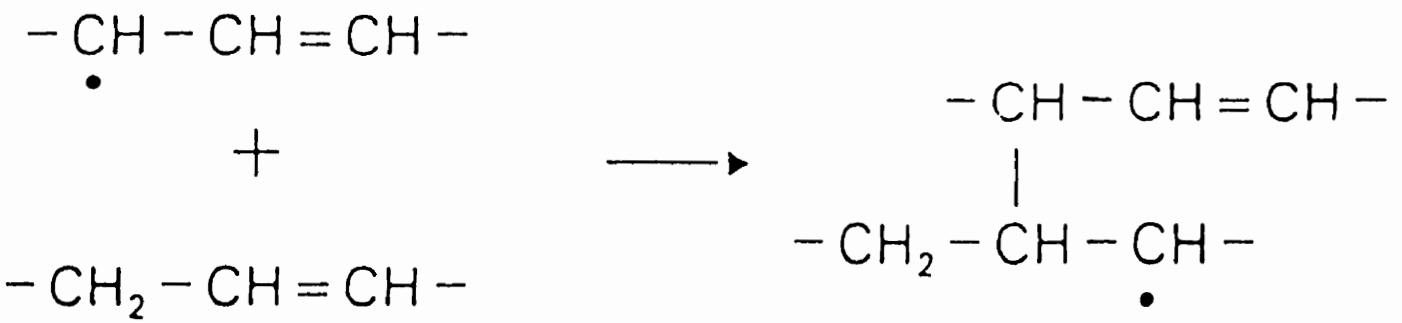
The two types of dimerization which can occur between fatty acids which are part of a triglyceride are shown in Figure 2.8 (Dobarganes and Marquez-Ruiz, 1996). Intermolecular dimerization occurs when the two fatty acids involved belong to separate triglycerides (Artman, 1969; Pokorny et al., 1976). When the fatty acids belong to the same triglyceride, the dimerization is referred to as intramolecular (Artman, 1969; Pokorny et al., 1976). A third type of dimerization can occur following intramolecular dimerization when one of the fatty acids involved becomes esterified to another triglyceride. The resulting dimer is identical to an intermolecular dimer but formed in a different manner (Artman, 1969). Further reactions of dimers can result in the formation of trimers and higher polymers (White, 1991).

Dimers and polymers can result from oxidative as well as thermolytic changes, although the mechanism of formation is different. Carbon to carbon bonds link together dimers and polymers formed thermolytically. Oxidative change can result in linkages formed by oxygen bridges (Artman, 1969). Therefore dimers produced by oxidation are more polar in nature (Paquette et al., 1985).

2.2 Methods to Monitor the Degradation of Frying Oils

Since frying is considered a complex process, a combination of methods are usually employed to determine the extent of degradation of a frying oil (Fritsch, 1981). Melton et al. (1994) have identified a number of measurements that are based on the formation of unstable or VDPs, including: conjugated dienes (CDs), FFAs, peroxide value (PV), total carbonyls and dienals, acid value and total volatiles (TVs). Measurements based on stable or NVDPs include total polar components (TPCs), fatty acid composition, polymeric triglycerides and iodine value (IV) (Melton et al., 1994).

a) Intermolecular addition:



b) Intramolecular addition:

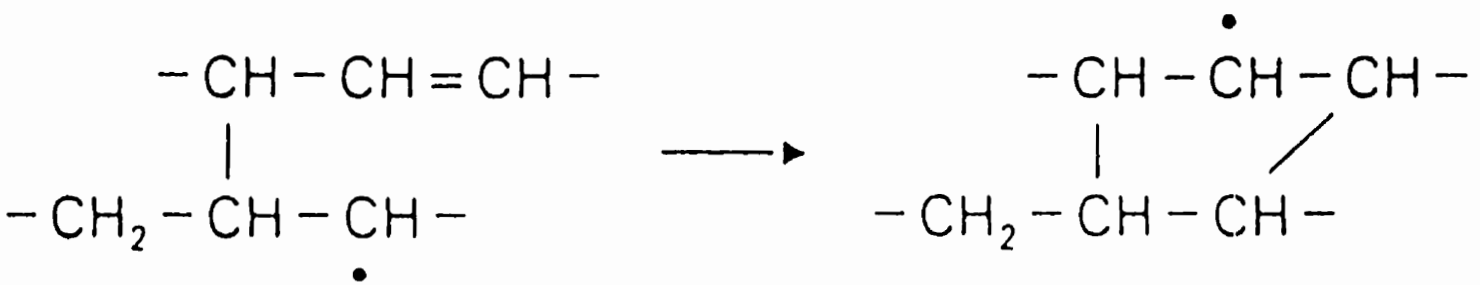


Figure 2.8. Formation of dimers between fatty acids by a) intermolecular and b) intramolecular dimerization (Dobarganes and Marquez-Ruiz, 1996)

Methods measuring NVDPs are generally viewed as being more reliable and dependable indicators of oil degradation than those based on VDPs (Melton et al., 1994). One further method of measuring oil deterioration is by sensory evaluation of the color, flavor and acceptability of the oil (Melton et al., 1994). Sensory evaluation is the method many restaurants rely on, and usually one person will decide if the oil is still acceptable for use (Melton et al., 1994).

2.2.1 Peroxide Value (PV)

Hydroperoxides (or peroxides) are one of the primary products of oxidation. PV determination using AOCS official method Cd 8-53 (1990) involves dissolving the fat in an acetic acid:chloroform solution and adding potassium iodide, which will react with bound oxygen. The iodine liberated during this reaction is then titrated with sodium thiosulphate until the endpoint is reached, as indicated by a color change (Kirk and Sawyer, 1991). Although still used, PV determination is not recommended for assessing frying oil deterioration as peroxides are unstable and levels may change between heating and analysis (Fritsch, 1981). In addition, peroxides quickly break down at the high temperatures of frying to form secondary oxidation products (Peled et al., 1975).

2.2.2 Free Fatty Acids (FFAs)

FFAs are considered poor indicators of frying oil degradation (Fritsch, 1981) since they are volatile and can be lost from the frying oil in the steam produced (Melton et al., 1994). In addition, the titration method does not distinguish between FFAs resulting from hydrolytic and oxidative changes (Fritsch, 1981). Formation of FFAs results mainly from hydrolysis but also can arise from the formation of acidic oxidation products (Berger, 1988). However, the level of acidity present in a frying oil is due to the presence

of both FFAs and carboxylic groups in polymeric products (Peled et al., 1975). Peled et al. (1975) displayed these separate sources of acidity by removing the FFAs from used frying oil and remeasuring the level of acidity. Nonetheless, FFA determination is a commonly used method to assess frying oil deterioration. AOCS official method Ca 5a-40 (1989) for determination of FFAs involves an acid-base titration and an indicator solution to identify the endpoint. This method lacks precision and is subjective as the color change at the endpoint is not easily detectable with the designated indicator solution. An alternative method for FFA determination is the Veri-Fry® Pro FFA-75 quick test (Test Kit Technologies, Inc., Metuchen, New Jersey, USA). The Veri-Fry® method is a much simpler, quicker and more objective method for determining FFAs in frying oils. The hot oil is placed in the reagent gel tube, mixed, heated, and its absorbance read at 610 nm. FFA levels are then determined using a calibration curve, developed from samples of known concentrations, relating FFA content to absorbance reading.

2.2.3 Fatty Acid Composition

If heating time, frying temperature, oil replenishment level and all other factors are held constant, a frying oil's stability will decline as the initial level of unsaturation of its fatty acids increases (Melton et al., 1994). The fatty acid profile of an oil is usually determined using gas chromatography (GC). Prior to injection on the column, the triglycerides are transformed into methyl esters to increase their volatility. The changes in particular fatty acids can be monitored by looking at the oil's fatty acid composition over time. A decline in unsaturated fatty acids over time indicates that the oil is being broken down.

2.2.4 Iodine Value (IV)

IV is a measure of the total level of unsaturation of an oil (Rossell, 1986). IV determination is based on the ability of iodine to react at the sites of unsaturation in an oil, and is defined as the grams of iodine that will combine with 100 g of oil (Potter, 1986; Orthoefer and Cooper, 1996). As an oil is exposed to frying temperatures, its level of unsaturation and corresponding IV will decrease as oxidation occurs at the double bonds (Potter, 1986). Oils with high IVs tend to oxidize more rapidly during deep frying (Orthoefer and Cooper, 1996). Waltking and Zmachinski (1970) determined that a decrease in IV does in fact correspond to a decrease in PUFA content of heated oils.

2.2.5 Conjugated Dienes (CDs)

CDs are formed when PUFAs are oxidized and one of the double bonds shifts (White, 1991). CDs, like hydroperoxides, are primary oxidation products. The level of CDs can be measured as these structures absorb ultraviolet light at a wavelength of 232 nm (White, 1991). Absorbance will increase during the initial phase of frying and then plateau as a balance between CD formation and conversion to secondary oxidation products occurs (White, 1991). CD levels eventually decrease as the primary product is destroyed (Perkins, 1967).

2.2.6 Total Polar Components (TPCs) and Polymers

Blumenthal (1991) provides a definition of the TPCs of an oil as being "the sum total of those materials which are not triglycerides". Melton et al. (1994) point out that TPCs includes all materials present in the oil which are more polar than triglycerides. TPC levels are commonly used to indicate the extent of oil degradation during frying (Melton et al., 1994). Paradis and Nawar (1981) compared four methods of assessing the

extent of frying oil degradation. Of the four methods compared (i.e. GC of triglyceride dimer esters, PV, dielectric constant and column chromatography of TPCs), the column chromatography method was the most sensitive to variations in the oil. TPCs increased as frying time continued (Paradis and Nawar, 1981). However, the column chromatography method for TPCs determines the total level of polar material without distinguishing between specific compounds (Paradis and Nawar, 1981). High performance size exclusion chromatography (HPSEC) separates molecules based on their molecular weight and shape (Christopoulou and Perkins, 1989). HPSEC is a method which monitors TPCs in general, as well as specific alteration products such as oxidized triglycerides and triglyceride dimers and polymers (Lopez-Varela et al., 1995). Polymers are high molecular weight compounds which accumulate in an oil during frying (Fedeli, 1988), causing foaming and increased viscosity (O'Brien, 1993). A measurement of the specific alteration compounds is important as it provides information about what types of reactions have occurred and to what extent. For example, levels of diglycerides and FFAs indicate the level of hydrolytic alteration which has occurred (Dobarganes et al., 1988; Lopez-Varela et al., 1995). Using HPSEC, Lopez-Varela et al. (1995) determined that thermoxidative reactions, as indicated by high levels of triglyceride dimers and polymers, were far more extensive than hydrolytic reactions when frying french fries in sunflower oil. Polar material and polymers have been identified as the chief products of frying oil degradation (Lopez-Varela et al., 1995).

In a laboratory experiment, Marquez-Ruiz et al. (1995) heated sunflower oil (SO), high oleic sunflower oil (HOSO) and palm olein (PO) in 1-L capacity electric fryers for a period of five hours, with intermittent frying of potatoes. Although levels of TPCs were

similar among the oils at five hours, analysis by HPSEC revealed differences between the oils in terms of specific alteration compounds. In all oils, they found that the levels of FFAs and diglycerides did not change significantly, whereas levels of dimers and polymers increased over frying time. Quantification of their results allowed the researchers to conclude that SO, which contained the highest level of PUFAs, developed the highest levels of dimers and polymers.

2.3 Modification of Vegetable Oils

One method of modifying vegetable oils uses transgenic techniques. In general, these techniques focus on altering triglyceride composition through modification of chain length and degree of unsaturation of fatty acids (Diehl, 1998). A better understanding of these techniques can be achieved by reviewing the pathway for triglyceride production in plants. Diehl (1998) has simplified this process into four key steps:

1) the appropriate chain length of the fatty acid is achieved, two carbon atoms at time, using additions of acetyl-CoA,
2) unsaturation or production of double bonds in the fatty acid,
3) attachment (by enzymes) of fatty acids to the sn-1 and sn-3 carbons of glycerol, and
4) attachment (by different enzymes than in step 3) of fatty acids to the sn-2 carbon of the glycerol molecule.

A step by step process for triglyceride production is shown in Figure 2.9 (Diehl, 1998). Figure 2.9 also displays enzymes which can be manipulated to alter the fatty acid composition. Briefly, the low linolenic property has been produced in the seed by blocking the delta-15-desaturase (D15D) enzyme which normally converts linoleic to

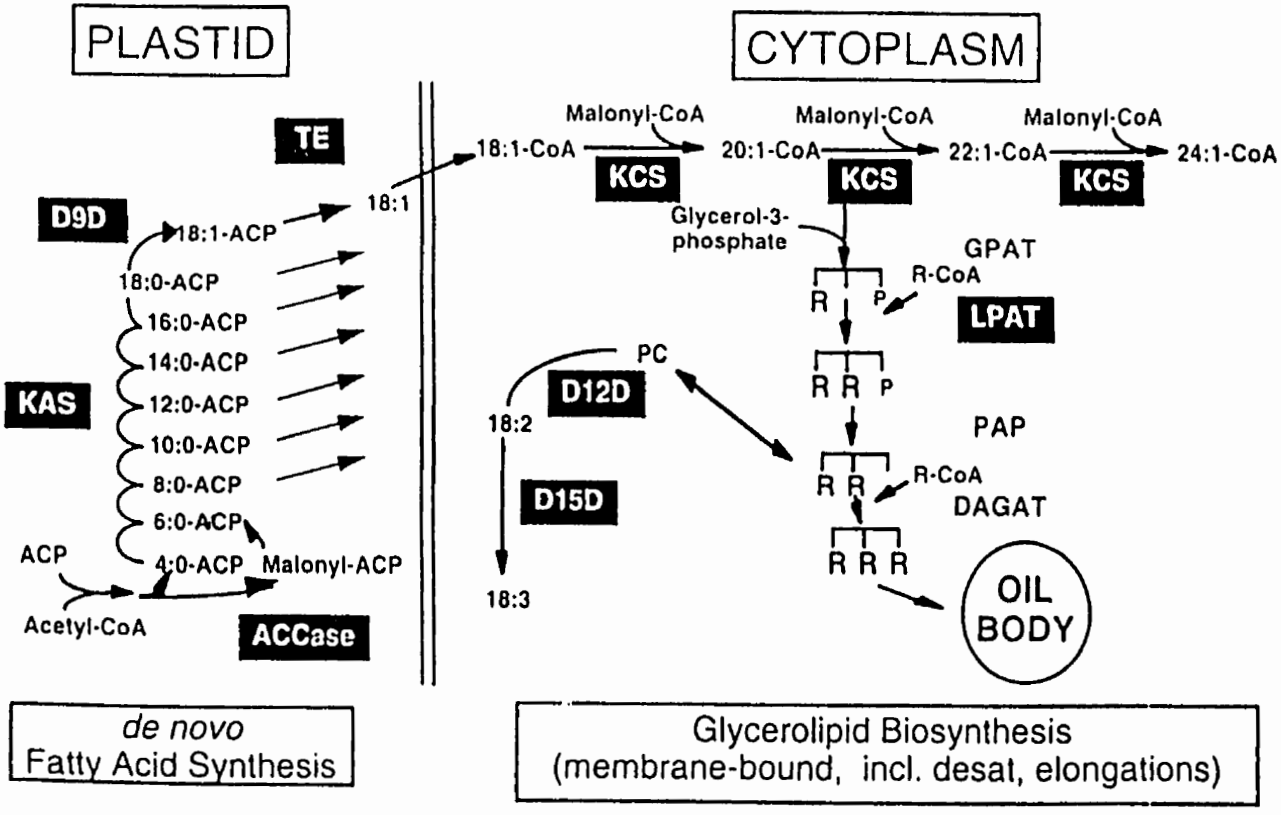


Figure 2.9. Outline of the biosynthetic pathway of seed oil (Diehl, 1998); where ACP = acyl carrier protein; KAS = keto-acyl ACP; D9D = delta-9 desaturase or stearoyl-ACP desaturase; TE = thioesterase; D12D = delta-12 desaturase; D15D = delta-15 desaturase; KCS = keto-acyl CoA synthase; LPAT = lysophosphatidic acid acyl transferase; ACCase = acetyl CoA carboxylase. Enzymes depicted with white lettering on a black background have been manipulated to alter the oil composition of transgenic crops.

linolenic acid by addition of a double bond (Eskin et al., 1996; Diehl, 1998). Similarly, the high oleic property has been produced by blocking the delta-12-desaturase (D12D) enzyme which converts oleic to linoleic acid (Eskin et al., 1996; Diehl, 1998). Many high oleic and low linolenic oils have been developed since the 1980s. For example, high oleic sunflower oil (specified as 77% oleic on a crude basis) has been produced in the United States since 1984 (Haumann, 1994). The first low linolenic canola cultivar (Stellar) was developed at the University of Manitoba and registered in 1987 (Scarth et al., 1988).

Two common method of stopping the expression of a gene, and therefore altering the composition of an oil, are "anti-sense" and "co-suppression" (Fader et al., 1995). The basic process of gene expression is as follows:

transcription translation
DNA ----------> mRNA ----------> protein (Fader et al., 1995)

Basically, the DNA code is "read" to produce complementary mRNA. In turn, the code for the amino acid sequence of a protein, such as an enzyme, is translated from the mRNA sequence. The code to transcribe DNA is read in what is called the sense direction and results in the production of sense mRNA (Fader et al., 1995).

The anti-sense method of modification is described by Fader et al. (1995). When an anti-sense gene is introduced, it is transcribed to produce an anti-sense mRNA. This anti-sense mRNA has a base sequence complementary to the sense mRNA. The two strands of mRNA interact with each other, remain in the nucleus, and degrade with no translation into protein. Using the anti-sense method, the production of a particular enzyme can be blocked to varying extents.

The method of co-suppression for modification is not as well understood as the anti-sense method. This method involves the introduction of a gene which is homologous to the one for which suppression is desired. The result is often suppression of both genes, although the mechanism is unclear (Fader et al., 1995).

2.4 Frying Stability of Modified Oils

A number of researchers have studied the stability of modified oils to frying either by frying food in the fat or by conducting simulated frying studies. In simulated frying experiments, the oil is heated to a typical frying temperature and held at this temperature for a period of time.

2.4.1 Canola Oil

Warner et al. (1994) examined the stability of regular, hydrogenated and four modified canola oils to frying at 192°C for 18 hours (nine hours/day for two days) with intermittent frying of potato chips. The fatty acid compositions of the fresh oils are given in Table 2.1. Results of FFA analysis indicated that the higher the oleic acid level of the oil, the higher the FFA level in the heated oil. The exception was the hydrogenated oil which had the lowest level of oleic acid but had an intermediate FFA level. The hydrogenated oil and one of the modified oils had the highest levels of TPCs after heating. The lowest TPC level was found in the modified oil with the largest amount of oleic acid. These results indicated that the high oleic canola oils were more stable to frying based on TPC measurements (an indication of the extent of polymerization) but less stable to frying using FFA values (an indication of the extent of hydrolysis).

Eskin et al. (1989) used a simulated frying study to determine the stability of three types of canola oil - laboratory refined, deodorized (RD) low linolenic canola oil,

Table 2.1. Summary of fatty acid compositions (% of total fatty acid composition) of oils in various studies

Oil	18:1[a]	18:2[b]	18:3[c]
Warner et al. (1994)			
Canola Oils:			
Regular	61.9	20.6	7.7
Hydrogenated	74.1	11.7	0.8
Modified #1	64.2	23.6	2.8
Modified #2	66.8	21.3	2.9
Modified #3	78.3	8.5	4.2
Blend of two modified #4	68.4	19.7	3.1
Eskin et al. (1989)			
Canola Oils:			
Lab RD[d] low linolenic	67.0	21.5	1.6
Lab RD regular	62.7	17.5	9.0
Commercial RD regular	65.0	18.5	8.5
Petukhov (1996)			
Canola Oils:			
Regular	56.5	22.3	10.8
Hydrogenated	73.7	8.0	-
Low linolenic	58.2	27.9	3.7
High oleic	75.2	8.0	5.5
Warner and Mounts (1993)			
Canola Oils:			
Regular	60.5	20.8	10.1
Low linolenic	65.6	24.0	1.7
Hydrogenated low linolenic	86.3	1.6	0.7
Brush hydrogenated low linolenic	72.9	16.8	0.8
Soybean Oils:			
Regular	24.2	54.4	6.2
Low linolenic	25.0	55.3	3.7
Hydrogenated low linolenic	64.4	20.6	0.4

[a]oleic acid
[b]linoleic acid
[c]linolenic acid
[d]refined, deodorized

Table 2.1.(cont'd). Summary of fatty acid compositions (% of total fatty acid composition) of oils in various studies

Oil	18:0[a]	18:1[b]	18:2[c]	18:3[d]
Liu and White (1992)				
Soybean Oils:				
Regular #1	n.r.[e]	22.6	56.7	6.8
Regular #2	n.r.	25.2	54.8	5.9
Low linolenic #1	n.r.	29.3	49.4	1.5
Low linolenic #2	n.r.	31.8	50.7	1.9
Low linolenic #3	n.r.	29.1	54.7	1.8
Low linolenic #4	n.r.	21.5	40.4	4.1
Canola Oils:				
Regular	n.r.	63.0	21.3	10.3
Mounts et al. (1994b)				
Soybean Oils:				
Commercial regular	n.r.	24.2	54.4	6.2
Commercial hydrogenated #1	n.r.	69.2	10.7	<0.2
Commercial hydrogenated #2	n.r.	37.6	44.2	4.1
Lab reduced linolenate #1	n.r.	49.5	35.0	1.9
Lab reduced linolenate #2	n.r.	28.8	52.3	2.9
Lab reduced linolenate #3	n.r.	26.0	51.6	5.5
Mounts et al. (1994a)				
Soybean Oils:				
Regular	n.r.	23.9	55.0	6.5
Lab low linolenic #1	n.r.	29.6	51.6	1.7
Lab low linolenic #2	n.r.	25.2	50.3	1.9
Lab low linolenic #3	n.r.	26.7	55.9	2.5
Miller and White (1988)				
Soybean Oils:				
Lab regular #1	4.8	24.1	53.7	6.9
Lab regular #2	5.2	22.2	54.5	8.1
Lab low linolenate	4.2	32.6	49.7	3.5
Lab high stearate	20.2	22.0	43.4	5.9

[a]stearic acid
[b]oleic acid
[c]linoleic acid
[d]linolenic acid
[e]not reported

Table 2.1.(cont'd). Summary of fatty acid compositions (% of total fatty acid composition) of oils in various studies

Oil	18:1[a]	18:2[b]	18:3[c]
Romero et al. (1995b)			
Sunflower Oil:			
High oleic	78.3	10.9	n.r.[d]
Cuesta et al. (1993)			
Sunflower Oil:			
Regular	32.4	55.5	n.r.
Dobarganes et al. (1993)			
Sunflower Oils:			
Regular	22.3	65.0	n.r.
High oleic #1	60.3	28.3	n.r.
High oleic #2	75.2	15.2	n.r.
High oleic #3	80.8	9.5	n.r.
Olive Oil:			
Regular	75.7	9.5	n.r.

[a]oleic acid
[b]linoleic acid
[c]linolenic acid
[d]not reported

laboratory RD regular canola oil and commercially RD regular canola oil. The fatty acid compositions of the canola oils are provided in Table 2.1. The oils were subjected to heating at 175 - 185°C for 10 minutes in air. The low linolenic canola oil was found to have the smallest changes in PVs, thiobarbituric acid (TBA), FFAs, total carbonyls and dienals after heating compared to the two regular canola oils, indicating greater stability to frying temperatures.

Petukhov (1996) compared the frying stability of regular (RCO), hydrogenated (HYCO), low linoienic (LLCO) and high oleic (HOCO) canola oils used for frying potato chips over five days (eight hours/day). The fatty acid compositions of the oils are listed in Table 2.1. Rates of formation of FFAs, CDs, polymers and TPCs were compared among the oils. HYCO had a significantly ($p<0.05$) faster rate of FFAs accumulation than LLCO. LLCO and RCO had significantly faster rates of CDs accumulation than HOCO. HYCO had a significantly lower rate of CDs accumulation than all other oils. No significant differences were found among the oils in rates of TPCs or polymers formation. However, the HOCO had a slower rate of TPCs formation than the other oils. Overall, the results indicated that none of the oils was consistently more stable.

2.4.2 Canola and Soybean Oils

Warner and Mounts (1993) conducted a study to compare the frying stabilities of regular, modified and hydrogenated soybean and canola oils. The specific oils and their fatty acid compositions are listed in Table 2.1. The oils were heated to 190°C (eight hours/day for five days) for a total heating time of 40 hours with intermittent frying of french fries. RSB oil showed the greatest degree of hydrolysis of fatty acids, as measured by FFAs, followed by HLLSB and then LLSB oils. For the canola oil samples, the

amount of hydrolysis was significantly higher in the RCA and HLLCA oils compared to the LLCA and BrHLLCA. The researchers also measured the level of TPCs in the oils, which they thought would be related to the level of unsaturation of the oil. This assumption did not hold true, however, since significantly higher levels of TPCs were found in RSB compared to the other two soybean oils even though levels of unsaturation were similar. TPCs in the four canola oils were found to be similar throughout the frying period, but LLCA had the lowest levels of TPCs at every frying time evaluated. Warner and Mounts (1993) found that all modified oils had at least a modest increase in stability over their unmodified counterpart. In addition, they concluded that hydrogenation of low linolenic oils did not further improve the stability of the oil.

2.4.3 Soybean Oil

Liu and White (1992) examined the stability of six soybean oils - four modified to reduce linolenic content and two regular. A regular canola oil was also included in the study. Oils were heated to 175-185°C with intermittent frying of bread cubes. Two 20-hour frying periods, with a 10 hour cooling down period between, were carried out. The oils were evaluated for their initial and final fatty acid compositions, as well as for levels of CDs at 0, 20 and 40 hours of frying. Initial fatty acid compositions of the oils are listed in Table 2.1. After heating for 20 and 40 hours, no significant differences in CD levels were found between oils. Soybean oils with a greater PUFA content (i.e. linolenic + linoleic acid) tended to have higher CD values after 40 hours of frying time. A positive and significant correlation was identified between CD values at 40 hours of frying and initial PUFA content ($r = 0.87$, $p = 0.01$). Canola oil had a significantly higher CD value at 0 hours (likely because of its higher linolenic acid content), but this trend was not

evident at 20 and 40 hours of frying. This may be due to the lower levels of linoleic acid compared with the soybean oils. To summarize, oils lower in linolenic acid content tended to have lower CD values after frying indicating greater stability to frying, although these differences were not significant ($p<0.05$). Significant correlations were found between initial PUFA content and CD value at 40 hours of frying.

Mounts et al. (1994b) examined the stability of low linolenic soybean oils. Oils used were as follows: three laboratory refined reduced linolenate soybean oils, two commercially prepared hydrogenated soybean frying oils, and a commercially prepared regular soybean oil. Fatty acid compositions of the oils are listed in Table 2.1. The oils were heated to 190°C for three days (approximately seven hours each day) with intermittent frying of french fries. No significant differences were found between the oils in terms of FFAs or TPCs at any point evaluated during the study.

Mounts et al. (1994a) also investigated the stability of three laboratory refined low linolenic acid soybean oils and one regular soybean oil. Fatty acid compositions of the oils are listed in Table 2.1. Frying was carried out at 190°C for a total of 20 hours (four days for five hours/day) with intermittent frying of french fries. In agreement with their previously cited study (Mounts et al., 1994b), no significant differences in FFAs or TPCs were found between the oils over the 20 hour frying period.

Miller and White (1988) compared the frying stability of a low linolenate and a high stearate soybean oil with regular soybean oils. Fatty acid compositions of these oils are listed in Table 2.1. All of the oils were laboratory refined. Oils were heated to 175-185°C for a total time of 40 hours (ten hours/day for four days) with intermittent frying of bread cubes. At ten hours of heating time, the two modified oils had significantly

($p<0.05$) lower CD values than the regular oils, indicating less deterioration during heating. At 40 hours of frying time, CD values for all four oils were significantly different from each other ($p<0.05$). The high stearate oil had the lowest CD value, followed by the low linolenate oil and then the two unmodified oils. The modified oils were concluded to be more stable to frying than the unmodified oils.

2.4.4 Sunflower Oil

Romero et al. (1995b) examined the frying stability of high oleic sunflower oil. The oil was heated to 180°C for a total of 16.5 hours with intermittent frying of french fries. To evaluate the extent of deterioration of the oil, they determined TPCs and used HPSEC to determine the amount of specific altered compounds such as polymeric triacylglycerides, oxidized triacylglycerides, and diacylglycerides. A level of 9.2 mg/100 mg oil was achieved for TPCs after 75 fryings of french fries, which they considered to be low in comparison to values reported by Cuesta et al. (1993). Cuesta et al. (1993) found levels of TPCs to be 19.1 mg/100 mg oil for regular sunflower oil after 75 fryings of french fries. In addition, levels of altered compounds as measured by HPSEC were found to be higher in the regular sunflower oil (Cuesta et al., 1993) than in the high oleic oil studied by Romero et al. (1995b). After 75 fryings, levels of triacylglyceride polymers, triacylglyceride dimers and oxidized triacylglycerides were 0.72, 2.64 and 3.60 mg/100 mg oil, respectively, for high oleic oil (Romero et al., 1995b) and 3.44, 7.51 and 6.26 mg/100 mg oil, respectively for regular oil (Cuesta et al., 1993). These lower levels of TPCs and specific altered compounds in high oleic sunflower oil compared to the regular sunflower oil indicated increased stability of the modified oil to frying temperatures.

Fatty acid compositions of the oils used by Romero et al. (1995b) and Cuesta et al. (1993) are listed in Table 2.1.

Dobarganes et al. (1993) investigated the behaviour of three high oleic sunflower oils compared with regular sunflower oil and olive oil upon heating with and without the presence of food in the frying medium. Simulated frying was done by heating the oils to 180°C for five and 10 hours. In the actual frying studies, the oils were maintained between 140 and 200°C over a five hour period with intermittent frying of french fries. Fatty acid compositions of the oils are listed in Table 2.1. In both simulated and actual frying experiments, FFA levels did not change substantially from their initial levels. However, after five and 10 hours of heating in the absence of food, the regular sunflower oil had a significantly ($p \leq 0.05$) higher level of TPCs than the three modified oils. Also after five hours of frying, the modified oil with the highest oleic acid content was found to have a significantly ($p \leq 0.05$) lower level of TPCs than the other three oils. These findings indicated that the modified sunflower oils were more stable to heating at frying temperature than was the regular sunflower oil.

Dobarganes et al. (1993) expected to find lower levels of TPCs in the oils in which french fries were cooked due to the absorption of degradation products into the food and the pattern of lower temperatures on addition of french fries. However, results for these oils paralleled those for the same oils heated for five hours in the absence of food. These results indicated that moisture from the french fries did not affect frying oils to a significant degree.

Dobarganes et al. (1993) also compared the three modified sunflower oils with an olive oil since two of the modified oils had similar oleic and linoleic acid levels to olive

oil. Two of the modified sunflower oils had higher total unsaturated fatty acids and linoleic acid levels than the olive oil. No significant differences, however, were found between these oils in relation to the levels of TPCs formed after heating for five or 10 hours. A third modified sunflower oil, which had a similar level of linoleic acid as the olive oil, had significantly lower TPC levels compared to olive oil heated for five hours. These findings suggest that the fatty acid composition and degree of unsaturation are not the sole predictors of thermal stability of an oil. The authors suggested that the differences in stability may also be attributed to differences in fatty acid triglyceride distribution or in unsaponifiables. Unsaponifiables consist of hydrocarbons, higher alcohols, oil-soluble vitamins, cholesterol and phytosterols (Kirk and Sawyer, 1991).

2.4.5 Conclusions

From these studies, it is clear that many modified oils have improved stability to frying temperatures compared to regular oils. These results, however, are not consistent for all modified oils and are not always significant. Differences between studies may be due to variations in frying procedures and duration of frying. Some of the research indicates that factors other than the fatty acid composition of the oil may contribute to its frying stability. It has been suggested that minor components of the oil may play a role in determining stability to frying. Minor components of oil include phospholipids (PLs), unsaponifiable matter, FFAs, trace metals and pigments (Goh et al., 1985; Orthoefer and Cooper, 1996).

2.5 Role of Minor Oil Components in Stability

2.5.1 Oxidative Activity

Food antioxidants are substances present naturally or added to foods which prevent or inhibit oxidation of lipid components (Labuza, 1971). The presence of antioxidants helps a food maintain its quality in terms of oxidative deterioration (Labuza, 1971). Antioxidants function in very small amounts, usually at 0.01% or less (Hudson, 1990; Madhavi et al., 1996). This ability to function at very low levels is important because antioxidants are susceptible to being oxidized and thus can enhance oxidation when present in high levels (Hudson, 1990).

There are three main types of antioxidants based on their mode of action (Shahidi and Wanasundara, 1992). These include free radical terminators, chelators, and oxygen scavengers (Shahidi and Wanasundara, 1992; St. Angelo, 1996). Free radical terminators are known as primary antioxidants as they react directly with lipid radicals to inhibit oxidation (Gordon, 1990). Secondary or preventive antioxidants, which include chelators and oxygen scavengers, will generally only act as antioxidants if another substance is present (Gordon, 1990; Jadhav et al., 1996).

Free radical terminators or chain stoppers are effective at low concentrations of even 0.01% or less (Madhavi et al., 1996) and, up to a point, antioxidant activity increases with the concentration of the antioxidant (Labuza, 1971). Free radical terminators, or chain-breaking antioxidants, halt oxidation by donating hydrogen atoms to lipid radicals (Gordon, 1990). The mechanism of antioxidant activity is best described in the following reaction by Burton and Ingold (1981):

$$ROO^* + ArOH \rightarrow ROOH + ArO^*$$

where ROO* is the peroxy radical, ArOH is the antioxidant, ROOH is the hydroperoxide and ArO* is the antioxidant radical. To be an effective antioxidant, donation of a hydrogen atom should occur quickly and result in an antioxidant radical which is more stable than the lipid radical or can be transformed into another stable product (Gordon, 1990). Free radical terminator antioxidants have a structure such that the radical formed is low in reactivity and does not react further with lipids (Jadhav et al., 1996). The following reaction results in the termination phase of lipid oxidation and the removal of the antioxidant radical (Burton and Ingold, 1981):

$$(n\text{-}1)ROO^* + ArO^* \rightarrow \text{nonradical products}$$

where ROO* is the peroxy radical and ArO* is the antioxidant radical.

If the antioxidant concentration is increased too much, its reaction with hydroperoxides to produce free radicals occurs more often and can reduce the antioxidant's protective effect (Labuza, 1971). In addition, if the antioxidant is added when the hydroperoxide level is already high, it may show no antioxidant effect (Labuza, 1971). In both of these situations, the tendency is for the antioxidant to act as a prooxidant.

One common type of primary antioxidants are phenolics, which follow the free radical terminator mechanism (Shahidi and Wanasundara, 1992). When the phenolic donates a hydrogen atom to a lipid radical, an unpaired electron remains on the oxygen molecule. The phenoxy radical is then stabilized by delocalization of the unpaired electron around the ring structure (refer to Figure 2.10). The presence of substituent groups on the ring structure of the phenol molecule is required in order for antioxidant

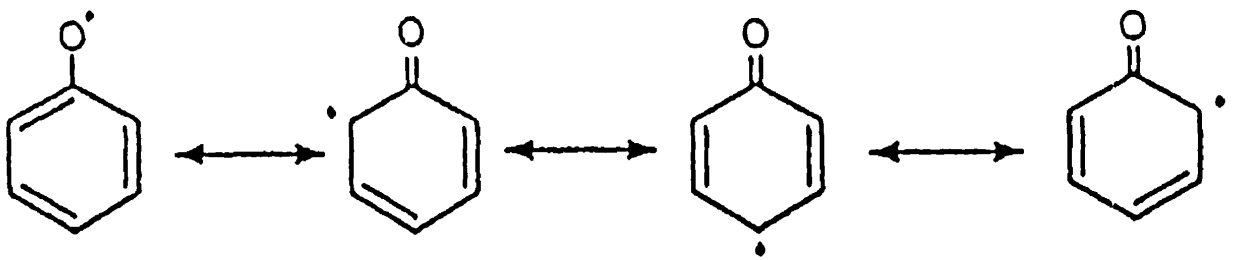


Figure 2.10. Delocalization of an unpaired electron around a phenoxy radical (Shahidi and Wanasundara, 1992)

activity to occur (Gordon, 1990; Shahidi and Wanasundara, 1992). When alkyl groups (as opposed to hydrogen atoms) are present in the 2, 4 or 6 positions, the electron density on the hydroxyl group is increased and so is the ability to react with lipid radicals (Gordon, 1990). The presence of large, bulky groups in the 2 and 6 positions will increase the stability of the antioxidant radical by providing steric hindrance around the radical (Gordon, 1990). However, the presence of these bulky groups will also limit the ability of the phenol to react with lipid radicals (Gordon, 1990). Therefore, bulky substituent groups are both advantageous and detrimental in the ability of phenolics to act as antioxidants. Phenolic antioxidants become prooxidants and are thus involved in the initiation process of oxidation when their concentration is increased to a high level (Jadhav et al., 1996). The following reaction mechanism of a phenolic acting as a prooxidant has been proposed (Schuler, 1990):

$$ROOH + AH \rightarrow ROO^* + AH_2$$

where AH is the antioxidant radical, ROOH is the hydroperoxide, ROO* is the peroxy radical and AH_2 is the antioxidant.

A mechanism for the antioxidant activity of certain phytosterols has also been suggested (Gordon and Magos, 1983). Certain phytosterols have been suggested to act as antioxidants by the donation of a hydrogen atom from an allylic carbon atom, as shown in Figure 2.11. The "sterol radical" formed from this donation is then stabilized by isomerization to a tertiary free radical (Gordon and Magos, 1983). Sims et al. (1972) reported that only phytosterols with a double bond structure in their side chain are capable of acting as antioxidants.

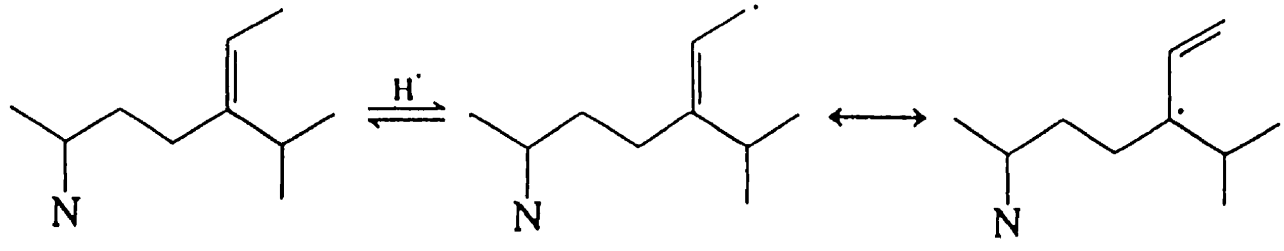


Figure 2.11. Mechanism for the antioxidant activity of sterols (N = sterol ring) (Gordon, 1990 as adapted from Gordon and Magos, 1983)

Secondary antioxidants include metal chelators and oxygen scavengers. Metals such as iron and copper are able to act as prooxidants by reducing the activation energy required to initiate oxidation (Jadhav et al., 1996). Metal chelators act as antioxidants by binding metal ions, thus preventing them from acting as prooxidants (Gordon, 1990). The binding of metal ions by chelating agents increases the activation energy required for the initiation of oxidation (Jadhav et al., 1996). Chelating agents contain an unshared pair of electrons which promotes their ability to form a complex (Dziezak, 1986). Citric acid is an example of a chelating agent which is often added to vegetable oils (Sherwin, 1972; Jadhav et al., 1996). Oxygen scavengers inhibit oxidation by reacting with oxygen, thus reducing the level of oxygen available to induce further oxidation (Kochhar and Rossell, 1990; Jadhav et al., 1996). Ascorbic acid (vitamin C) acts as an oxygen scavenger by reacting with oxygen to produce dehydroascorbic acid (Kochhar and Rossell, 1990; Jadhav et al., 1996).

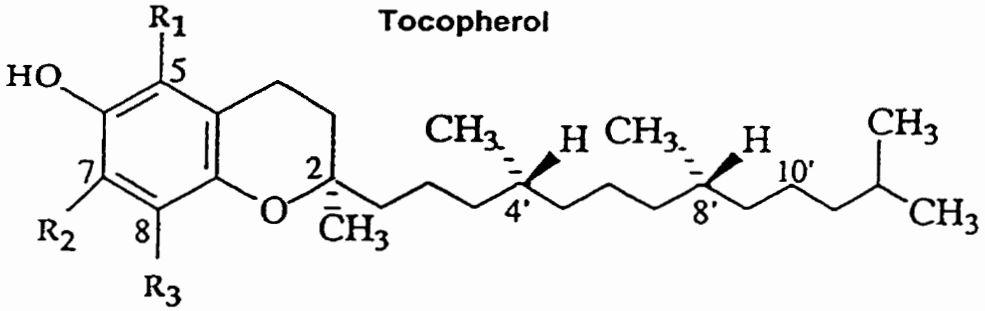
2.5.2 Tocopherols and Tocotrienols

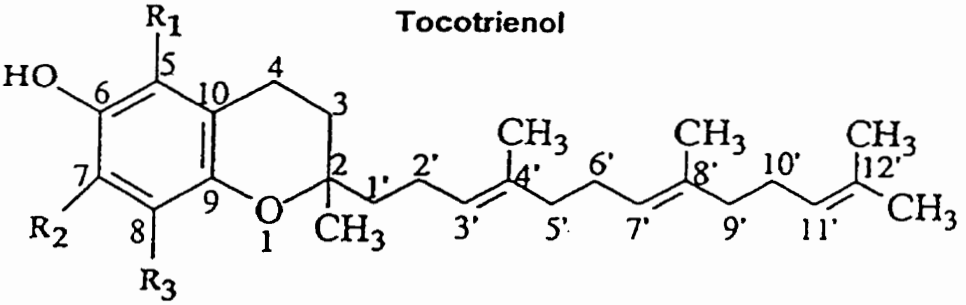
There are eight structurally different compounds in the tocopherol family - four known as tocopherols (or tocols) and four known as tocotrienols (Tomassi and Silano, 1986; Madhavi et al., 1996). Tocopherols are minor components of vegetable oils present in refined oils at a level of about 60 to 110 mg/100 g (Eskin et al., 1996). Tocotrienols do not appear or are present in very small amounts in most vegetable oils, with the exception of a few oils such as palm oil (Tan, 1989; Clark et al., 1990). The basic structure of all eight of these compounds is similar, consisting of a 6-chromanol aromatic ring system containing a hydroxyl group and a 16 carbon phytol side chain (Bauernfeind, 1977; Tomassi and Silano, 1986). The difference between tocopherols and tocotrienols is the

presence of three double bonds in the phytol side chain of tocotrienols (Bauernfeind, 1977; Tomassi and Silano, 1986). Each of the two groups in the tocopherol family consists of an α, β, δ, and γ isomer. These isomers differ in the number of methyl groups present on the aromatic ring (Bauernfeind, 1977; Tomassi and Silano, 1986). The structures of tocopherols and tocotrienols are presented in Figure 2.12.

Tocopherols most commonly act as natural antioxidants by donation of a hydrogen atom from the hydroxyl group on the ring system to a free radical (Sleeter, 1981). Therefore the presence of tocopherols in oil generally increases its stability. Tocotrienols also show antioxidant activity as they contain a hydroxyl group in the same location as occurs in tocopherols (Madhavi et al., 1996). The mechanism of antioxidant activity of tocotrienols is expected to be similar to tocopherols (Kamal-Eldin and Appelqvist, 1996). The various isomeric forms of tocopherols and tocotrienols have varying degrees of antioxidant effectiveness. The activity of each tocopherol isomer also varies with temperature (Madhavi et al., 1996) and concentration (Jung and Min, 1990; Huang et al., 1995; Blekas et al., 1995). The order of antioxidant activity of tocopherols is reported to be $\alpha > \beta > \gamma > \delta$ at 37°C, but reverses at higher temperatures of 50 to 100°C (Madhavi et al., 1996).

Under certain conditions, such as high concentrations or during early stages of oxidation, tocopherols have been reported to exert prooxidant activity in oil (Jung and Min, 1990; Schuler, 1990; Blekas et al., 1995; Huang et al., 1995). Excluding extremely high concentrations of tocopherols, prooxidant activity occurs mainly with α- and β-tocopherol, not with γ- and δ-tocopherol (Schuler, 1990).





Position of methyl groups	Tocopherols	Tocotrienols
5, 7, 8 - Trimethyl	α	α
5, 8 - Dimethyl	β	β
7, 8 - Dimethyl	γ	γ
8 - Monomethyl	δ	δ

Figure 2.12. Structures of tocopherols and tocotrienols (Eitenmiller, 1997)

Oils lower in unsaturated fatty acids generally have a lower content of tocopherols than oils higher in unsaturates. Kamal-Eldin and Andersson (1997) used the technique of principle component analysis (PCA) to identify correlations between tocopherol content and fatty acid composition in 101 oils samples of 14 types. They discovered a significant positive correlation ($r = 0.549$, $p<0.05$) between α-tocopherol and linoleic acid content. Results also suggested a positive, but non-significant, correlation between γ-tocopherol and linolenic acid content. The authors reached the final conclusion, however, that factors other than PUFA content, such as lipid structure and the presence of antioxidants and/or prooxidants, are important in determining tocopherol level in an oil (Kamal-Eldin and Andersson, 1997).

Mounts et al. (1996) compared levels of tocopherols in five low linolenic and one regular crude soybean oil. They found that total tocopherols were lower in four out of the five low linolenic oils than in the regular oil. Also, the relative contribution of individual tocopherols varied between the oils. For example, in one of the low linolenic oils, the percentage contribution of γ-tocopherol to total tocopherols was lower compared to the regular oil. The researchers therefore concluded that modification does appear to alter the tocopherol content and composition of soybean oil. Dolde et al. (1996) examined oil extracted from soybean, sunflower and canola seeds (with diverse genetic backgrounds) for fatty acid composition and tocopherol content. The researchers reported no significant correlation between degree of unsaturation (based on IV) and tocopherol concentration. They suggested that any apparent correlation between these two components in unmodified seed may be due to external factors, such as environmental parameters, which influence their synthesis.

Tocopherols are heat sensitive and readily destroyed by frying (Bauernfeind, 1977). Therefore studies looking at the oxidative activity of tocopherols in oils have focused on autoxidation rather than frying stability. However, Li (1996) investigated changes in tocopherols of oils during heating of oils at a simulated frying temperature of 190 ± 2°C for 72 hours. The oils studied included regular, high oleic, low linolenic and hydrogenated canola oils. The decomposition rates of α- and γ-tocopherols were thought to be related to the amount of unsaturated fatty acids in the oil. High oleic and low linolenic oils, with relatively low levels of unsaturates, experienced slower rates of decomposition of both types of tocopherol than the other two oils during simulated frying. The hydrogenated oil may have experienced decomposition rates similar to the regular oil, even though its level of unsaturation was lower, due to the presence of metals involved in hydrogenation (which may have increased the oxidation of tocopherol). Li (1996) also determined that γ-tocopherol decomposition rate was faster than that of α-tocopherol. However, it was suggested that the particular rates of decomposition would vary with conditions such as temperature, oil type and food type. Gordon and Kourimska (1995a) compared the decomposition rates of α-, β-, and γ-tocopherol in low erucic acid rapeseed oil used for deep frying of potato chips. Results indicated that α-tocopherol was lost at a significantly faster rate than β- or γ-tocopherol. Miyagawa et al. (1991) compared the decomposition rates of α-, δ- and γ-tocopherol during deep frying of potatoes coated with wheat flour, egg and water; or of uncoated french fries; in a mixture of soybean and rapeseed oils. The researchers reported greater losses of all tocopherol isomers during frying of french fries as opposed to frying of coated potatoes. Overall, the decomposition rates of the isomers over both frying processes were $\gamma > \delta \geq \alpha$. However,

it was stated that tocopherol losses would vary from one experiment to another due to differences in oil, type of food fried and heating conditions.

Gordon and Kourimska (1995b) evaluated the effectiveness of added δ-tocopherol (0.2 g/kg) as an antioxidant in rapeseed oil during heating at 80°C and deep fat frying of french fries at 162°C. The added δ-tocopherol was reported to be a poor antioxidant under both heating conditions. The authors suggested that levels of tocopherols naturally present in the rapeseed oil were at optimum levels to prevent oxidation prior to the addition of δ-tocopherol.

As stated previously, most of the studies which examined the oxidative activity of tocopherols used temperatures lower than those employed during frying. The antioxidant and prooxidant activities of tocopherols will likely be different at frying temperatures due to the more aggressive conditions under which oxidation is occurring. However, due to a lack of research in the area of frying, studies which examined the activity of tocopherols at lower oxidative temperatures will be reviewed. It is recognized that these findings will not be directly applicable to the frying process.

Blekas et al. (1995) investigated the effects of addition of various levels of α-tocopherol to purified olive oil on its oxidative stability to 40°C for 120 days. They purified olive oil by removing unidentified minor components. α-Tocopherol was added to the purified oil at the following levels: 0, 100, 500 and 1000 mg/kg. The researchers monitored the PVs and CDs of the oils at several intervals throughout the 120 day storage period. Results from both measurements indicated that α-tocopherol was prooxidative (indicated by higher levels of PVs and CDs compared to the control of 0 mg/kg α-

tocopherol added) in the oil at ≤ 20 days of storage. After 20 days, α-tocopherol acted as an antioxidant at all levels tested. The antioxidant effect of α-tocopherol was found to decrease with increasing concentration.

Blekas et al. (1995) performed a second experiment to further examine the apparent prooxidant role of α-tocopherol during the initial stages of autoxidation. They looked at the effect of addition of 100 mg/kg α-tocopherol on purified olive oil samples varying in their initial degree of autoxidation. At early stages of autoxidation (identified as PV < 15 meq/kg), α-tocopherol acted as a prooxidant. In moderately oxidized oil (identified as PV > 15 meq/kg), 100 mg/kg α-tocopherol acted as an antioxidant. Therefore the authors concluded that α-tocopherol can act as either an antioxidant or a prooxidant depending on the level of hydroperoxides present.

Jung and Min (1990) examined the addition of various concentrations of pure tocopherols to purified soybean oil on the oxidative stability of soybean oil. The purified soybean oil was reported to contain no peroxides, chlorophylls, FFAs, tocopherols or PLs (although no data was provided to substantiate this claim). These researchers added α-, δ- and γ-tocopherol to the soybean oil at concentrations of 0, 100, 250, 500 and 1000 mg/kg. The oils were stored at 55°C in the dark for a total of six days and PVs determined on each day. A PV above that of the control was interpreted as a prooxidant behavior and a PV below that of the control was identified as antioxidant behavior (even though the differences between the control and the experimental sample may not have been significant). Results indicated that α-tocopherol acted as an antioxidant at a level of 100 mg/kg, but as a prooxidant at levels of ≥ 250 mg/kg in the oil. Looking at the mean

PV for the six days, only the differences between the control and concentrations of ≥ 500 mg/kg were found to be significant ($p<0.05$). For γ-tocopherol, PV decreased with increasing concentration up to 250 mg/kg (significantly lower than control, $p<0.05$), indicating antioxidant behavior. Prooxidant activity was displayed at concentrations of ≥ 500 mg/kg, with significant differences ($p<0.05$) from the control at 1000 mg/kg. δ-Tocopherol displayed antioxidant behavior at concentrations of ≤ 500 mg/kg. PV decreased with increasing concentration up to 500 mg/kg, with significant differences ($p<0.05$) from the control being present at concentrations of 250 and 500 mg/kg. At a concentration of 1000 mg/kg, a significant ($p<0.05$) increase in PV occurred, indicating a prooxidative effect. Overall, the results indicated that α-, δ- and γ-tocopherols could act as both antioxidants and prooxidants depending on the concentration present in the oil. Of the concentrations tested, optimum antioxidant activity was displayed at 100 mg/kg for α-, 250 mg/kg for γ- and 500 mg/kg for δ-tocopherol. The researchers related these varying optimum antioxidant concentrations to stability of each tocopherol isomer. The conclusion reached was that the less stable the tocopherol was to oxidation, the lower was the concentration of that tocopherol required to achieve optimum antioxidant activity.

Recognizing that tocopherols occur in oils as a mixture of several isomeric forms, Huang et al. (1995) investigated the oxidative stability of mixtures of tocopherols in addition to pure isomers of individual tocopherols. The researchers added various concentrations of δ, γ, 1:1 mixtures of α and γ, and soybean tocopherol concentrate (12% α, 1% β, 58% γ , 19% δ and 10% vegetable oil) to corn oil which had been stripped of

tocopherols. Oils were stored at 60°C for six days and monitored for CDs and hexanal (a VDP of n-6 PUFA) production. Results can be summarized as follows:

a) Effect of δ-tocopherol. δ-Tocopherol was added at levels of 0, 100, 250, 500, 750, 1000 and 2000 mg/kg. Very little activity in terms of peroxide formation and hexanal production was seen on day one. After day one, the control oil displayed higher levels of peroxides and hexanal than any of the samples with added δ-tocopherol. Concentrations of 100 and 250 mg/kg δ-tocopherol produced rapid increases in oxidation after day two, but still displayed antioxidant activity. At higher concentrations (>250 mg/kg), oxidation was inhibited to a fairly large extent. Overall, oxidation decreased with increasing concentration of δ-tocopherol.

b) Effect of γ-tocopherol. γ-Tocopherol was studied at a single level of 5000 mg/kg. Significant ($p<0.05$) prooxidant behavior, in terms of peroxide formation, was observed on days two and four. At day six, however, γ-tocopherol displayed a significant ($p<0.05$) reduction in PV from the control, indicating an antioxidant effect. In addition, γ-tocopherol displayed significant ($p<0.05$) reduction in hexanal production, indicating antioxidant activity.

c) Effect of 1:1 (α:γ) mixtures. Concentrations of 1:1 (α:γ) of 500 to 3000 mg/kg displayed prooxidant behavior in terms of peroxide development before day three, with activity increasing with concentration. At a concentration of 250 mg/kg, the mixture showed no effect on the oil until day three. At day three, antioxidant behavior of this concentration became apparent. After day

four, all concentrations displayed significantly lower ($p<0.05$) PVs than the control, indicating antioxidant activity. The tendency to inhibit peroxide formation increased with oxidation time for all concentrations. In addition, after day four, antioxidant activity increased with decreasing concentration. The control oil encountered marked increased in hexanal production after day three. For the samples with 1:1 (α:γ) added, no hexanal production occurred until day six. These results indicated an inhibition of peroxide decomposition to hexanal at all concentrations. At day six, a 250 mg/kg mixture displayed the greatest hexanal production. However no significant differences ($p<0.05$) between the various concentrations were discovered in terms of hexanal production.

d) Soybean tocopherol mixtures. At day one, concentrations of $\geq$1000 mg/kg promoted hydroperoxide formation. Overall, no inhibition of oxidation (as measured by PV) was found on day one. After day one, an inhibitory effect was present in samples containing 750 mg/kg of the mixture or less. After day three, the inhibition was present for all concentrations except 3000 mg/kg. At day four and later, however, an inhibition occurred for all concentrations tested and tended to increase with length of oxidation. The greatest inhibition throughout the study occurred when 500 mg/kg of the soybean tocopherol mixture was added. Hexanal formation did not occur to a great extent in the oils with added tocopherol mixtures. In fact, only concentrations of < 500 mg/kg displayed any production of hexanal. Greatest inhibition of hexanal

formation occurred with the 250 mg/kg samples, even though no significant differences ($p<0.05$) were calculated between any of the samples.

Clark et al. (1990) added high levels of tocopherols (i.e. 1000 mg/kg) to refined peanut, cottonseed and hydrogenated soybean oil to test for prooxidant activity. Degree of oxidation was measured as the time to reach a PV of 100 meq/kg. Prooxidant activity was not detected in any of the oils despite total tocopherol concentrations of up to 2000 mg/kg. These results do not agree with the findings of Huang et al. (1995).

Marinova and Yanishlieva (1992) heated purified lard triglycerides at 25, 50, 75 and 100°C. Results indicated that when α-tocopherol was added to the lard, its ability to block oxidation increased with temperature but not substantially with concentration (within a range of 0.2 to 2.0 g/kg of α-tocopherol added). The researchers suggested that the added α-tocopherol became involved in side reactions rather than acting as an antioxidant. As concentration of α-tocopherol increased, the oxidation rate increased. However, with increasing temperature at a constant concentration of α-tocopherol, oxidation rate decreased.

When tocopherols become involved in antioxidant reactions, they themselves are oxidized. Jung and Min (1992) studied the effect of oxidized tocopherols on stability of purified soybean oil to oxidation. The purified oil was reported to contain no peroxides, chlorophylls, FFAs, tocopherols or PLs. The researchers added oxidized α-, γ- or δ-tocopherol individually at levels of 0, 100, 250, 500 and 1000 mg/kg. The soybean oil with added oxidized tocopherols was stored in the dark at 55°C. Results of PV analysis indicated that oxidized tocopherols showed prooxidant activity which increased with increasing concentration. Reductions in headspace-oxygen content with increasing

concentration of oxidized tocopherols added confirmed their prooxidant activity. Oxidized α-tocopherol showed stronger prooxidant activity than oxidized γ- and δ-tocopherol.

In conclusion, the oxidative activities of the pure tocopherols and their mixtures depends on the concentration of the pure tocopherols, the stage of oxidation, oil type, the type of food being fried and the type of product monitored. To summarize, tocopherols are reported to act as antioxidants or prooxidants during oxidation depending mainly on concentration. Oxidized tocopherols also appear to have a role in oil oxidation. However, the role of tocopherols (and also oxidized tocopherols) in oxidation, specifically during the frying process, has yet to be fully explored.

2.5.3 Phytosterols

Among the minor components of oils which may influence frying oil stability are phytosterols. These plant sterols are one of the most abundant minor components in oils, making up between 0.53 to 0.97% of the oil by weight (Eskin et al., 1996). Phytosterols are almost identical in structure to cholesterol. The only difference between them is in the side chain, where phytosterols have an extra branch which is missing in cholesterol (refer to Figure 2.13). Phytosterols have oxidation products similar to those of cholesterol (Finocchiaro and Richardson, 1983). Since cholesterol oxidation products have been determined to be harmful in the body, the potential clearly exists for similar effects of phytosterol oxidation products (Finocchiaro and Richardson, 1983).

The most abundant or commonly found sterol in vegetable oils is β-sitosterol (Finocchiaro and Richardson, 1983). However, the total sterol content and composition varies between oils (Eskin et al., 1996). Other common phytosterols include campesterol,

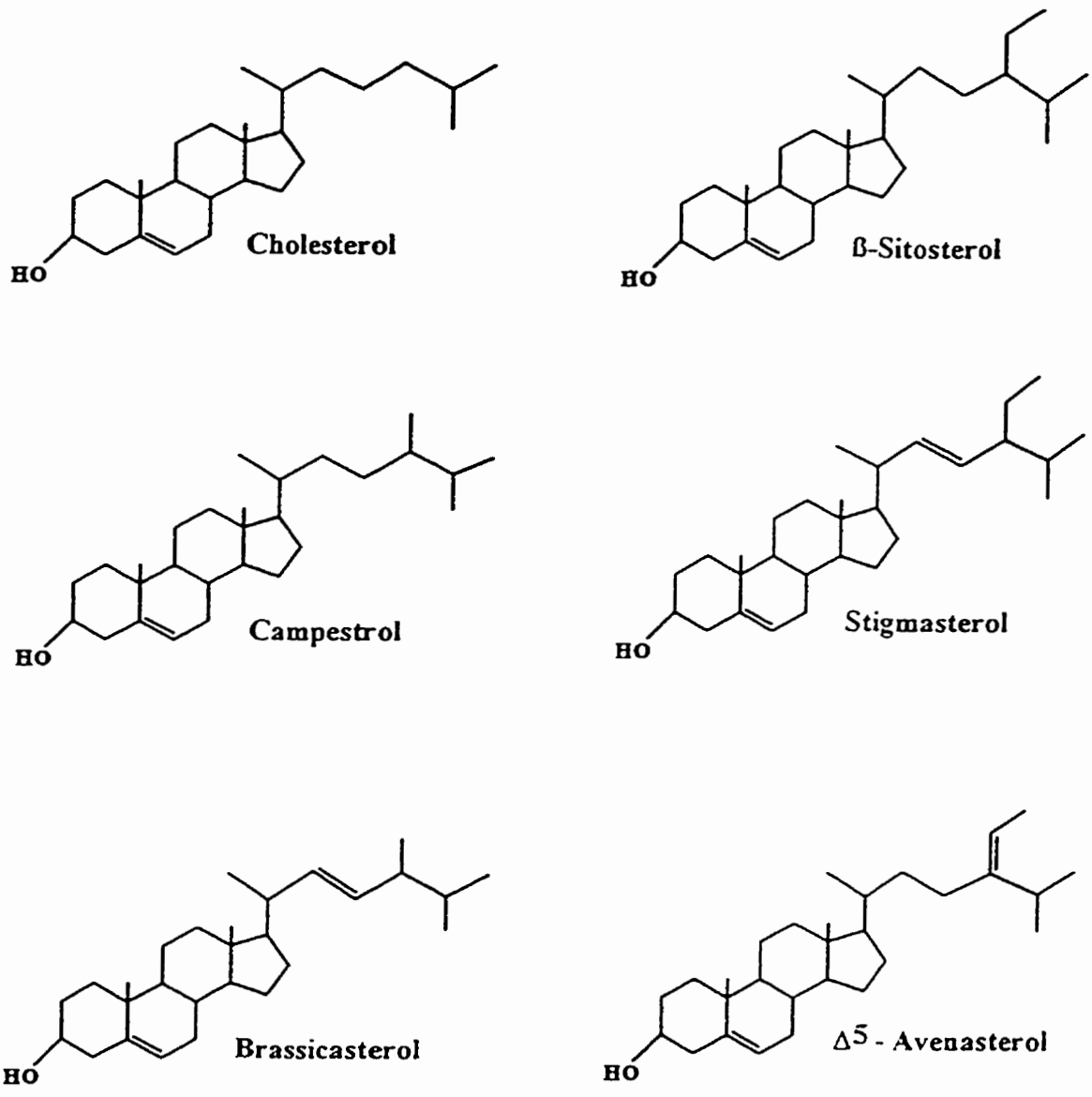


Figure 2.13. Structures of cholesterol and some common phytosterols (Dutta et al., 1996)

stigmasterol and brassicasterol (Kochhar, 1983). The structures of some common phytosterols are shown in Figure 2.13.

Certain phytosterols have been shown to have antioxidant activity while others do not (Sims et al., 1972). A few phytosterols not commonly found in vegetable oils have been reported to be prooxidative in heated oils (Sims et al., 1972). Different phytosterols, for example Δ^5-avenasterol and β-sitosterol, are very similar to each other in structure. In this case, the only difference between these two structures is the double bond in the side chain of Δ^5-avenasterol (known as an ethylidene group) (see Figure 2.13). It has been shown that only phytosterols containing this double bond will perform as antioxidants (Sims et al., 1972). For example, Δ^5-avenasterol shows antioxidant activity in heated oils, while β-sitosterol has no reported effect on oxidation (White and Armstrong, 1986). The stability of phytosterols at frying temperatures varies, with stigmasterol being the most stable followed by β-sitosterol, campesterol and brassicasterol (Li, 1996).

There are several studies which look at the behavior of phytosterols during heating of oils to frying temperatures. Sims et al. (1972) looked at the effect of sterols from different sources on oxidation of safflower oil heated to 180°C for a total of 28 hours. Various sterols had been added to the oils at a level of 0.05% by weight. IV was monitored as a measure of oil deterioration. Results indicated that some sterols acted as prooxidants, some as antioxidants, while others exerted no effect. Spinasterol acted as a prooxidant, while vernosterol, Δ^7-avenasterol and fucosterol were reported to be antioxidative. Other sterols, including some common plant sterols, were determined to be either slightly prooxidative or ineffective. This group of sterols included β-sitosterol,

stigmasterol, cholesterol, ergosterol and lanosterol. Overall, only sterols with a double bond in their side chain acted as antioxidants.

Boskou and Morton (1976) studied the effect of various sterols on oxidative activity by adding individual sterols and sterol mixtures to cottonseed oil. The oils were heated to 180°C for 72 hours (eight hours/day) and IV monitored after each 24 hour heating period. The sterols added to the oil included pure Δ^5-avenasterol and two sterol mixtures. One sterol mixture (A) consisted of 87% β-sitosterol, 9% Δ^5-avenasterol and 3% campesterol. The second sterol mixture (B) contained 94% β-sitosterol, 4% campesterol and 2% unidentified sterols. Δ^5-avenasterol and each of the two mixtures were added separately to oil samples at a level of 0.4%. Their results indicated that pure Δ^5-avenasterol, and to a lesser extent mixture A, were antioxidative. Mixture B had no effect on oxidation. The researchers suggested that Δ^5-avenasterol was the main factor responsible for the antioxidant activity.

Gordon and Magos (1983) heated triglyceride mixtures, with a composition similar to olive oil, to 180°C for 72 hours. To these triglyceride mixtures, various sterols were added at a level of 0.1%. IV was monitored as a measure of the extent of oxidation. Results indicated that Δ^5-avenasterol and fucosterol were antioxidative. Cholesterol and stigmasterol were found to have practically no effect on oxidation. In a second portion of the study, Δ^5-avenasterol was added to the triglyceride mixture in various concentrations (i.e. 0.01, 0.05 and 1.0%). A small antioxidant effect was observed at each concentration, which increased with increasing concentration. These results indicate that individual sterols may have an antioxidant effect in oils even when present at very low levels.

White and Armstrong (1986) followed CD and polymer formation in soybean oil heated to 180°C for 28 hours. The oils contained added sterols designated as sterol fractions A and B, as well as pure β-sitosterol. Sterol fractions A and B contained mainly β-sitosterol and some Δ^5-avenasterol with smaller amounts of other unidentified sterols present. Sterol fraction A was reported to contain 5% Δ^5-avenasterol, while no other information was provided as to the sterol composition of the two fractions. Sterol fraction A (added at a level of 0.05%) and sterol fraction B (added at a level of 0.25%) both acted as antioxidants. Sterol fraction B (added at a level of 0.17%) had only a small antioxidant effect. To determine which of the sterols, β-sitosterol or Δ^5-avenasterol, was responsible for the antioxidant effect, pure β-sitosterol was heated in the oil under the same conditions but was found to have no effect on oxidation. This finding suggested that Δ^5-avenasterol was responsible for the antioxidant effect present in the oils containing sterol fractions A and B. These results provided further confirmation of the ability of Δ^5-avenasterol and the inability of β-sitosterol to retard oil deterioration.

Thus, only those phytosterols containing an ethylidene group in their side chain appear to be capable of acting as antioxidants in oils heated to frying temperatures. In addition, the degree of antioxidant activity appears to be concentration dependent (Gordon and Magos, 1983).

2.5.4 Metals

Metals or catalysts containing metals accelerate the oxidative breakdown of hydroperoxides (Lea, 1960). Therefore adding trace amounts of metals such as iron, copper, cobalt or nickel leads to the development of rancidity by catalyzing reactions between oxygen and unsaturated fatty acids (Benjelloun et al., 1991). One reaction

mechanism showing the ability of metals to catalyze oxidation reactions is as follows (Schaich, 1992):

$$M^{(n+1)+} + LH \rightarrow M^{n+} + L^{*} + H^{+}$$

where M is the metal (which transfers an electron), LH is the unsaturated lipid molecule, L* is the radical formed, and H^{+} is hydrogen.

Benjelloun et al. (1991) reported that oxidative stability of rapeseed oil, heated at 180°C for eight hours, improved once copper and iron were removed from the oil. Gordon et al. (1994) assessed the oxidative stability of rapeseed oil with added copper and iron. Iron contents ranged from 0.07 mg/kg to 0.35 mg/kg, while copper was added at levels ranging from 0.05 to 0.25 mg/kg. Results indicated that oxidative stability decreased with increasing concentrations of copper and iron. Copper was reported to reduce oil stability to a greater extent than iron (Gordon et al., 1994). These two studies support the general belief that metals play a prooxidant role in oil oxidation. Goh et al. (1985) also reported that both copper and iron acted as prooxidants in oil, with copper being the stronger prooxidant of the two metals.

2.5.5 Other Factors Affecting Oil Stability

In addition to tocopherols, phytosterols and metals, a number of other oil components, such as FFAs, PLs and pigments, have the potential to affect frying oil stability. These components may act as antioxidants, prooxidants or synergistically with other oil components. However, these components cause problems only in poorly refined oils from which they have not been removed. It has recently been reported that compounds which model chlorophyll degradation products are capable of acting as prooxidants, even at levels of <1 mg/kg, and may not be completely removed by refining

(Ramamurthi and Low, 1998). In addition, the distribution of fatty acids on the triglyceride molecule may also be important in determining oil stability.

Chapter 3. Frying Stability of Regular and Modified Vegetable Oils

3.1 Introduction and Objectives

High levels of PUFAs have been implicated as the main factor responsible for poor frying stability of vegetable oils. Consequently, efforts to produce a more stable frying oil have focused on reducing PUFA levels by either blending with a more saturated oil; hydrogenation; interesterification; and by genetic modification. Genetic modification can involve the introduction of anti-sense genes, which block the production of desaturase enzymes responsible for adding double bonds to fatty acids (Diehl, 1998).

Modification has resulted in the production of oils with lower levels of linolenic (C18:3) and linoleic (C18:2) acid. In addition, oils with high oleic acid (C18:1) content have also been developed. Despite reductions in PUFA levels, modified oils do not consistently show significant improvement in frying stability compared to the original oils (Warner and Mounts, 1993; Mounts et al., 1994a; Mounts et al., 1994b). It has been suggested that minor oil components such as tocopherols, phytosterols and metals may play a significant role in the frying stability of vegetable oils.

This study was designed to compare the composition and frying stability of regular and modified canola, soybean and sunflower oils. In addition, an attempt was made to relate differences in composition, particularly the minor components, to frying stability. The specific objectives of the study were:

1. to characterize regular and modified oils in terms of fatty acid composition, tocopherols, phytosterols and metals;
2. to determine the frying stability of these oils;

3. to relate differences in frying stability to one or more of the following factors: fatty acid composition; initial tocopherol level; rate of tocopherol degradation; initial phytosterol level; rate of phytosterol degradation; and metal content.

3.2 Materials and Methods

3.2.1 Frying Oils

Oils used in this study included four types of canola oil, two types of sunflower oil, and two types of soybean oil. Canola oils included regular (RCO), high oleic (HOCO), high oleic low linolenic (HOLLCO) and low linolenic (LLCO) oils. Regular (RSY) and low linolenic (LLSY) soybean oils were also included. Sunflower oils consisted of a regular (RSO) and a high oleic (HOSO) variety. With the exception of LLSY and HOSO, all of the oils were commercially refined. LLSY and HOSO were both pilot plant processed oils. All of the oils contained citric acid, but no other preservatives were added to any of the oils. LLSY and RSY were refined by American processors, whereas HOSO was refined by a Canadian processor. The remaining oils were refined at the following locations: HOCO and HOLLCO (InterMountain, Idaho Falls, Indiana, USA), RCO and LLCO (CanAmera, Altona, Manitoba, Canada), and RSO (CanAmera, Edmonton, Alberta, Canada).

3.2.2 Frying Procedure

The frying procedure involved heating 2 L of each oil at 175 ± 2°C for 12 hours/day (for a total of six days) in 2-L capacity domestic deep fryers (one Tefal® and two SEB® brands). Fresh oil samples were placed in three 30 mL glass vials, flushed with nitrogen and stored at -20°C until required for analysis. In addition, two 30 mL samples of each oil were taken at predetermined intervals throughout frying and stored in

a similar manner. Sampling points are identified in Figure 3.1. Each day, after 12 hours of heating, the fryers were shut off and left to sit overnight for 12 hours. This type of intermittent heating has been found to be more abusive than continuous heating. In intermittent heating, hydroperoxides continue to form when the fryer is shut off (Artman, 1969). When heating is resumed, these hydroperoxides decompose to produce a higher concentration of free radicals than would be achieved during continuous heating. The result is greater oxidative breakdown in the oil heated intermittently compared to continuously for the same period of time (Artman, 1969).

In addition to sampling, frying was carried out in the oils at certain times during each day. Russett type potatoes were peeled and sliced into french fries using a Starfrit® potato chipper. The sliced potatoes were rinsed with cold water prior to being submerged into the oil. A ratio of 1:6 of fries to oil was used in determining the amount of potatoes to fry. A constant volume of 2 L was assumed for this determination, and the corresponding weight of each batch of potatoes for frying was 307 ± 2 g. A 1:6 ratio of oil to food is the ratio recommended by Morton and Chidley (1988) and used by other researchers (Arroyo et al., 1992; Arroyo et al., 1995; Lopez-Varela et al., 1995). Each batch of potatoes was fried for six minutes, with the schedule of frying times provided in Figure 3.1.

Fryers were switched on 15 minutes prior to the beginning of frying each day. This 15 minutes was the time required for the oil to heat up to the desired temperature. In addition, oils were conditioned by heating at the frying temperature for 30 minutes on frying day one only. This "break-in" period for a fresh oil allows for greater contact time and resulting heat transfer between the food and the oil (Blumenthal, 1991). Contact time

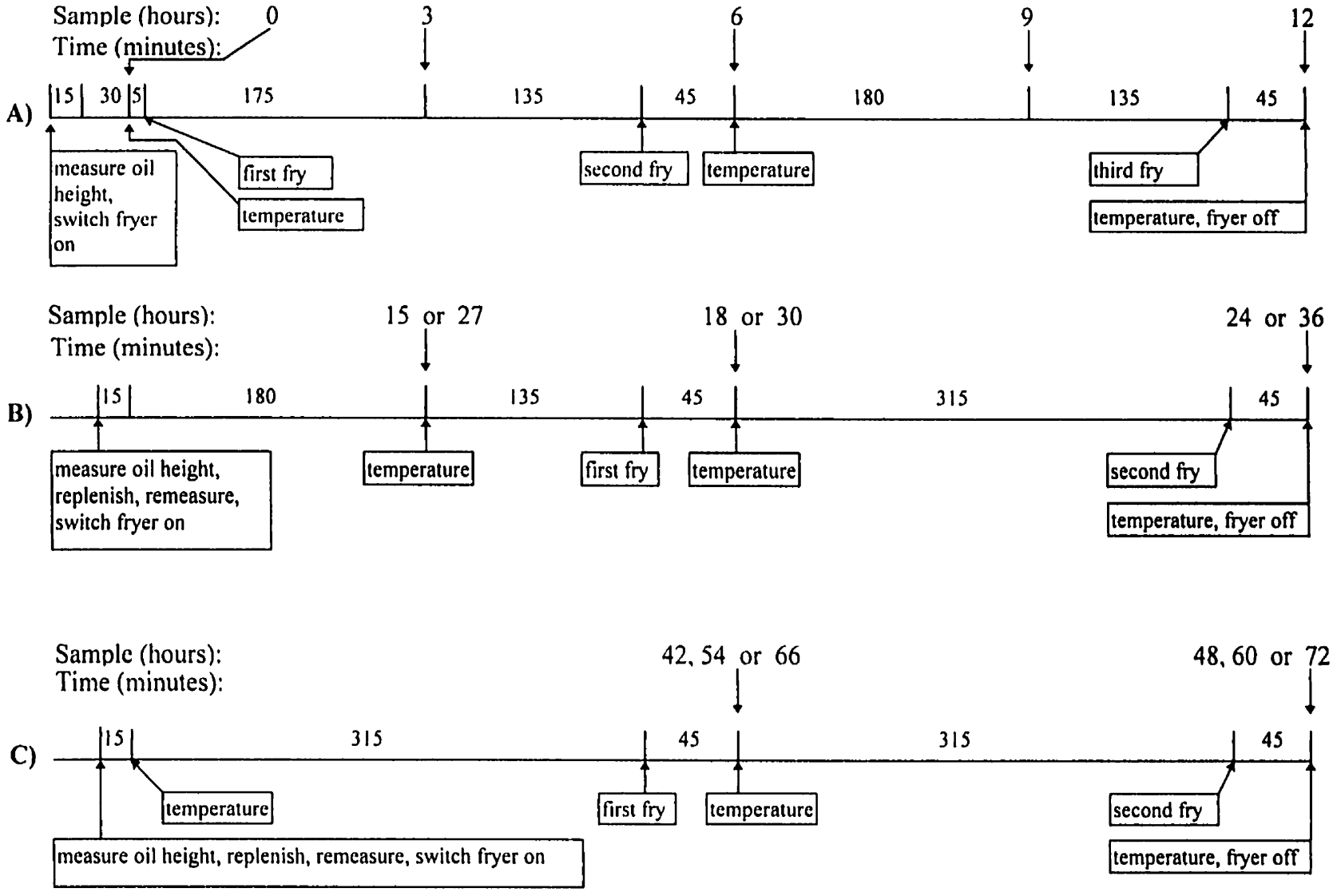


Figure 3.1. Schedule of frying procedure for A) day one, B) days two and three and C) days four to six

between the oil and the food is impaired due to the release of steam from the food, but will increase as frying progresses (Blumenthal, 1991). A "break-in" period also allows time for fried flavor to develop, as the fresh oil is often bland (Pokorny, 1989). In general, flavor will improve during initial stages of frying, reach an optimum level, and eventually become less acceptable in later stages of frying (Pokorny, 1989). On day one, a 0 hour sample was identified as the sample taken at the point after 15 minutes of warming up and 30 minutes of conditioning of the oil.

Oil temperatures were monitored three times each day for each oil, and the points at which temperatures were determined are also listed in Figure 3.1. Pokorny (1989) stated that the specific frying temperature, as long as it is below 200°C, is not of great concern as the extent of oxidation is limited by the amount of surface area in contact with air. When temperature reading and sampling were scheduled for the same time, temperature was read first so that the presence of the metal ladle did not result in a momentarily lower temperature.

3.2.3 Determination of Oil Replenishment Level

Each morning, the oil was replenished with fresh oil to re-establish the initial volume of 2 L. Oil replenishment helps maintain the oil in good quality over a longer period of time by diluting the products of oxidation and hydrolysis (Pokorny, 1989). A standard curve was developed to indicate the volume of oil in the fryer which corresponded to its height as measured on a metal ruler placed in the center of the fryer. Since all three fryers used in the study were of similar dimensions, one curve was used for all three fryers. Starting at a volume of 400 mL, the height of the oil in the fryer was measured (in cm) as oil continued to be added in 100 mL volumes to reach a total of 2 L.

Regression analysis of the data provided the following equation for use in determining the amount of oil required for replenishment each morning:

volume (mL) = -364 + 491 * height (cm), with an r value of 0.999 (equation 3.1)

The height of the oil in each fryer was measured each day prior to heating. Using the regression equation, the volume present was calculated and subtracted from the initial volume at the beginning of the previous day. The difference between these two volumes indicated the volume of fresh oil required to re-establish the previous day's starting level. Total oil replenishment for the six day frying period ranged from 42 to 49% of the original 2 L volume. In one of the fryings of RSY, replenishment level was 61% due to excessive foaming which resulted in loss of oil on day five.

3.3 Analytical Methods

3.3.1 Peroxide Value (PV)

PVs were determined in duplicate on fresh oils using AOCS official method Cd 8-53 (1990).

3.3.2 Free Fatty Acids (FFAs)

FFAs were measured in duplicate on fresh oils and throughout frying using two methods of analysis.

FFAs were determined for RCO, HOCO and HOLLCO throughout the frying period by titration, using AOCS official method Ca 5a-40 (1989). Only these three oils were evaluated by this method as the color change at the end point was difficult to identify using this procedure.

The Veri-Fry® Pro FFA-75 quick test method (Test Kit Technologies, Inc., New Jersey, USA) was designed to evaluate the quality of frying oils. This method was used

for all eight oils in the fresh state and throughout frying. The test measures FFAs ranging from 0.02 to 0.7% (as % oleic acid). This method was performed by adding hot oil to the reagent gel tubes provided. Hot oil was added up to the line marked on the tube, which was capped and shaken to dissolve the gel in the oil completely. The tube was then placed in a 65°C water bath until a clear layer formed on the bottom. The tube was placed in a Milton Roy Spectronic 3000 spectrophotometer and the absorbance of the clear bottom layer determined at 610 nm. The higher was the absorbance reading, the lower was the level of FFAs present in the oil sample. A standard curve was developed based on the absorbance readings of five reference samples with known concentrations of FFAs.

3.3.3 Total Polar Components (TPCs)

TPCs were determined using Sep-Pak® Vac 6cc (1g) silica cartridges (Water Chromatography Division, Millipore Corporation, Milford, MA, USA) to separate the polars from the nonpolars. The procedure was performed as described by Petukhov (1996), based on the method of Sebedio et al. (1986). Approximately 50 mg of oil was weighed out in a 5 mL vial, with the exact weight of the sample recorded. 4.5 mL of petroleum ether was added to the vial to dissolve the sample.

The cartridge was activated by running 5 mL of hexane through, ensuring the cartridge never went dry. The dissolved oil sample was added to the cartridge using a small funnel once a small amount of hexane was still visible above the solid silica. Collection of the solvent into a labeled, disposable aluminum tray began immediately upon addition of the sample to the column. The exact weight of the empty tray was

measured and recorded. Nitrogen gas was applied to the cartridge to produce a constant flow rate of 2 mL/minute.

The vial which contained the original dissolved sample was washed with 10 mL of petroleum ether. These washes were added successively to the column, being careful to never let the column go dry. To complete the elution of the nonpolar fraction, a total of 20 mL of petroleum ether:diethyl ether (90:10 v/v) was added to the column. The metal tray was then exchanged with an empty 30 mL test tube. The column was then washed with 30 mL of methanol to elute the polar fraction. The test tube was capped and refrigerated until required for use in HPSEC of the polar fraction.

The solvent containing the nonpolar fraction (in the metal tray) was evaporated under nitrogen, and the empty tray weighed. The difference between the weight of the tray prior to the elution and the weight of the tray at the end of the elution and evaporation of solvent equaled the weight of the nonpolar fraction (equation 3.2). The percentage TPCs in the oil was determined by subtracting the weight of the nonpolar fraction from the initial weight of the oil, dividing this number by the initial weight of the oil, and multiplying by 100 (equation 3.3 - based on AOAC method 982.27, 1990).

Weight of nonpolars =weight of tray plus nonpolars - weight of empty tray (equation 3.2)

% TPCs ={(weight of oil - weight of nonpolars)/weight of oil} * 100 (equation 3.3)

3.3.4 High Performance Size Exclusion Chromatography (HPSEC)

HPSEC was performed on the polar fractions collected during analysis of TPCs. The method was based on that of Dobarganes et al. (1988). Briefly, methanol was evaporated (under nitrogen) from the test tubes containing the polar fraction. Next, 1 mL of tetrahydrofuran (THF) was added to the test tube and the polar fraction dissolved by

mixing on a vortex. A sample containing 0.1 mL of the polar solution was combined with 9.9 mL of THF in a vial and mixed on a vortex.

The analysis was performed on a Shimadzu LC-10AD liquid chromatograph with a Shimadzu SIL-10A auto injector and a Shimadzu SCL-10A system controller. The system was connected to a guard column and two size exclusion columns joined in series (Phenogel 5 Guard, 30 x 4.60mm; Phenogel 5 100A pore size, 300 x 4.60 mm; Phenogel 5 500A pore size, 300 x 4.60 mm; all from Phenomenex®, Torrance, CA, USA). The Phenogel packing material in the columns consisted of styrene divinylbenzene. Samples were analyzed with a mobile phase of 100% THF, a flow rate of 0.300 mL/minute, a run time of 40 minutes and an injection volume of 30 ul. A Sedex 45 evaporative light scattering detector was connected to the system and operated at a temperature of 50°C and a pressure of 2.4 bars.

3.3.5 Fatty Acid Composition

The relative % contributions of various fatty acids to fatty acid composition were determined using GC based on AOCS official method Ce 1-62 (1990). Prior to analysis, the samples were methylated according to the method of Przybylski (1995, personal communication). The methylation procedure involved placing 0.020 g of oil in a threaded test tube and dissolving it in 1 mL of petroleum ether. This solution was mixed on a vortex to achieve a monophase system. Next, 12 mL of 0.5 N methanolic HCl was added to the test tube, mixed and placed in a 65°C oven for one hour. The test tube was then placed in a dark cupboard overnight. The following day, the sample was placed in the oven again for one hour at 65°C to ensure complete methylation of the oil sample. During both heating periods, the contents of the test tube were mixed every five minutes

until clear. On the second day, the test tubes were removed from the oven and allowed to cool to room temperature. 6 mL of isooctane and 6 mL of distilled water were added to the tubes. The content of the tubes were mixed by turning them upside down gently two or three times. The tubes were then allowed to stand for the contents to separate into two layers. The clear upper layer was then transferred to a GC vial in preparation for injection.

Analysis of fatty acid composition was carried out using a Hewlett Packard 5890A GC. The column attached was a Supelcowax 10 column (30 m x 0.25 mm i.d., Supelco, Oakville, ON, Canada). The mode of detection was flame ionization with a Hewlett Packard 3392A Integrator. An autosampler was used to inject 0.5 µl samples onto the column, with hydrogen as the carrier gas. A standard containing a mixture of fatty acid methyl esters was used to identify the peaks (#403, Nu-Chek-Prep, Elysian, USA). The GC program was as follows:

Initial temperature	175°C
Initial time	2 minutes
Rate	3°C/minute
Final temperature	235°C
Final time	5 minutes
Injection temperature	250°C
Detector temperature	260°C

3.3.6 Tocopherols

The method for tocopherol analysis was based on AOCS official method Ce 8-89 (1990). Samples were prepared by weighing 100 mg (exact weight recorded) of oil directly into a threaded high performance liquid chromatography (HPLC) vial and diluting with 1.5 mL hexane. Analysis was performed on a Shimadzu LC-10AD liquid chromatograph, with Shimadzu SIL-10A auto injector and Shimadzu SCL-10A system

controller. A Hewlett Packard HP 1046A programmable fluorescence detector was attached with excitation λ = 288 nm and emission λ = 331 nm. The column was a normal phase Prodigy 5µ silica column (250 x 3.20 mm, Phenomenex®, Torrance, CA, USA). 40 µl of each sample was injected with a flow rate of 0.800 mL/minute and a run time of 15 minutes. The mobile phase was 5% methyl-tert-butyl-ether in hexane. Levels of tocopherols were quantified using separate calibration curves for α- , β- , γ- and δ-tocopherol.

3.3.7 Phytosterols

Prior to HPLC analysis of sterols, samples were saponified as described by Li (1996), based on the method of Lozano et al. (1993). Oil was weighed into threaded test tubes in amounts ranging from 300 to 500 mg (exact weight recorded). 10 mL of 1 N methanolic KOH was added to the test tube, which was capped and vortexed to achieve a monophase system. The tubes were then placed in a dark cupboard for 16 to 18 hours. The contents of the test tube were placed in 125 mL separatory funnels, to which 10 mL of water and 10 mL of diethyl ether were added. The funnel was then capped, flipped back and forth a few times, and allowed to stand until the contents separated into two layers. The bottom water layer was drained off and washed with another 10 mL of diethyl ether. After separation, the second water layer was drained off and discarded. The two ether layers were then combined and washed with 10 mL of water. The entire water layer was then drained. The ether layer was transferred to a vial and the solvent evaporated under nitrogen. Any water remaining was evaporated by adding 0.5 mL of benzene or isopropanol. Four mL of ethanol:methanol (1:1 v/v) was added to the dry vial. When flakes occurred in the vial, it was heated to 60°C until clear.

The sample was then transferred to an HPLC vial and analyzed on a Shimadzu LC-10AD liquid chromatograph with SIL-10A auto injector and SCL-10A system controller. The method for analysis was based on Holen (1985) as modified by Li (1996). The column was a reverse phase C18 column (5μm, 250 mm x 4.6 mm i.d., Beckman). The mobile phase was methanol, run time 30 minutes, flow rate of 0.800 mL/minute and injection volume of 20 μl. Detection occurred at a wavelength of 205 nm on a Shimadzu SPD-10AV UV/VIS detector. Phytosterols were quantified using separate calibration curves for β-sitosterol, stigmasterol, campesterol and brassicasterol.

For sunflower oils, 1 g of oil sample was used during saponification steps due to the lower levels of phytosterols present. Twice the amount of methanolic KOH and water were used at the appropriate steps although the amount of ether used remained unchanged.

3.3.8 Metals

The amount of Cu, Fe and Ni in the fresh oils was determined using the AOCS official method Ca 18b-91 (1992). This method involved vaporizing a sample of the oil in a graphite furnace, followed by detection of each metal at a certain wavelength using atomic absorption spectrophotometry. This analysis was performed at the Grain Research Laboratory of the Canadian Grain Commission (Winnipeg, Manitoba, Canada).

3.4 Statistical Analysis

The rates for TPCs formation, FFAs accumulation and individual and total phytosterol degradation were compared using analysis of covariance (ANCOVA) with frying time as the covariate or continuous variable. The model included the variables of specific oil type, frying time and the interaction between them. ANCOVA was performed

using SAS statistical software, and allowed for the comparison of rates (i.e. slopes) between oils. To compare rates between oils, t-tests for multiple comparisons were used with an adjusted α level of 0.05/ the number of comparisons being made.

TPCs and FFAs are both measures of oil stability. Therefore, correlation analysis was performed to determine the relationship between FFAs determined by two methods (i.e. AOCS method and Veri-Fry® method), and between TPCs and FFAs by both methods separately. Correlation coefficients (r) were obtained from regression analysis of the data (Sigma Plot scientific graphing software). Strong relationships between any of these methods would be revealed by high r values.

Chapter 4. Results and Discussion

4.1 Initial Oil Quality

4.1.1 Peroxide Values (PVs)

PVs were determined for each of the eight oils to establish initial quality. Freshly refined oils should have low peroxide values of <1 meq/kg if they are of good quality (Rossell, 1986; Orthoefer and Cooper, 1996). Stored oils, however, may reach PVs of up to 10 meq/kg before any flavor problems or major oxidative damage has occurred (Rossell, 1986). Fresh oils used in this study were all found to have PVs ≤1.1 meq/kg (Table 4.1), indicating satisfactory quality of the oils.

4.1.2 Free Fatty Acids (FFAs)

Freshly refined oils should have FFA contents of <0.05% as these components are removed during processing, primarily during degumming (Jung et al., 1989), alkali refining (Mounts, 1981) and deodorization (Mounts, 1981; Erickson, 1996). During storage, levels of FFAs will increase in the oil as a consequence of the breakdown of triglycerides during hydrolysis reactions, and less frequently as a product of oxidation reactions (Lopez-Varela et al., 1995). Using the Veri-Fry® Pro FFA-75 quick test, initial FFA contents of most oils fell at or below the recommended level of <0.05% FFAs for fresh oils (Table 4.1). One oil (HOLLCO) had a slightly higher level of FFAs than recommended, but this level was still within the limitation of good quality.

FFA content in the initial oils ranged from 0.01% in RSY to 0.07% in HOLLCO. The FFA content among the canola oils prior to frying ranged from 0.03% in LLCO to 0.07% in HOLLCO. Soybean oils ranged in FFA content from 0.01% in RSY to 0.02%

Table 4.1. Quality parameters in fresh oils[a]

Oil	Peroxide value (meq/kg)	Free fatty acids[b] (% oleic acid)	Free fatty acids[c] (% oleic acid)
Canola:			
RCO	1.1	0.05	0.07
HOCO	0.6	0.04	0.06
HOLLCO	0.6	0.07	0.11
LLCO	0.6	0.03	n.p.
Soybean:			
RSY	0.2	0.01	n.p.
LLSY	0.3	0.02[d]	n.p.
Sunflower:			
RSO	0.6	0.03[d]	n.p.
HOSO	0.7	0.02	n.p.

[a]all values are average of duplicate analysis
[b]values obtained by Veri-Fry® Pro FFA-75 quick test
[c]values obtained by AOCS official method Ca 5a-40 (1989)
[d]based on a single analysis only
n.p. = not performed

in LLSY . For the sunflower oils, the initial FFA content ranged from 0.02% in HOSO to 0.03% in RSO.

FFA content as determined by AOCS official method Ca 5a-40 (1989) produced slightly higher values compared to the Veri-Fry® Pro FFA-75 quick test (Table 4.1). One possible reason for this difference is that the AOCS method involves the visual assessment of a color change, which is difficult to distinguish using the recommended indicator solution.

4.1.3 Total Polar Components (TPCs)

Initial levels of TPCs were similar among all oils, ranging from 3.9% in LLSY to 6.6% in RSY (Table 4.2). These values corresponded to literature values for TPCs in fresh oils (Cuesta et al., 1993; Dobarganes et al., 1993; Warner et al., 1994). Initial levels of polars ranged from 4.8 to 6.5% for the canola oils; from 3.9 to 6.6% for the soybean oils; and from 4.5 to 5.4% for the sunflower oils. TPCs varied slightly among oils depending upon the polarity of minor components in the oil, such as FFAs and oxidation products. Polymerization of the oil may occur, to a certain extent, during deodorization of the oil, which is performed at high temperatures of between 210 and 270°C (Hopia, 1993b; Erickson, 1996). In addition, bleaching may lead to the production of a small amount of dimers (Hopia, 1993b).

4.1.4 High Performance Size Exclusion Chromatography (HPSEC)

Polar fractions of the fresh oils were analyzed by HPSEC. Amounts of specific polar components were analyzed qualitatively. All of the fresh oils, excluding HOSO, contained the peak representing monoglycerides and FFAs. Correspondingly, none of the fresh oils contained the peak representing polymers higher than dimers. This finding was

Table 4.2. Total polar components (TPCs) in fresh oils[a]

Oil	TPCs (%)
Canola:	
RCO	6.2
HOCO	5.8
HOLLCO	6.5
LLCO	4.8
Soybean:	
RSY	6.6
LLSY	3.9
Sunflower:	
RSO	5.4
HOSO	4.5

[a]all values are average of duplicate analysis

as expected since these components are typical products of thermoxidation reactions (i.e. oxidation reactions at high temperatures) (Dobarganes et al., 1988; Romero et al., 1995a). The absence of higher polymers further confirmed that none of the fresh oils had experienced a great deal of oxidative damage. Small amounts of dimers and oxidized triglycerides were identified in HOCO, HOLLCO, LLCO and RSO. LLSY contained oxidized triglycerides but not dimers. Diglycerides were present only in HOLLCO and LLCO.

4.1.5 Fatty Acid Composition

Vegetable oils modified to improve stability generally display lower levels of PUFAs, particularly linoleic and linolenic acid (Dobarganes et al., 1993; Erickson and Frey, 1994; Romero et al., 1995b). The oils evaluated in this study showed this pattern. In every case, the three regular oils (RCO, RSY and RSO) showed higher levels of PUFAs than did their corresponding modified counterpart(s) (Table 4.3). Takeoka et al. (1997) reported that, among seven common frying oils, oils with higher levels of PUFAs developed higher levels of TPCs. Thus the modified oils in the current study would be expected to show greater stability as compared to corresponding regular oils. One limitation of the study by Takeoka et al. (1997) was that the researchers compared only regular oils.

RSO had a considerably higher level of PUFAs (69.5%) than HOSO (3.5%). Differences in PUFA content of RSY and LLSY were much smaller (63.0% as compared to 59.0% PUFAs, respectively). PUFA content in canola oils ranged from 15.1 to 27.7% in modified oils compared to 31.5% in RCO.

Table 4.3. Fatty acid composition of fresh oils expressed as % of total fatty acid composition[a]

Oil	SFA[b]	MUFA[c]	PUFA[d]	18:1[e]	18:2[f]	18:3[g]
Canola:						
RCO	7.5	60.2	31.5	57.4	21.2	10.2
HOCO	7.0	76.4	16.0	74.4	9.3	6.7
HOLLCO	8.0	76.5	15.1	74.8	12.0	3.1
LLCO	7.2	64.7	27.7	62.7	24.6	3.0
Soybean:						
RSY	15.6	21.1	63.0	20.8	54.4	8.6
LLSY	15.8	24.7	59.0	24.4	55.7	3.3
Sunflower:						
RSO	11.2	19.0	69.5	18.7	68.3	1.2
HOSO	7.0	89.3	3.5	88.9	3.2	0.4

[a]all values are average of duplicate analysis
[b]saturated fatty acids
[c]monounsaturated fatty acids
[d]polyunsaturated fatty acids
[e]oleic acid
[f]linoleic acid
[g]linolenic acid

RCO fatty acid composition consisted of 57.4% oleic acid, 21.2% linoleic acid and 10.2% linolenic acid (Table 4.3). As expected, all three modified canola oils contained lower linolenic acid (3.0 to 6.7%) and higher oleic acid (62.7 to 74.8%) levels. With the exception of LLCO (C18:2: 24.6%), the modified oils also displayed lower levels of linoleic acid (9.3 and 12.0%). Although LLCO had a lower level of linolenic acid than RCO, total PUFA content of LLCO (27.7%) was not much different from RCO (31.5%).

RSY consisted of 20.8% oleic acid, 54.4% linoleic acid and 8.6% linolenic acid. In comparison, LLSY contained a higher level of oleic acid (24.4%) and a lower level of linolenic acid (3.3%). Linoleic acid content of LLSY (55.7%) was similar to that of RSY.

The modified sunflower oil, HOSO, contained a substantially higher level of oleic acid (88.9%) than did RSO (18.7%). Correspondingly, the linoleic acid content was much higher in RSO (68.3%) compared to HOSO (3.2%). Linolenate content in both sunflower oils was very low (0.4 and 1.2%).

4.1.6 Tocopherols

Tocopherol content and composition in an oil is important since tocopherols are capable of acting as antioxidants and, to varying degrees, can slow down oxidative deterioration. Therefore, all other factors being constant, oils with higher levels of tocopherols would be expected to show greater stability. Among the canola oils, HOLLCO contained the highest level of tocopherols in the fresh oil (Table 4.4). A level of 893 mg/kg tocopherols was found in HOLLCO compared to levels between 468 and 601 mg/kg for the other three canola oils. For both the soybean and sunflower oils, the regular oil displayed a higher level of tocopherols than did their corresponding modified

Table 4.4. Tocopherol content and composition of fresh oils[a]

Oil	Tocopherols (mg/kg)	α-Tocopherol (%)	γ-Tocopherol (%)
Canola:			
RCO	565	35	65
HOCO	601	30	70
HOLLCO	893	32	68
LLCO	468	32	67
Soybean:			
RSY	1012	5	94
LLSY	846	6	92
Sunflower:			
RSO	632	94	3
HOSO	358	96	2

[a]all values are average of duplicate analysis

counterparts. RSY had a tocopherol level of 1012 mg/kg compared to LLSY, which contained 846 mg/kg. In the case of the sunflower oils, the modified oil (HOSO) contained about half (358 mg/kg) the level of tocopherols as did RSO (632 mg/kg). Melton et al. (1998) also reported lower levels of α-tocopherol in high oleic sunflower oil as compared to regular sunflower oil. For both the soybean and sunflower oils, the regular oils were commercially processed whereas the modified oils were pilot plant processed. Therefore, differences in processing conditions may explain differences in tocopherol content of these oils. Bleaching and deodorization are the steps producing the most losses in tocopherols (Frankel, 1989). It is possible that temperature or pressure differences during pilot plant processing could explain the lower levels of tocopherols found in these oils. The tocopherol contents in RCO, RSY and RSO were typical of values reported for these oils (Yuki and Ishikawa, 1976; Syvaoja et al., 1986).

Tocopherol composition within an individual oil is also important. It has been shown that different forms of tocopherols have different degrees of antioxidant effectiveness. The order of antioxidant activity (*in vitro*) has been reported to be $\delta > \gamma > \beta > \alpha$ at temperatures between 50 and 100°C (Madhavi et al., 1996). In addition, the antioxidant activity of various combinations of tocopherols has yet to be fully investigated. Another area for exploration is how tocopherol content and composition changes when an oil is modified. Mounts et al. (1996) compared the content and composition of tocopherols in one regular and five low linolenic soybean oils. Analysis of the crude oils indicated that four of the five low linolenic oils had lower tocopherol levels than the regular oil. This finding was in agreement with results of the current study, where tocopherol content was determined to be lower in LLSY as compared to RSY.

With three of the modified oils, γ-tocopherol content was reduced and accounted for most of the difference in level of total tocopherols (Mounts et al., 1996). In the current study, a reduction in γ-tocopherol content also accounted for the difference in total tocopherol content of LLSY as compared to RSY. As γ-tocopherol has been found to be a more effective antioxidant than β- and α-tocopherol *in vitro* at higher temperatures (i.e. 50 to 100°C) (Madhavi et al., 1996), a reduction in its level in an oil would be expected to result in reduced stability of the oil. Therefore, this finding is significant and further research should identify how tocopherol content and composition are affected by modification of the oilseed.

Of the four forms of tocopherol, only α- and γ-tocopherol were present in substantial amounts in the canola, soybean and sunflower oils. Canola oils contained trace amounts of δ-tocopherol. β-tocopherol was found in RSO in small quantities. Soybean oils and HOSO contained small amounts of both δ- and β-tocopherol. The percentage contributions of each of these tocopherols was similar within each oil type (Table 4.4). Among the four canola oils, α-tocopherol content ranged from 30 to 35% and γ-tocopherol from 65 to 70% of total tocopherol composition. Soybean oils were made up of 5 to 6% α-tocopherol and 92 to 94% γ-tocopherol. Sunflower oils were composed of mainly α-tocopherol (94 to 96%) with much smaller contributions by γ-tocopherol (2 to 3%). The % contribution of tocopherol isomers to total tocopherol composition in canola and sunflower oils were typical of values reported previously (Syvaoja et al., 1986). The tocopherol composition of soybean oil reported in this study

differs from literature values, which indicate higher levels of δ-tocopherol, lower levels of γ-tocopherol, and slightly higher levels of α-tocopherol (Syvaoja et al., 1986).

Overall, the oils investigated in this study showed small variations in the relative percentage contributions of tocopherol isomers, but varied considerably with respect to total tocopherol content. With respect to soybean and sunflower oils, the modified oils had much lower levels of tocopherols than did their corresponding regular oils. Limitations of these findings are that the oils were not matched for factors including growing location, seed maturity at harvest and seed grade, and are thus not directly comparable. Therefore the effect of modification on level and composition of tocopherols cannot be determined from this experiment.

4.1.7 Phytosterols

Phytosterol content and composition of the oils was determined at 0 hours (i.e. after 30 minutes of conditioning) instead of on the fresh oils. As phytosterols were determined to be relatively stable in this study, it was assumed that 0 hour oils would display only very minor differences from fresh oils in terms of phytosterols. Phytosterol levels were highest among the canola oils (Table 4.5). Of the canola oils, HOCO contained a much lower level of phytosterols compared to the other three oils (6268 mg/kg as compared to 8692 to 8794 mg/kg). A reduction in phytosterols has been reported during the deodorization step of refining (Mounts, 1981; Eskin et al., 1996). It is possible that variation in the deodorization process (such as use of an abnormally high temperature), or the modification procedure itself, may have produced these differences. With the exception of HOCO, the modified oils were not substantially different from RCO in terms of total phytosterol content. LLSY contained a lower level of phytosterols

Table 4.5. Phytosterol content and composition of fresh oils[a]

Oil	Phytosterols (mg/kg)	β-Sitosterol (%)	Campesterol (%)	Stigmasterol (%)	Brassicasterol (%)
Canola:					
RCO	8794	50	41	-	9
HOCO	6268	51	38	-	10
HOLLCO	8692	53	37	-	10
LLCO	8748	51	40	-	9
Soybean:					
RSY	3616	57	29	14	-
LLSY	3067	59	30	12	-
Sunflower:					
RSO	4281	66	24	10	-
HOSO	1978	62	32	6	-

[a]all values are average of duplicate analysis

(3067 mg/kg) than did RSY (3616 mg/kg). The phytosterol content of HOSO (1978 mg/kg) was less than half that found in RSO (4281 mg/kg). LLSY and HOSO were both pilot plant processed oils. Therefore, lower levels of phytosterols in LLSY and HOSO compared with the corresponding regular oils may be due to differences in processing conditions, particularly during the deodorization process. Overall, values for phytosterol content in RCO and RSY were typical of values reported in the literature (Ferrari et al., 1996).

Four different types of phytosterols were identified in the oils. Soybean and sunflower oils contained β-sitosterol, campesterol and stigmasterol. In addition to β-sitosterol and campesterol, canola oils also contained brassicasterol. The main phytosterol present in each of the eight oils was β-sitosterol. This was consistent with literature reports stating β-sitosterol is the most abundant phytosterol in vegetable oils (Finocchiaro and Richardson, 1983). Only slight variations were found between RCO and modified canola oils in terms of phytosterol composition. β-Sitosterol content ranged from 50% of total phytosterol composition in RCO to 53% in HOLLCO. Campesterol content ranged from 37 to 41%, while brassicasterol content ranged from 9 to 10% (Table 4.5). In soybean oils, β-sitosterol content ranged from 57 to 59%, campesterol content from 29 to 30%, and stigmasterol content from 12 to 14%. The phytosterol composition varied to a greater extent between the two sunflower oils compared to canola and soybean oils. HOSO was composed of 62% β-sitosterol, 32% campesterol and 6% stigmasterol compared to 66%, 24% and 10% in RSO. In comparison, there were few differences between the regular and modified canola and soybean oils in terms of relative contributions of individual phytosterols. Larger differences existed between RSO and

HOSO, however no conclusions can be drawn from this study regarding the effect of modification on phytosterol composition as oilseeds were not selected for other influencing factors.

Mounts et al. (1996) investigated levels of phytosterols in the crude oil from one regular and five low linolenic soybean oils. Two of the modified oils had higher levels of phytosterols and one had a lower level of phytosterols than the regular oil. Overall, there were only small differences in the phytosterol compositions of the oils. However, two of the modified oils displayed lower levels of β-sitosterol than did the regular oil.

4.1.8 Metals

Traces of metals such as iron, copper, cobalt or nickel are known to act as prooxidants in oils, thus reducing the oxidative stability (Benjelloun et al., 1991). Fresh oil samples were analyzed for copper, iron and nickel content. Analysis was carried out at the Grain Research Laboratory, Canadian Grain Commission (Winnipeg, Manitoba, Canada). For most of the oils, the content of these three metals was below the detection limit of 0.010 mg/kg (Table 4.6). Nickel was not detected in any of the oils. Low levels of copper were found in one of the RCO (0.020 mg/kg), one of the HOCO (0.010 mg/kg) and one of the LLSY (0.020 mg/kg) replicates. RSY contained copper at a concentration of 0.020 mg/kg in both replicates. Only LLCO contained any trace of iron, with an average concentration of 0.014 mg/kg. In all cases, the content of copper, iron and nickel was either absent or very low. Since the levels of metals were similar within each oil group, particularly soybean and sunflower oils, their effect on oxidation would be similar. Therefore, metal content of the oils did not appear to contribute to differences in frying stability.

Table 4.6. Copper, iron and nickel content of fresh oils[a]

Oil	Copper (mg/kg)	Iron (mg/kg)	Nickel (mg/kg)
Canola:			
RCO	0.020[b]	n.d.	n.d.
HOCO	0.010[b]	n.d.	n.d.
HOLLCO	n.d.	n.d.	n.d.
LLCO	n.d.	0.014	n.d.
Soybean:			
RSY	0.020	n.d.	n.d.
LLSY	0.020[b]	n.d.	n.d.
Sunflower:			
RSO	n.d.	n.d.	n.d.
HOSO	n.d.	n.d.	n.d.

[a]all values are average of duplicate analysis
[b]element was detectable, at stated level, in one replicate only
n.d. = not detectable with minimum limit of quantification = 0.010 mg/kg

4.2 Frying Performance of the Oils

4.2.1 Free Fatty Acids (FFAs)

FFA contents of all oils were determined at certain intervals throughout frying by the Veri-Fry® Pro FFA-75 quick test method (Test Kit Technologies, Inc., Metuchen, New Jersey, USA). In addition, FFAs in RCO, HOCO and HOLLCO were determined at the same intervals using AOCS official method Ca 5a-40 (1989). A high correlation coefficient of $r = 0.98$ was found between the two methods for the three oils evaluated by both procedures (Figure 4.1). The Veri-Fry® Pro FFA-75 quick test was found to be a simpler and quicker method of determining FFA content. In this study, determination of FFA content using AOCS official method Ca 5a-40 (1989) was found to be subjective due to the lack of a clear change in color at the end point of titration using the recommended indicator solution. A strong correlation between the two methods indicated that the Veri-Fry® Pro FFA-75 quick test could be used to evaluate frying oils in place of the official method with a high degree of confidence in the results obtained.

As frying progressed, the FFA content, as determined by the Veri-Fry® method, increased in all oils (Figures 4.2 to 4.5). Figure 4.2 compares rates of FFAs accumulation among all eight oils, whereas Figures 4.3 to 4.5 compare rates within each oil group. ANCOVA in combination with t-tests for multiple comparisons revealed significant differences ($p<0.05$) between some of the oils in terms of rates of FFAs accumulation (Table 4.7). A comparison of all eight oils revealed that HOSO exhibited a greater rate of FFAs accumulation than all oils except HOCO and LLCO. In a frying study of regular and modified canola oils, Warner et al. (1994) reported that the higher the oleic acid content of the oil, the higher was its FFA content in the heated oil. A similar finding was

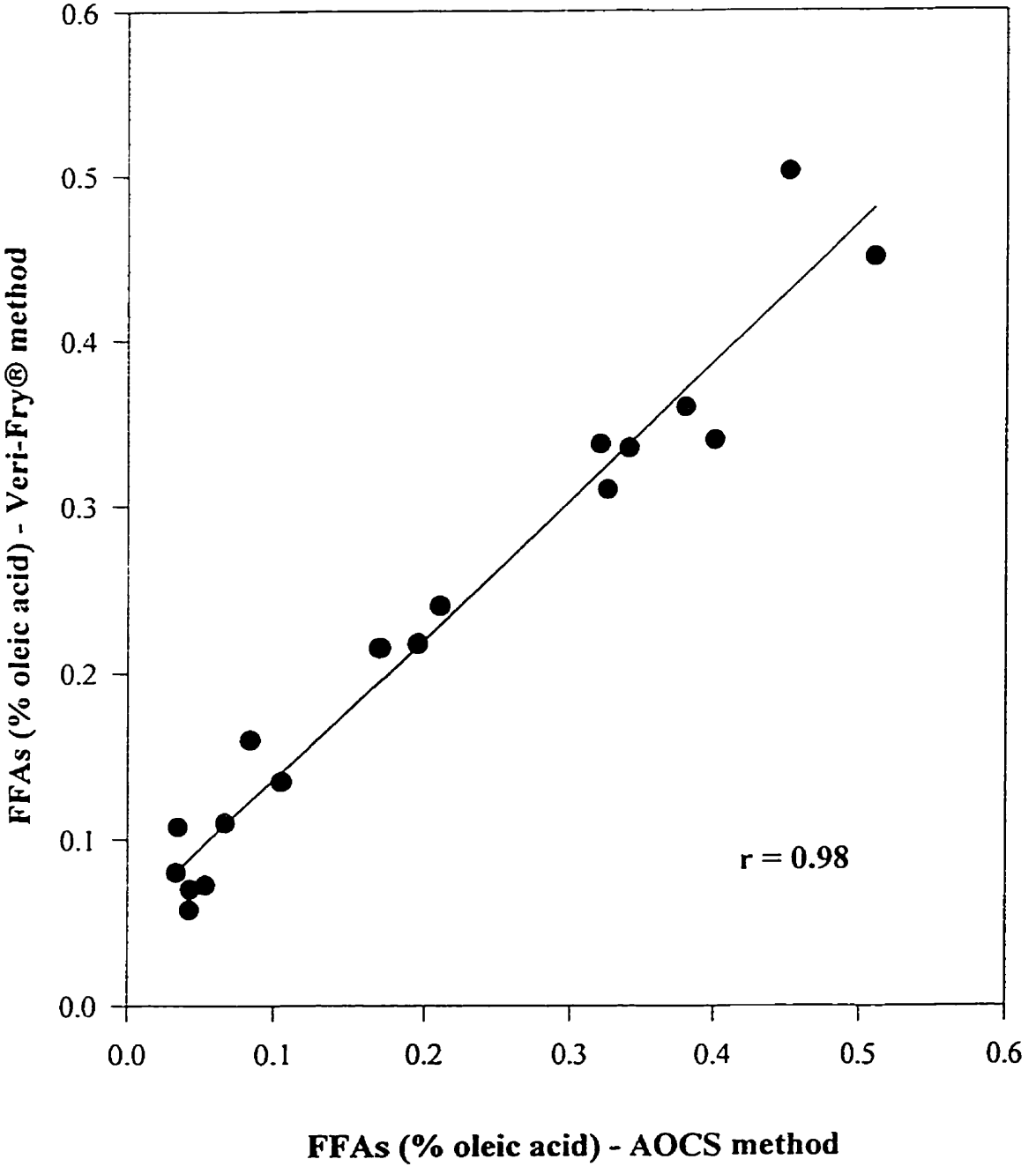


Figure 4.1. Relationship between free fatty acids (FFAs) determined by Veri-Fry® Pro FFA-75 quick test method and AOCS official method Ca 5a-40 for RCO, HOCO and HOLLCO

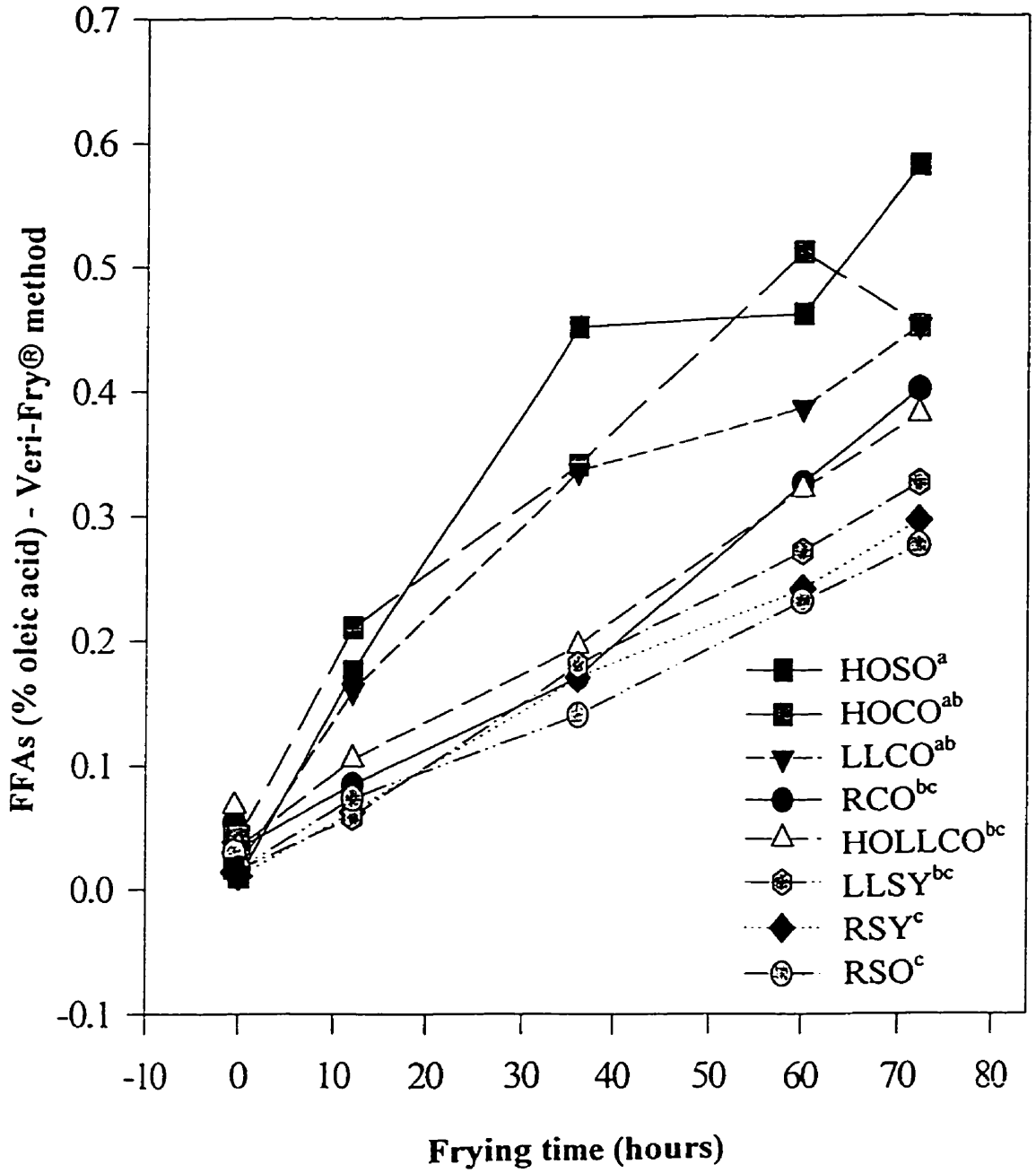


Figure 4.2. Free fatty acids (FFAs) determined by the Veri-Fry® Pro FFA-75 quick test method over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of FFAs accumulation

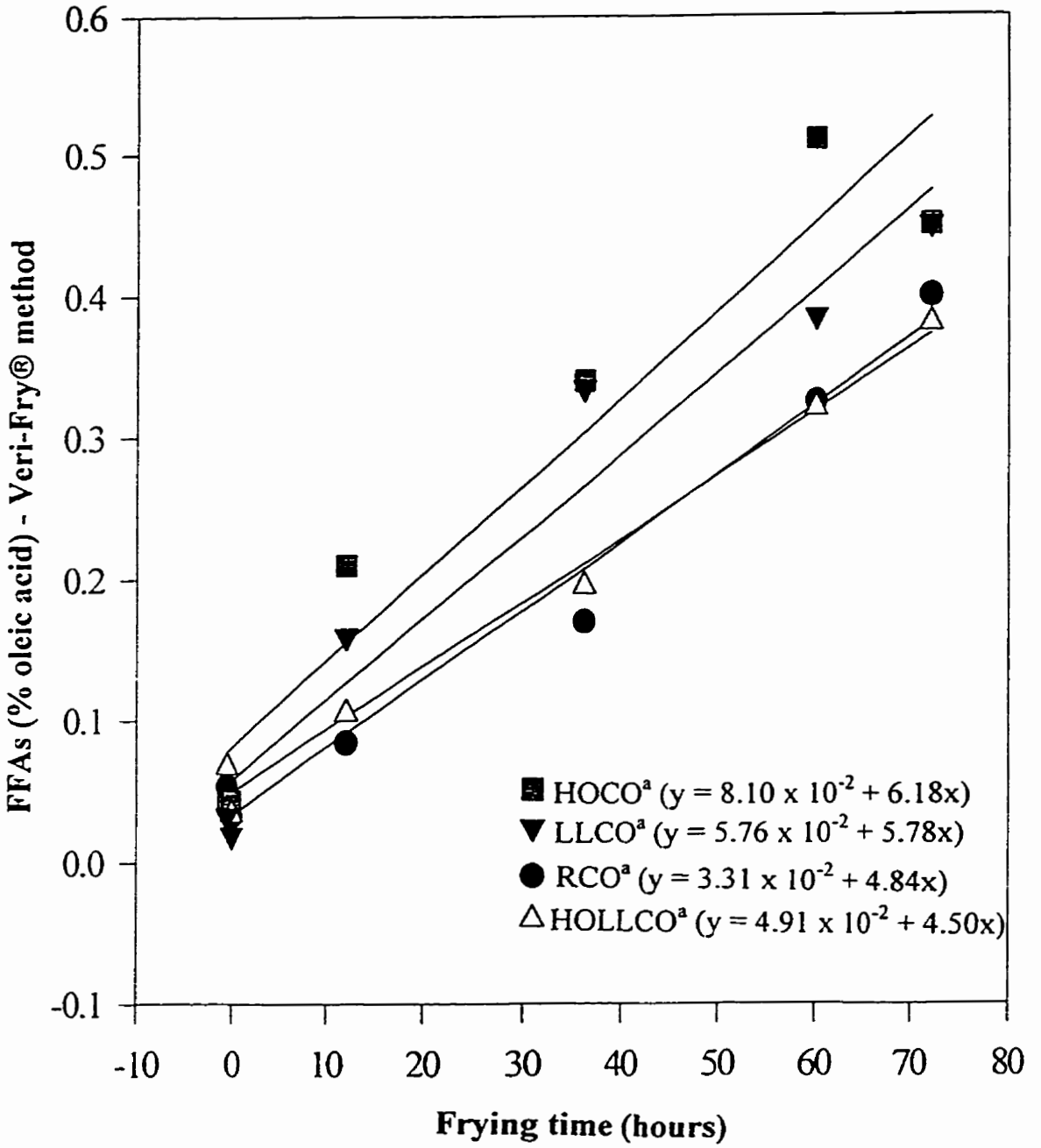


Figure 4.3. Free fatty acids (FFAs) determined by the Veri-Fry® Pro FFA-75 quick test method over frying time for canola oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of FFAs accumulation

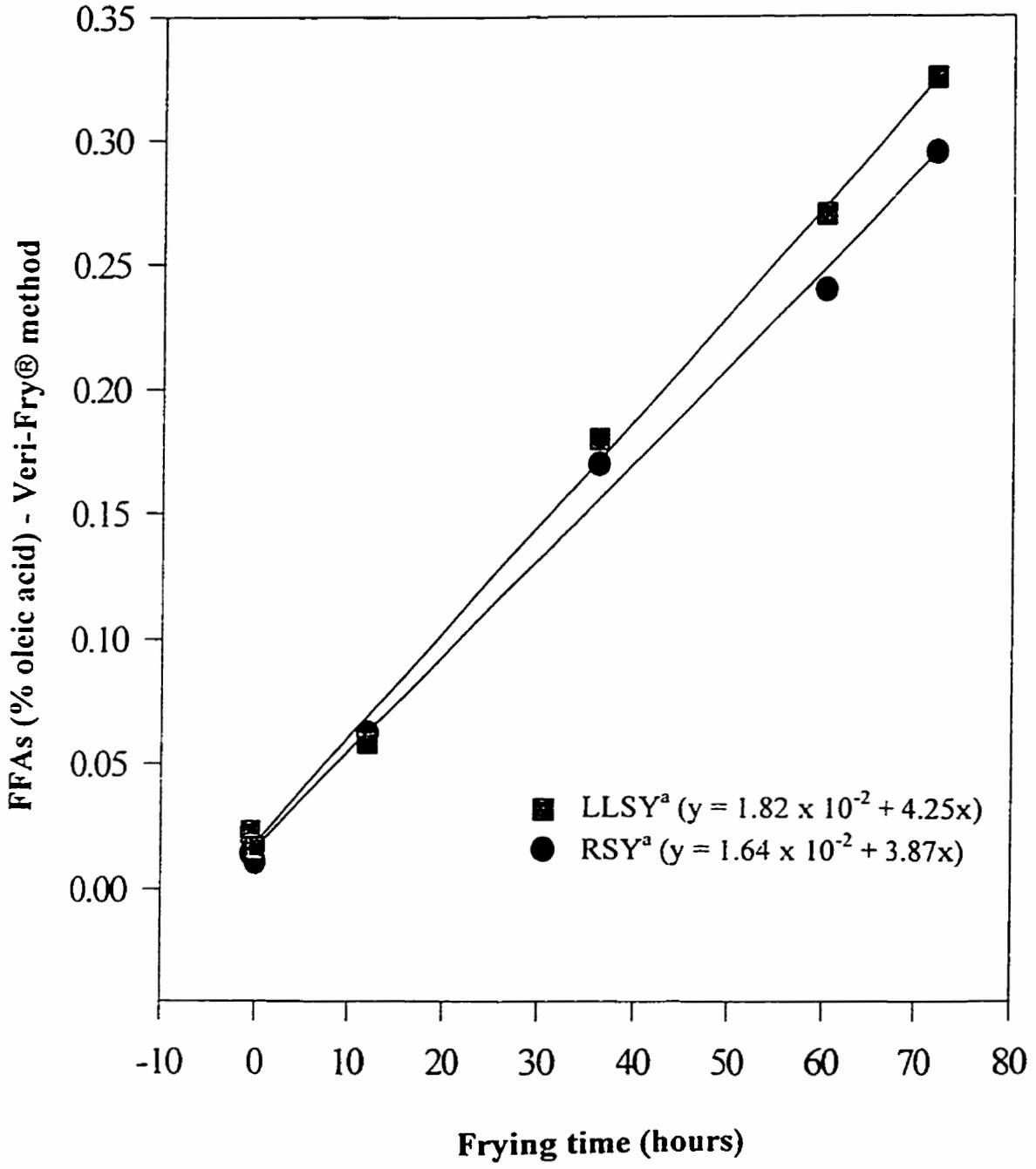


Figure 4.4. Free fatty acids (FFAs) determined by the Veri-Fry® Pro FFA-75 quick test method over frying time for soybean oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of FFAs accumulation

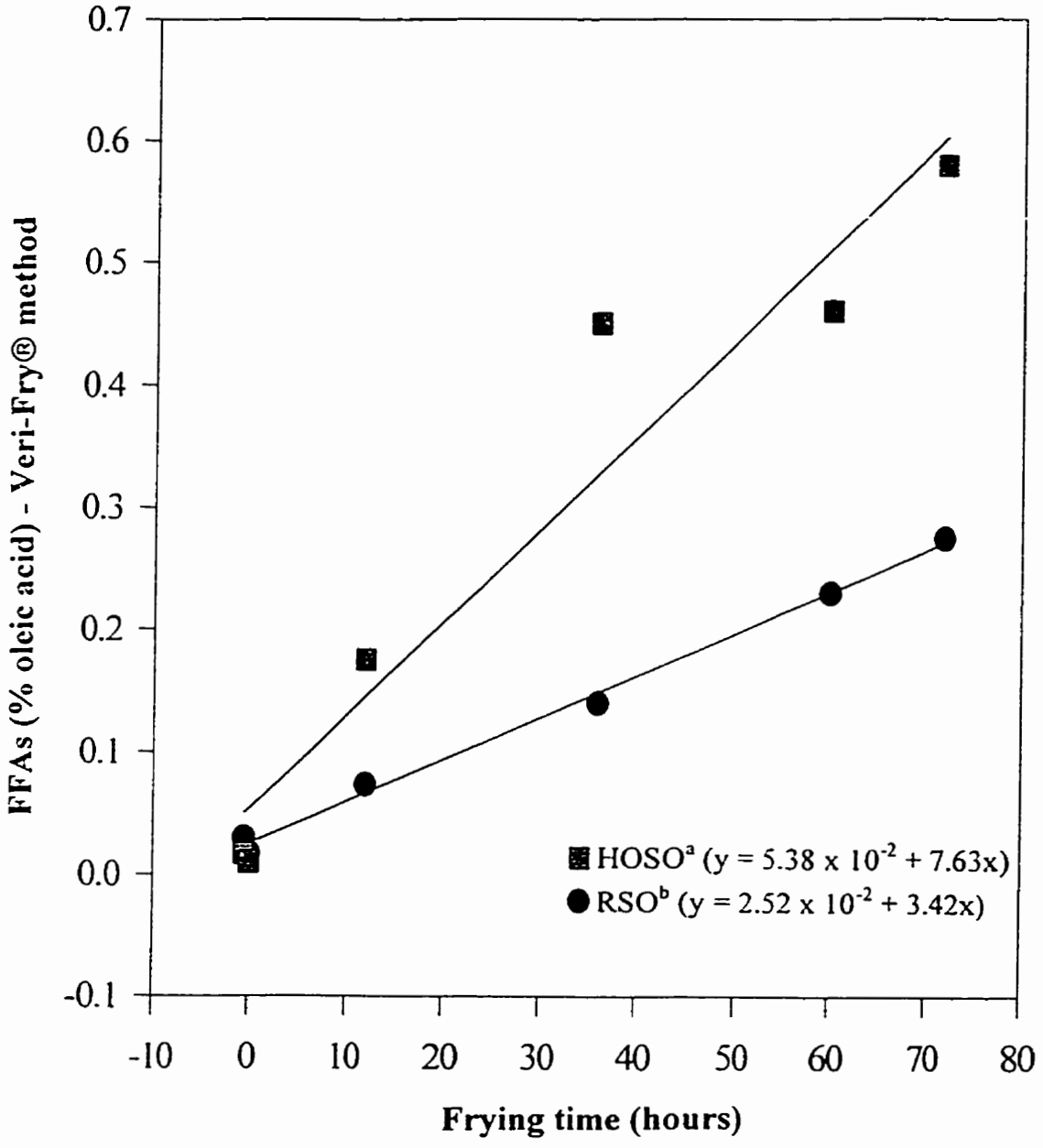


Figure 4.5. Free fatty acids (FFAs) determined by the Veri-Fry® Pro FFA-75 quick test method over frying time for sunflower oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of FFAs accumulation

Table 4.7. Statistical comparison of rates of free fatty acids (FFAs) accumulation among all oils

Oil	Rate of FFAs accumulation ($\times 10^{-3}$ % FFAs/hour)
HOSO	7.69^{a}
HOCO	6.18^{ab}
LLCO	5.78^{ab}
RCO	4.84^{bc}
HOLLCO	4.50^{bc}
LLSY	4.28^{bc}
RSY	3.56^{c}
RSO	3.45^{c}

N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of FFAs accumulation

found in the current study; where the two oils with the highest oleic acid contents, HOSO and HOCO, had the fastest rates of FFAs accumulation. However the study by Warner et al. (1994) was carried out for only 18 hours, looked at levels of FFAs rather than rates, and tocopherol levels were not reported.

No significant differences in rates of FFAs accumulation were found among the four canola oils or between the two soybean oils (refer to Table 4.7). Petukhov (1996) compared the frying stability of regular, hydrogenated, low linolenic and high oleic canola oils during frying of potato chips over a period of 40 hours. Results indicated that the hydrogenated canola oil had a significantly ($p<0.05$) faster rate of FFAs accumulation than low linolenic canola oil. No significant differences in rates of FFAs accumulation were reported among the other three canola oils. Warner and Mounts (1993) reported that FFA levels were higher in regular as compared to low linolenic soybean oil, and significantly ($p<0.05$) higher in regular as compared to low linolenic canola oil heated for 40 hours. However, the researchers examined levels rather than rates of FFAs accumulation. Mounts et al. (1994a; 1994b) performed two frying studies, each comparing the stability of three low linolenic soybean oils to a regular soybean oil. In agreement with the results of the current investigation, analysis of FFAs revealed no significant differences ($p\leq0.05$) in stability between the regular and low linolenic soybean oils. However, the studies by Mounts et al. (1994a; 1994b) were much shorter in duration (~20 hours) and looked at levels rather than rates of FFAs accumulation. The modified oils were all laboratory rather than commercially refined.

HOSO exhibited a significantly faster rate of FFAs accumulation than RSO. Since FFAs are products of frying oil degradation, these results indicate that HOSO is

degrading at a significantly faster rate than RSO. The absence of significant differences between canola oils and between soybean oils in this measure indicates that FFA determination revealed no stability differences among these oils.

4.2.2 Total Polar Components (TPCs)

TPCs increased in all oils as frying time progressed (Figure 4.6). LLSY had a significantly faster rate of TPCs formation than RSY, HOCO, HOLLCO and RCO. Rates of TPCs formation for HOLLCO and RCO were significantly slower than for all other oils. These results indicated that, using this measure of stability, HOLLCO and RCO were significantly more stable during frying than all other oils.

Although there are no internationally recognized guidelines to control the extent of abuse of frying oils, some European countries have set regulations concerning maximum levels of TPCs permitted (Firestone, 1996). The consensus among many European countries is that once an oil reaches a level of 25 to 27% TPCs, it is no longer fit for use (Firestone, 1996). In the current study, RCO and HOLLCO were the only oils which did not exceed these limits after 72 hours of frying.

Among the canola oils, LLCO and HOCO showed no significant differences in their rates of TPCs formation, however they both displayed significantly faster rates of TPCs formation than did HOLLCO and RCO (Figure 4.7). As TPCs are products of oil degradation, their faster rate of formation in these two oils indicated that LLCO and HOCO were significantly less stable during frying than the other two canola oils. The superior frying stability of RCO over LLCO and HOCO is an unexpected result as both modified oils contained lower levels of PUFAs, and thus should have been less susceptible to oxidative breakdown. Warner et al. (1994) reported that high oleic canola

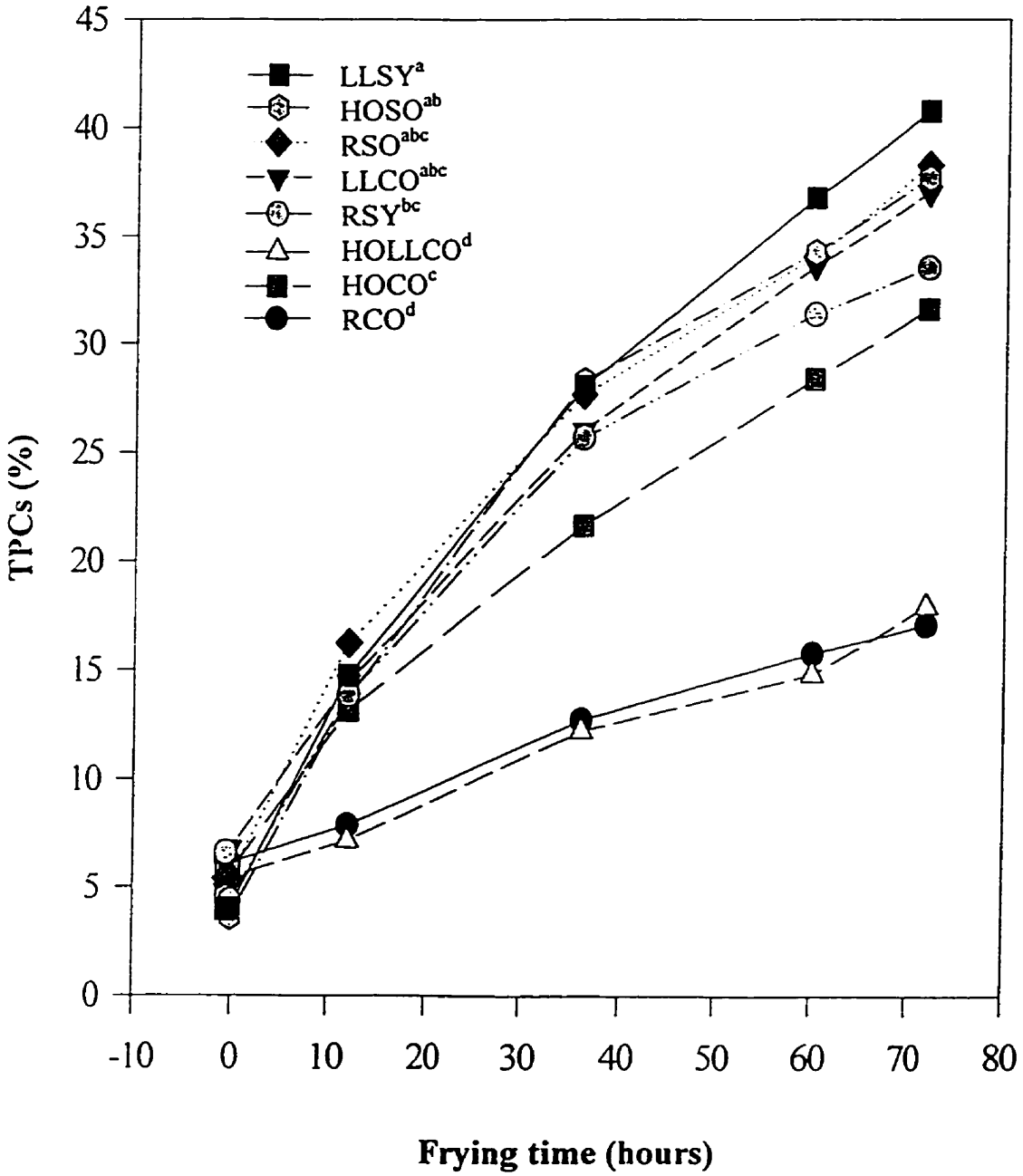


Figure 4.6. Total polar components (TPCs) over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of TPCs formation

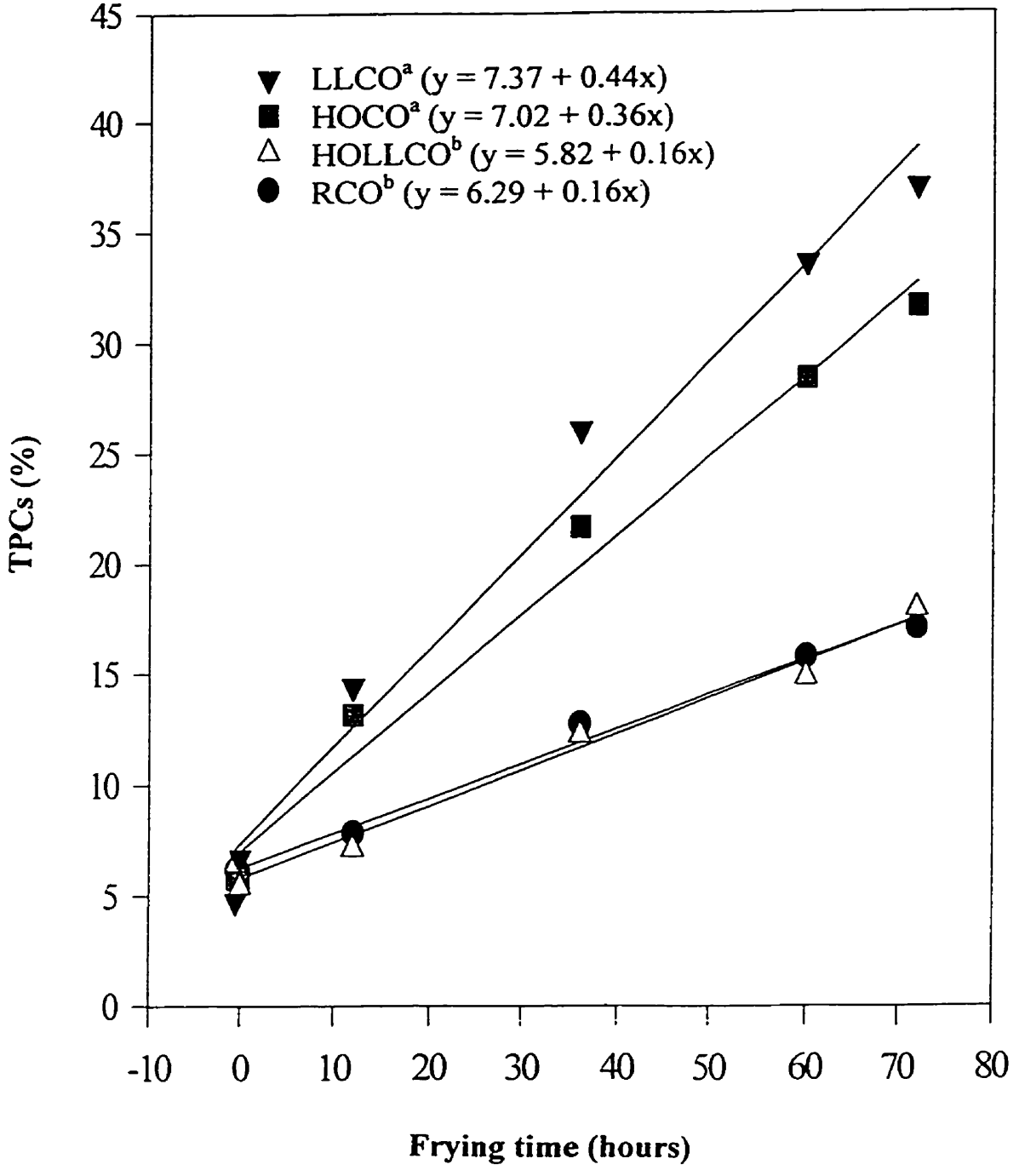


Figure 4.7. Total polar components (TPCs) over frying time for canola oils

N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of TPCs formation

oils were more stable than regular canola oils based on levels of TPCs formed during frying. However, their study was carried out for only 18 hours and looked at levels of TPCs rather than rates of formation. Warner and Mounts (1993) found no significant differences ($p<0.05$) in TPCs between regular and low linolenic canola oil in a 40 hour frying study. Petukhov (1996) also found no significant differences ($p<0.05$) in rates of TPCs formation among regular, hydrogenated, low linolenic and high oleic canola oils in a 40 hour frying study.

No significant differences were found between RCO and HOLLCO in terms of rates of TPCs formation, indicating no apparent differences in their frying stability. This result was also unexpected as HOLLCO contained a lower level of PUFAs (15.1% as compared to 31.5% in RCO - refer to Table 4.2) and also a greater tocopherol content (893 mg/kg as compared to 565 mg/kg in RCO - refer to Table 4.4), and thus would be anticipated to show greater stability than RCO. The fact that no significant difference in stability existed between HOLLCO and RCO suggests that factors other than PUFA content and tocopherol levels played significant roles in oil stability during frying. Two of the modified canola oils, HOCO and HOLLCO, contained similar levels of PUFAs (16.0% and 15.1%, respectively). Thus, using traditional reasoning which suggests that stability depends primarily on levels of PUFAs, these two oils would be expected to show similar stability. However, HOLLCO was found to be significantly more stable than HOCO as measured by rate of TPCs formation. This greater stability of HOLLCO may be a result of its higher level of tocopherols providing a protective effect against oxidative breakdown.

Among the soybean oils, LLSY had a significantly faster rate of TPCs formation than did RSY (Table 4.8, Figure 4.8). Again, this result was unexpected since LLSY contained lower levels of PUFAs and correspondingly would be predicted to show improved frying stability. However, LLSY also contained lower levels of tocopherols (846 mg/kg) than RSY (1012 mg/kg). Therefore, the higher level of tocopherols in RSY may have provided a protective effect in this oil which eliminated any improvement in stability that the lower PUFA level of LLSY may have produced. Warner and Mounts (1993) compared the frying stability of regular and low linolenic soybean oil over a 40 hour frying period. They reported significantly ($p<0.05$) higher levels of TPCs in regular compared to low linolenic soybean oil. In two separate 20 hour frying studies, Mounts et al. (1994a; 1994b) reported no significant differences ($p\leq0.05$) in TPCs among regular and low linolenic soybean oils. However, all three of these studies were performed for a much shorter period of time and levels rather than rates of TPCs formation were considered. In addition, Mounts et al. (1994a; 1994b) examined low linolenic soybean oils which had been refined in the laboratory.

No significant differences were found between RSO and HOSO in terms of rates of TPCs formation (Table 4.8, Figure 4.9). This result was unexpected as HOSO contained substantially lower levels of PUFAs than RSO (3.5% as compared to 69.5%, respectively), and thus would be expected to show improved frying stability. However, RSO had almost twice the level of tocopherols (632 mg/kg) as did HOSO (358 mg/kg). The higher level of tocopherols in RSO compared to HOSO may have compensated for its higher PUFA content, providing extra protection against oxidation. The combination of these two factors (i.e. PUFA and tocopherol contents) provides one possible

Table 4.8. Statistical comparison of rates of total polar components (TPCs) formation among all oils

Oil	Rate of TPCs formation (% TPCs/hour)
LLSY	0.51^{a}
HOSO	0.47^{ab}
RSO	0.45^{abc}
LLCO	0.44^{abc}
RSY	0.40^{bc}
HOCO	0.36^{c}
HOLLCO	0.16^{d}
RCO	0.16^{d}

N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of TPCs formation

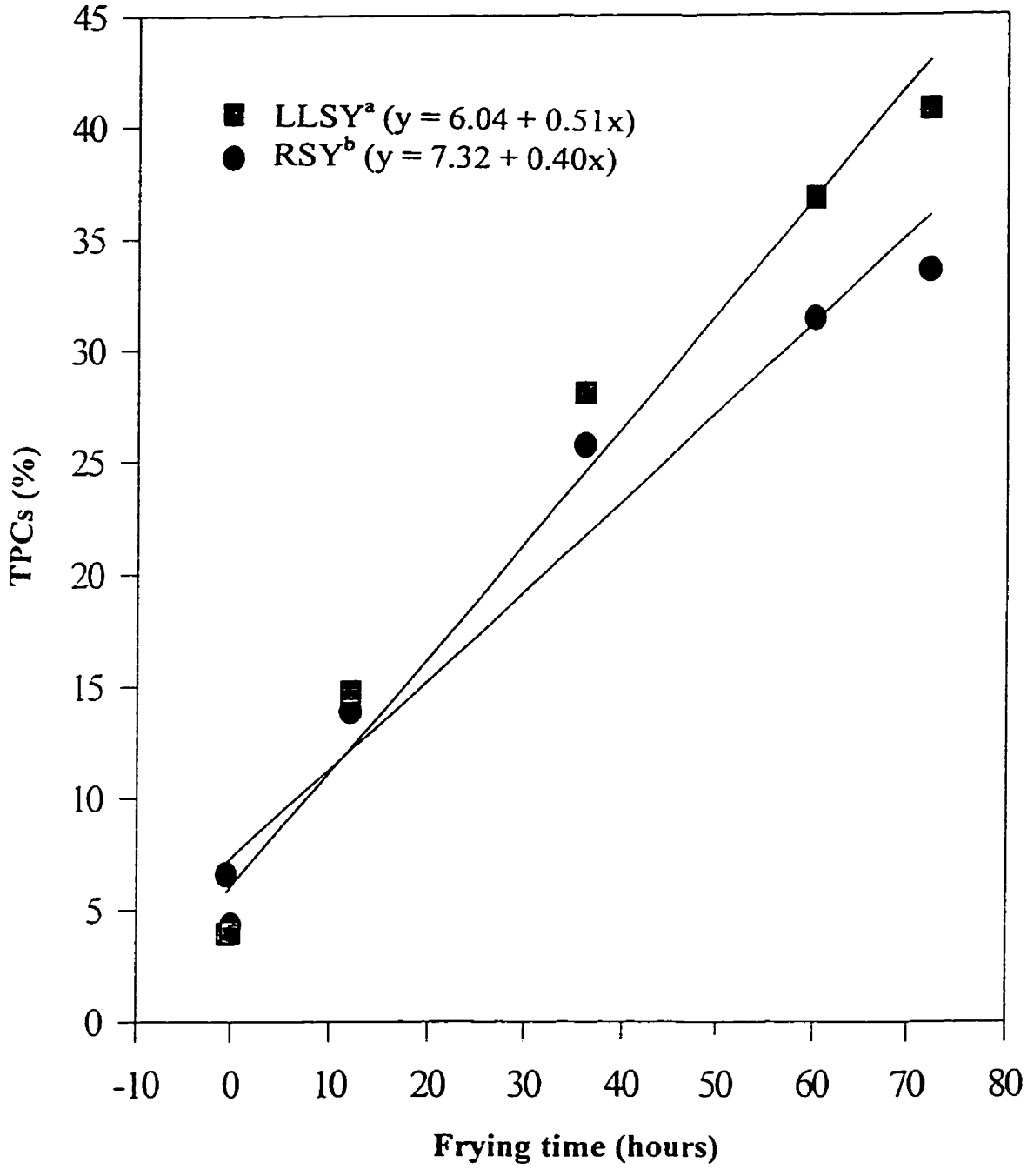


Figure 4.8. Total polar components (TPCs) over frying time for soybean oils

N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of TPCs formation

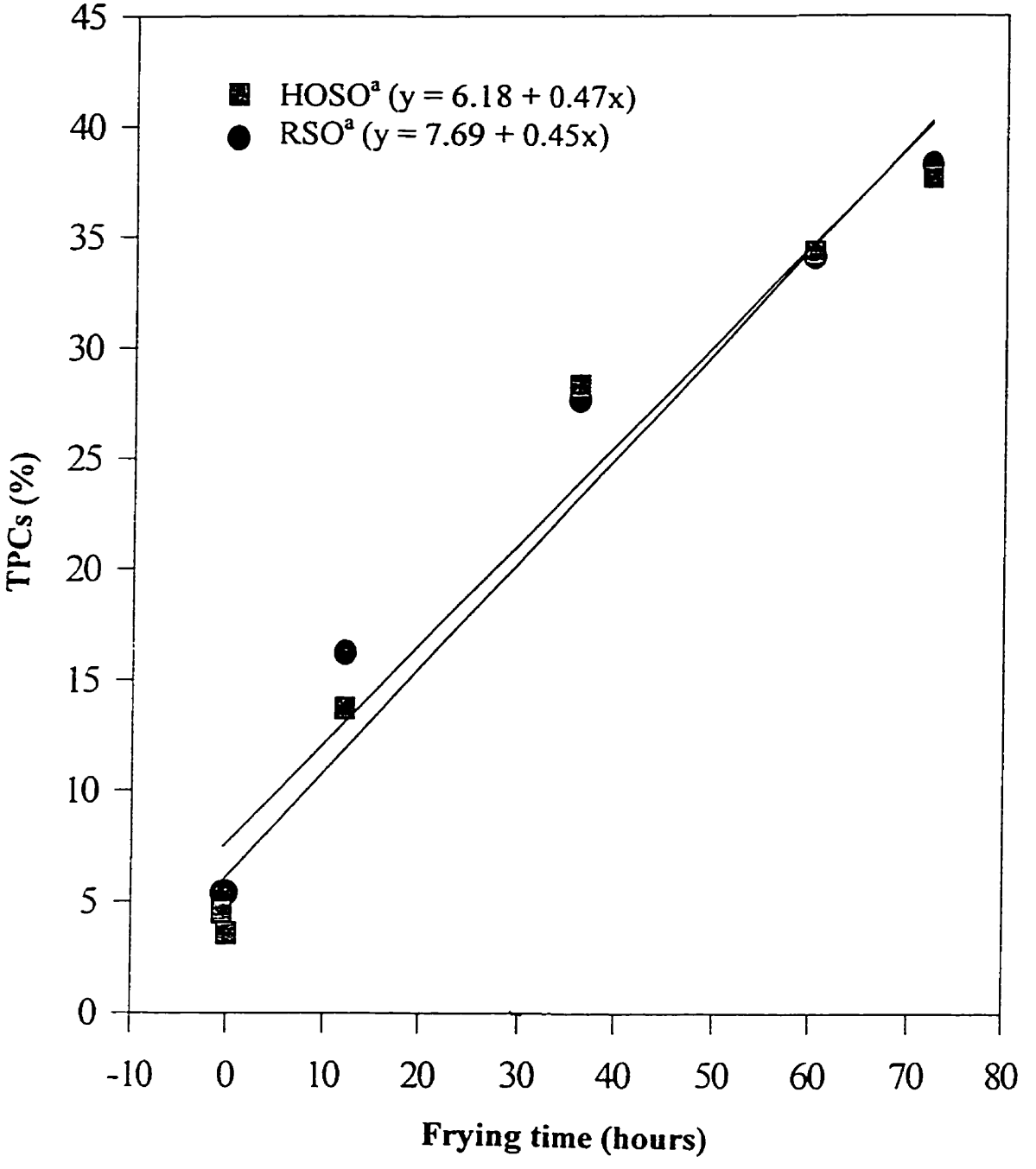


Figure 4.9. Total polar components (TPCs) over frying time for sunflower oils

N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of TPCs formation

explanation as to why these two oils showed similar stability. Romero et al. (1995b) reported much lower levels of TPCs in high oleic sunflower oil after frying for 16.5 hours when compared with a similar study (Cuesta et al., 1993) performed with a regular sunflower oil. Dobarganes et al. (1993) reported significantly ($p \leq 0.05$) higher levels of TPCs in regular as compared to three high oleic sunflower oils during a 10 hour simulated frying study. An actual frying experiment was also carried out for five hours, and results indicated that the modified oil with the highest oleic acid content had a significantly lower level of TPCs than all other oils after five hours of frying. However these studies were performed for only a very short period of time, and levels rather than rates of TPCs formation were compared. In addition, the studies conducted by Romero et al. (1995b) and Cuesta et al. (1993) involved frying many more batches of french fries than in the current study.

Overall, none of the modified oils displayed any significant improvement in stability when compared with the corresponding regular oil, despite reduced levels of PUFAs in each instance. In several cases, the modified oil was found to be significantly less stable than its regular counterpart. The absence of any improvement in frying stability of the modified oils provides strong evidence that fatty acid composition alone cannot fully explain differences in frying stability. In several situations, lower initial tocopherol levels provided one possible explanation as to why the modified oils did not show improvements in stability over their regular counterpart.

Since FFAs and TPCs are both measures of frying oil degradation, correlation analysis was carried out to determine whether there was a strong relationship between the results obtained by these two methods. Plots of the relationship and corresponding

correlation coefficients between TPCs and FFAs (either by the Veri-Fry® Pro FFA-75 quick test or the AOCS method) are provided in Figures 4.10 to 4.12. A correlation coefficient of r = 0.80 existed between TPCs and FFAs determined for all eight oils using the Veri-Fry® Pro FFA-75 quick test (Figure 4.10). A strong correlation also existed between TPCs and FFAs determined for RCO, HOCO and HOLLCO by the AOCS method, with r = 0.96 (Figure 4.11). When results for these same three oils were considered, a correlation coefficient of r = 0.94 existed between TPCs and FFAs determined using the Veri-Fry® Pro FFA-75 quick test (Figure 4.12). These results indicated that a strong relationship existed between TPCs and FFAs determined by either method. This finding is contrary to the results of a study by Lopez-Varela et al. (1995), who evaluated sunflower oils used in frying and found a much lower correlation coefficient of r = 0.41 between TPCs and FFAs. However, these researchers used a shorter frying period (~25 hours) and fried 75 batches of french fries as compared to the 13 batches fried in the current study.

4.2.3 High Performance Size Exclusion Chromatography (HPSEC)

HPSEC has become an important technique in the analysis of polymers in oils (Christie, 1995). When levels of TPCs are similar, HPSEC can still reveal any differences in the particular breakdown products present (Dobarganes et al., 1988). Such differences in these products and their relative amounts may indicate differences in the breakdown patterns or reactions occurring in a specific oil during the frying process (Dobarganes et al., 1988). For example, the amount of hydrolysis can be determined by quantification of diglycerides by HPSEC, as these compounds are less volatile than FFAs and remain in the oil (Dobarganes et al., 1988; Arroyo et al., 1995). The amount of

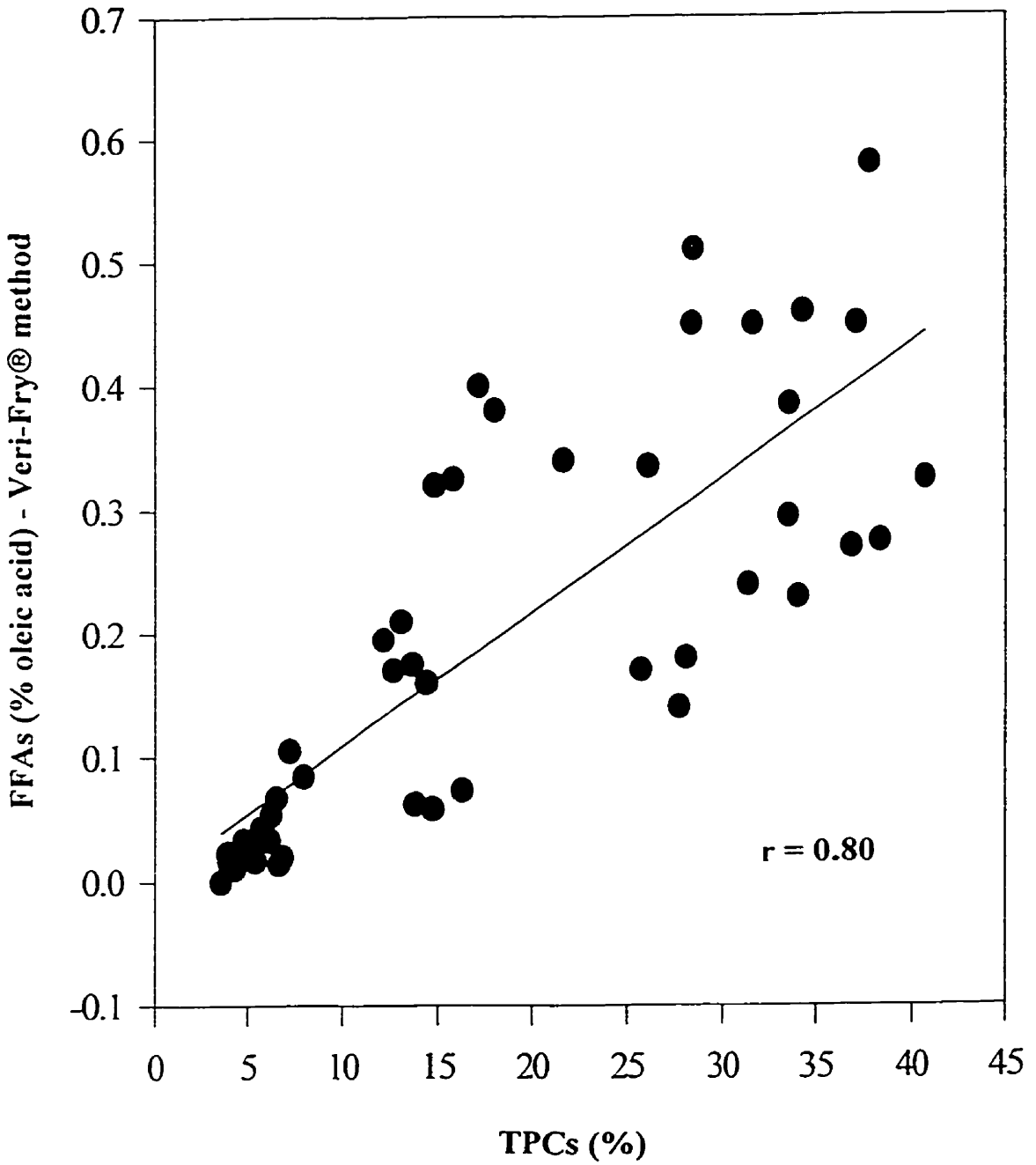


Figure 4.10. Relationship between free fatty acids (FFAs) determined by the Veri-Fry® Pro FFA-75 quick test method and total polar components (TPCs)

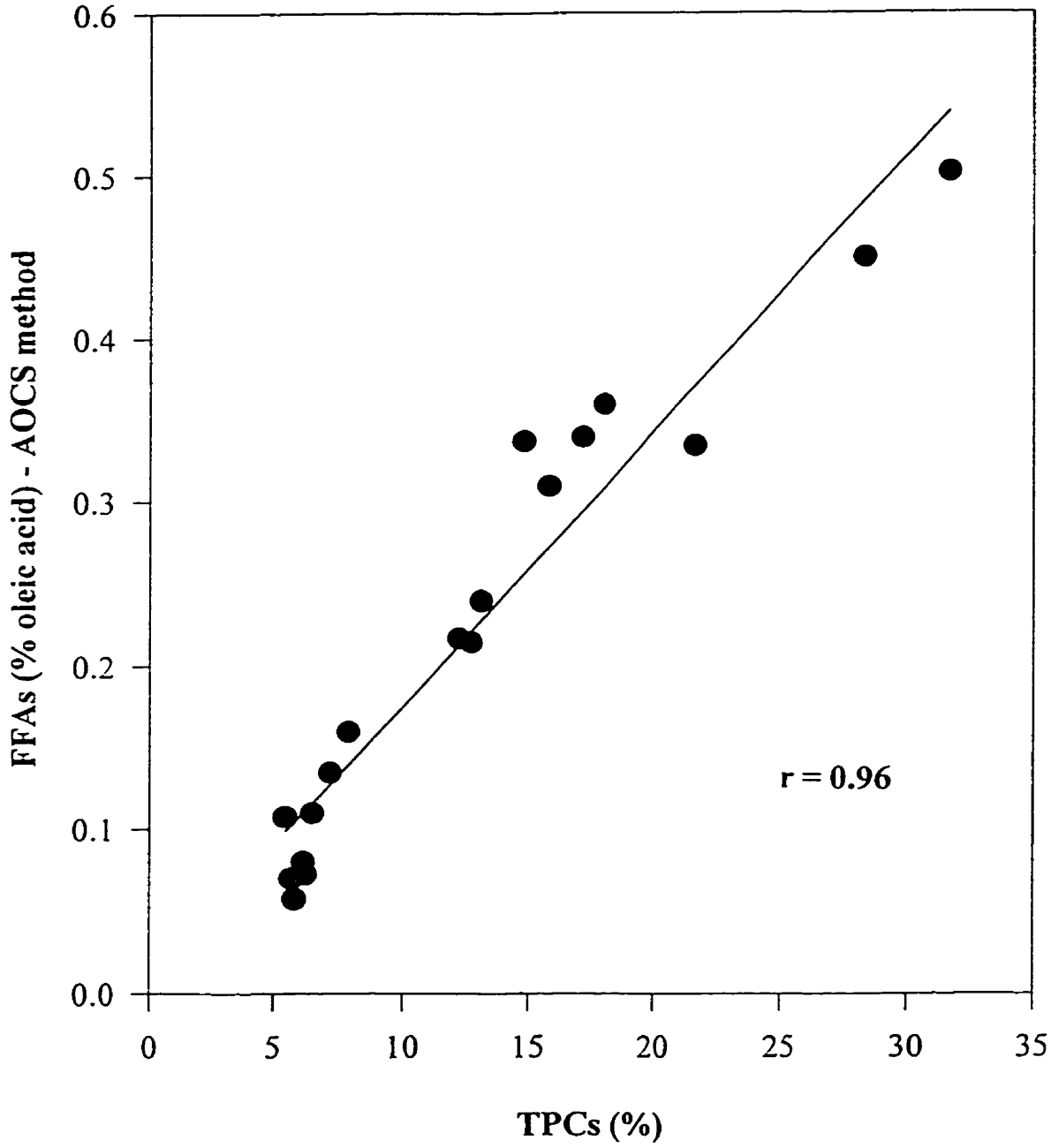


Figure 4.11. Relationship between free fatty acids (FFAs) determined by AOCS official method Ca 5a-40 and total polar components (TPCs) for RCO, HOCO and HOLLCO

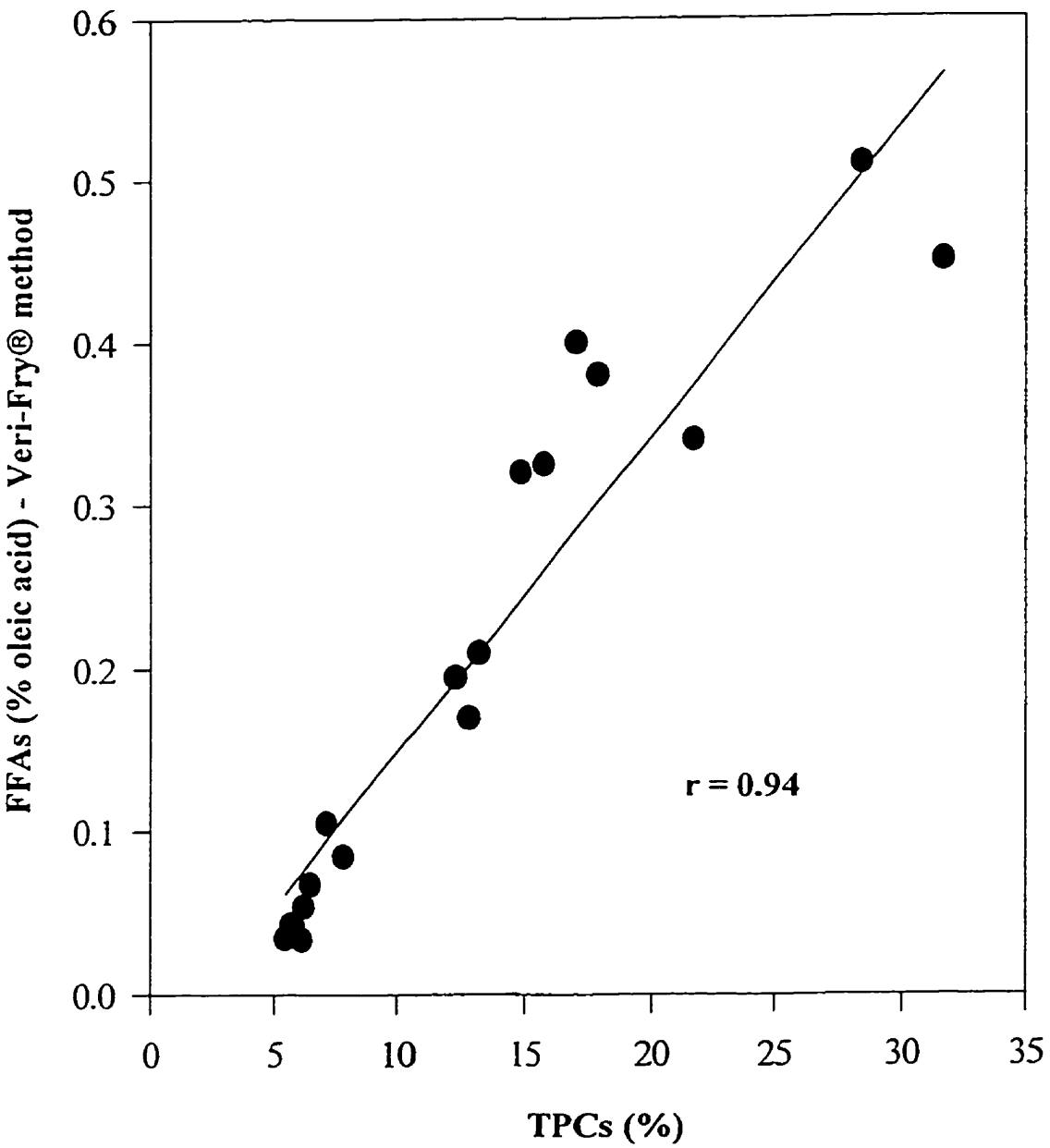


Figure 4.12. Relationship between free fatty acids (FFAs) determined by the Veri-Fry® Pro FFA-75 quick test method and total polar components (TPCs) for RCO, HOCO and HOLLCO

thermoxidation which has occurred is indicated by levels of polymers, dimers and oxidized triglycerides (Dobarganes et al., 1988; Romero et al., 1995a). Furthermore, the nutritional implications of ingesting specific alteration compounds may differ even when levels of TPCs are similar (Dobarganes et al., 1988). For example, when a fat is metabolized in the body, diglycerides and FFAs are normal breakdown products. However, polymers and oxidized triglycerides are not encountered during fat metabolism and thus are more of a nutritional concern (Romero et al., 1995a).

Results for HPSEC analysis of the oils over frying time are reported in terms of changes in peak area due to the heterogeneity of the fractions (Hopia et al., 1993) and the lack of appropriate standards. The lack of uniformity in fractions also resulted in a range of standard deviations from between 9 to 13% (Hopia et al., 1993). The five major fractions identified in the oils in the current study were monoglycerides and FFAs (peak 5), diglycerides (peak 4), oxidized triglycerides (peak 3), dimeric triglycerides (peak 2) and higher polymeric triglycerides (peak 1). Identifications of the fractions were based upon the retention time of the unaltered triglycerides in fresh oil samples. Trends, rather than specific changes in peak areas, will be emphasized in the discussion as these indicated overall which types of reactions - oxidative or hydrolytic - occurred to a greater extent.

Figures 4.13 to 4.20 show the separation of polar components by HPSEC for each of the oils. Levels of dimers and higher polymers (peaks 1 and 2) increased steadily over frying time for HOCO, HOLLCO, LLCO and HOSO (Figures 4.14 to 4.16, 4.20). An exception to this pattern was found in RCO, where dimers and higher polymers increased gradually over time until 60 hours, at which point they increased dramatically by 72 hours

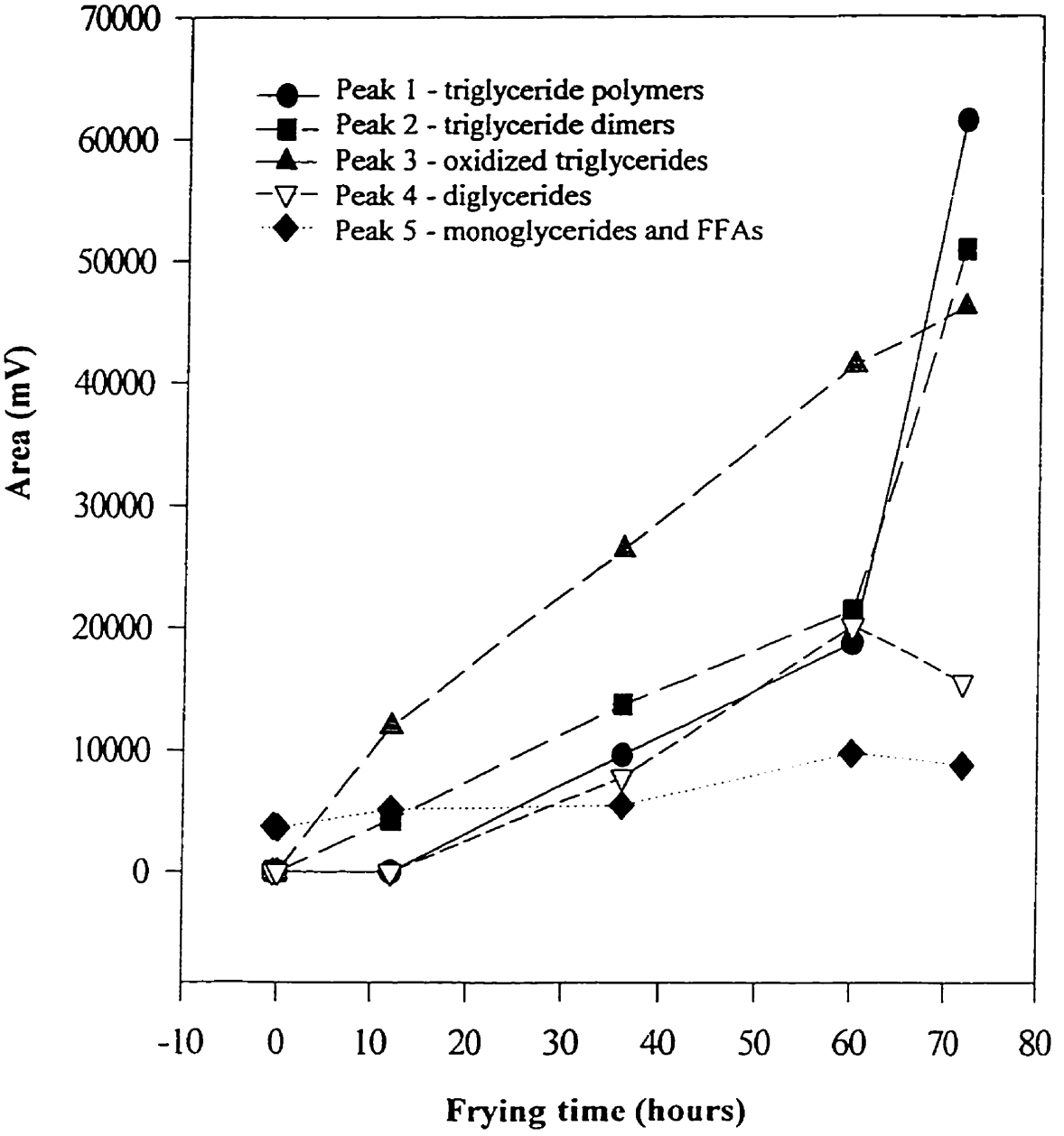


Figure 4.13. Separation of polar components of RCO over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

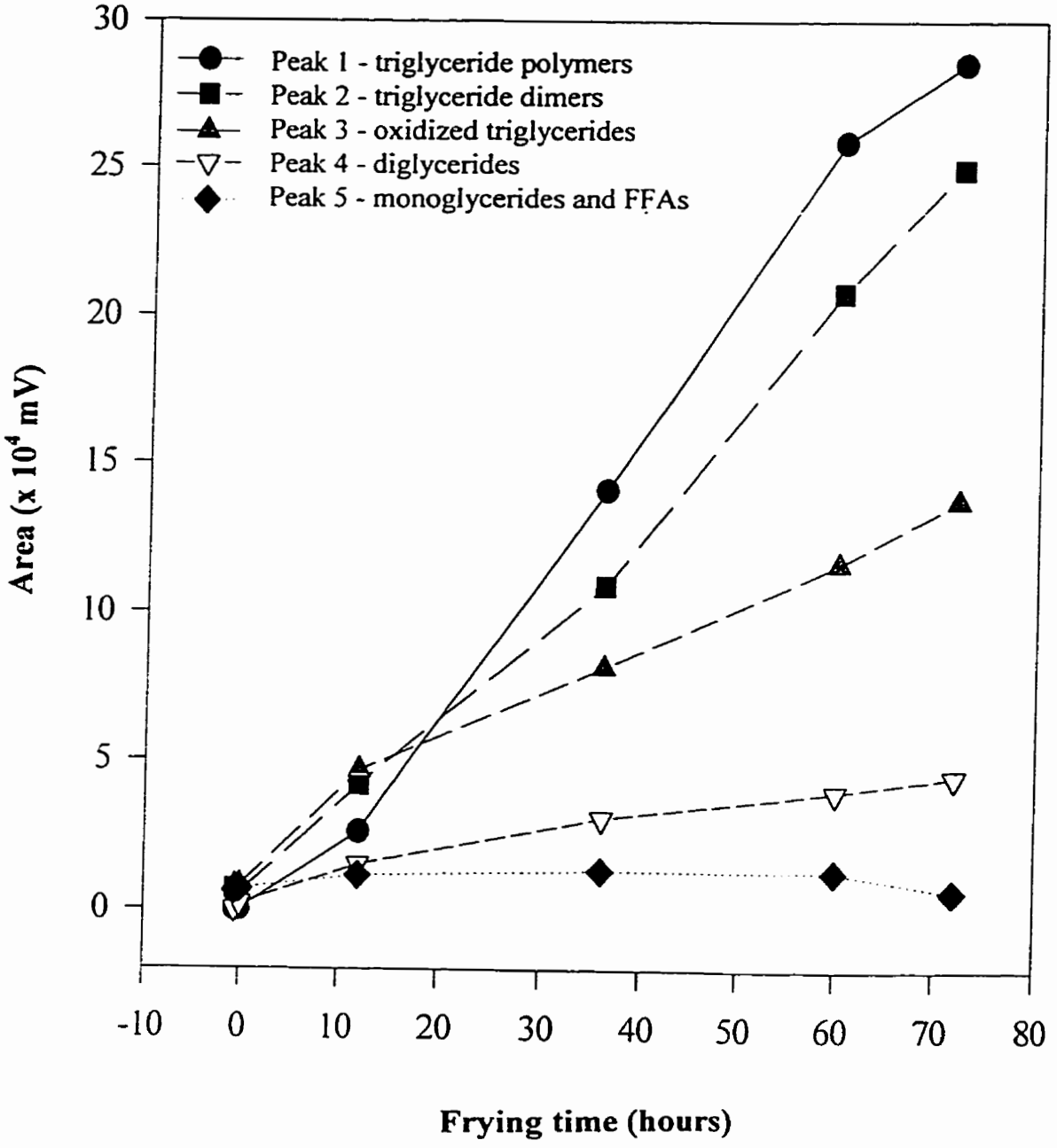


Figure 4.14. Separation of polar components of HOCO over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

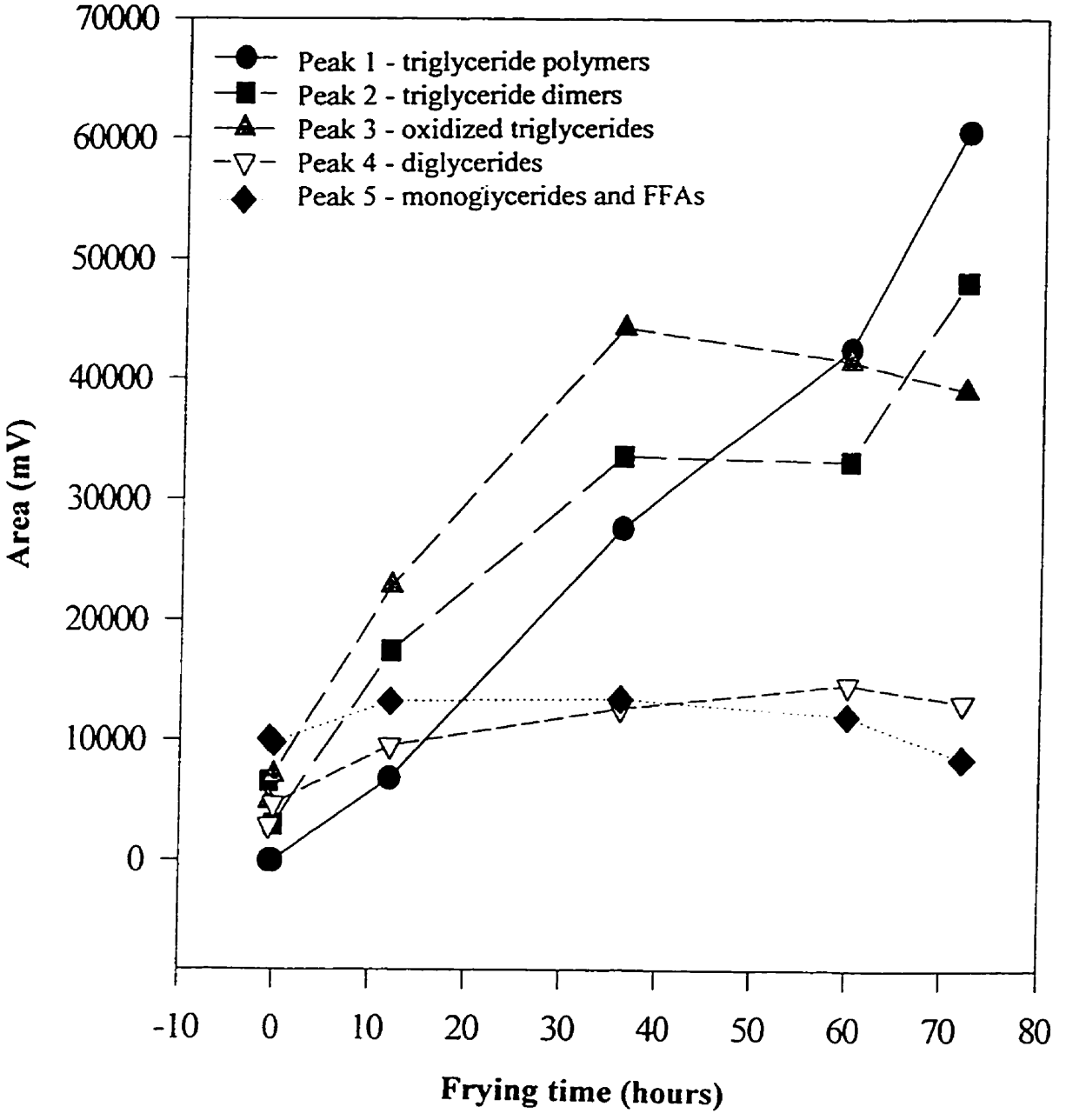


Figure 4.15. Separation of polar components of HOLLCO over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

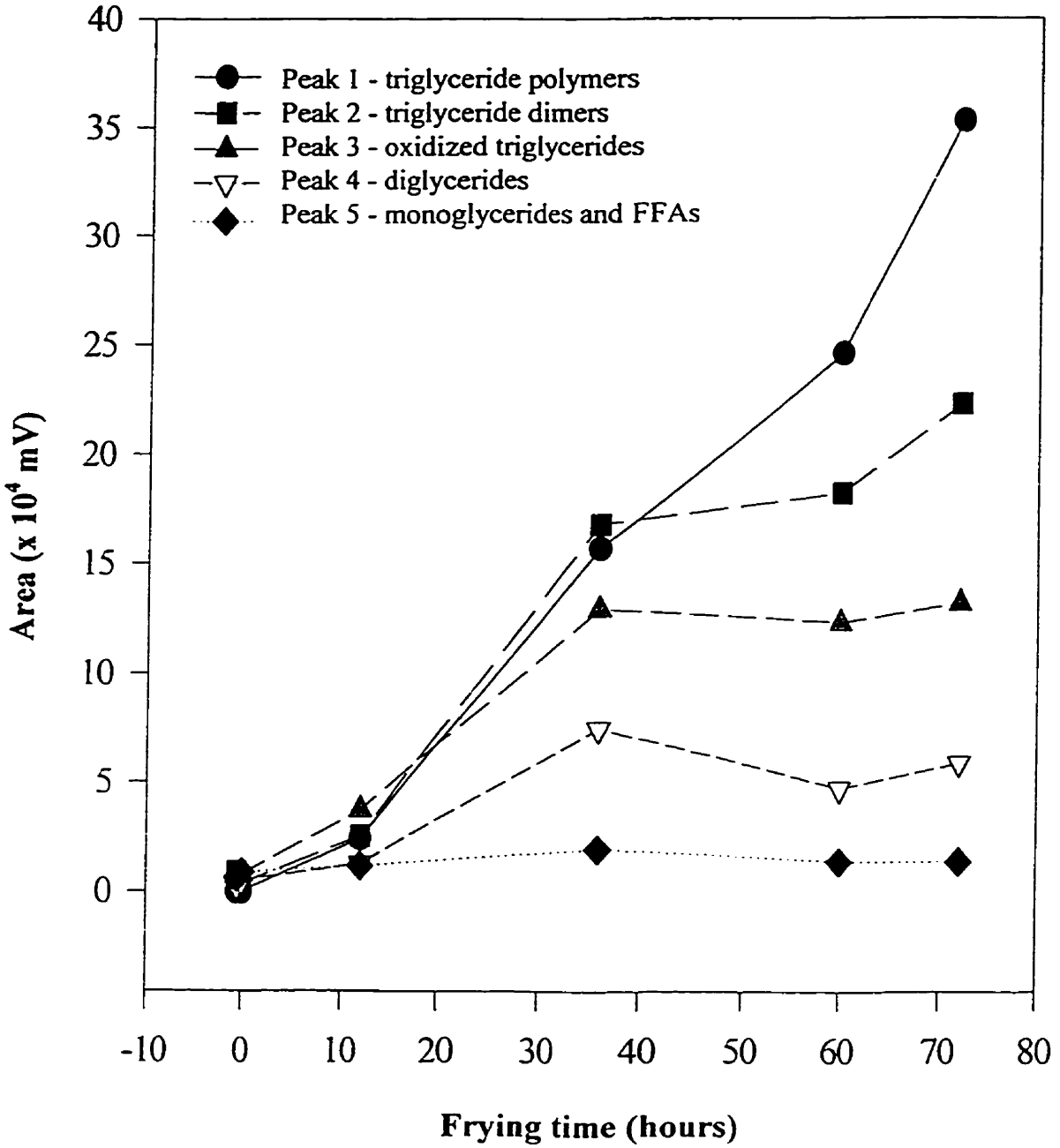


Figure 4.16. Separation of polar components of LLCO over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

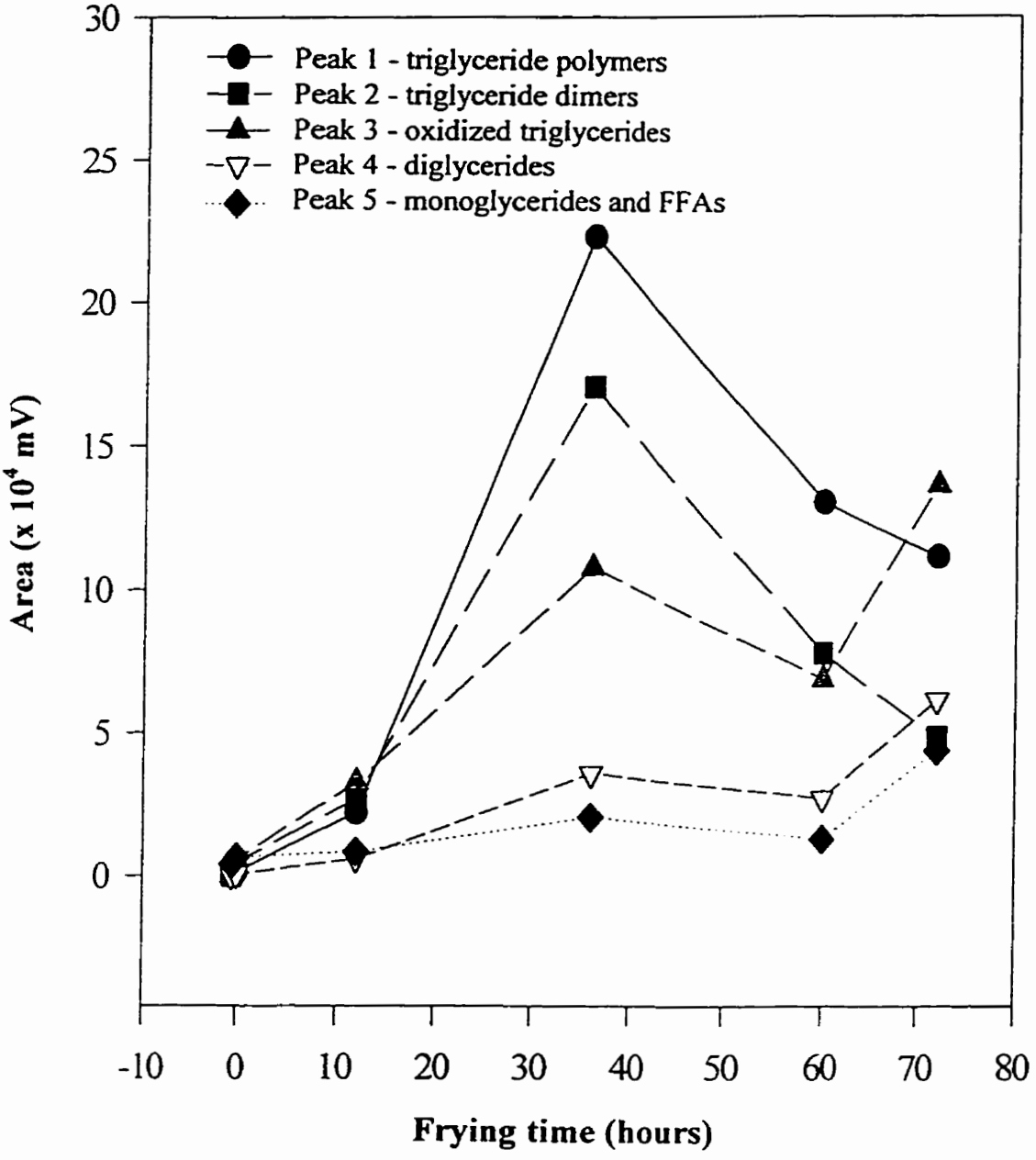


Figure 4.17. Separation of polar components of RSY over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

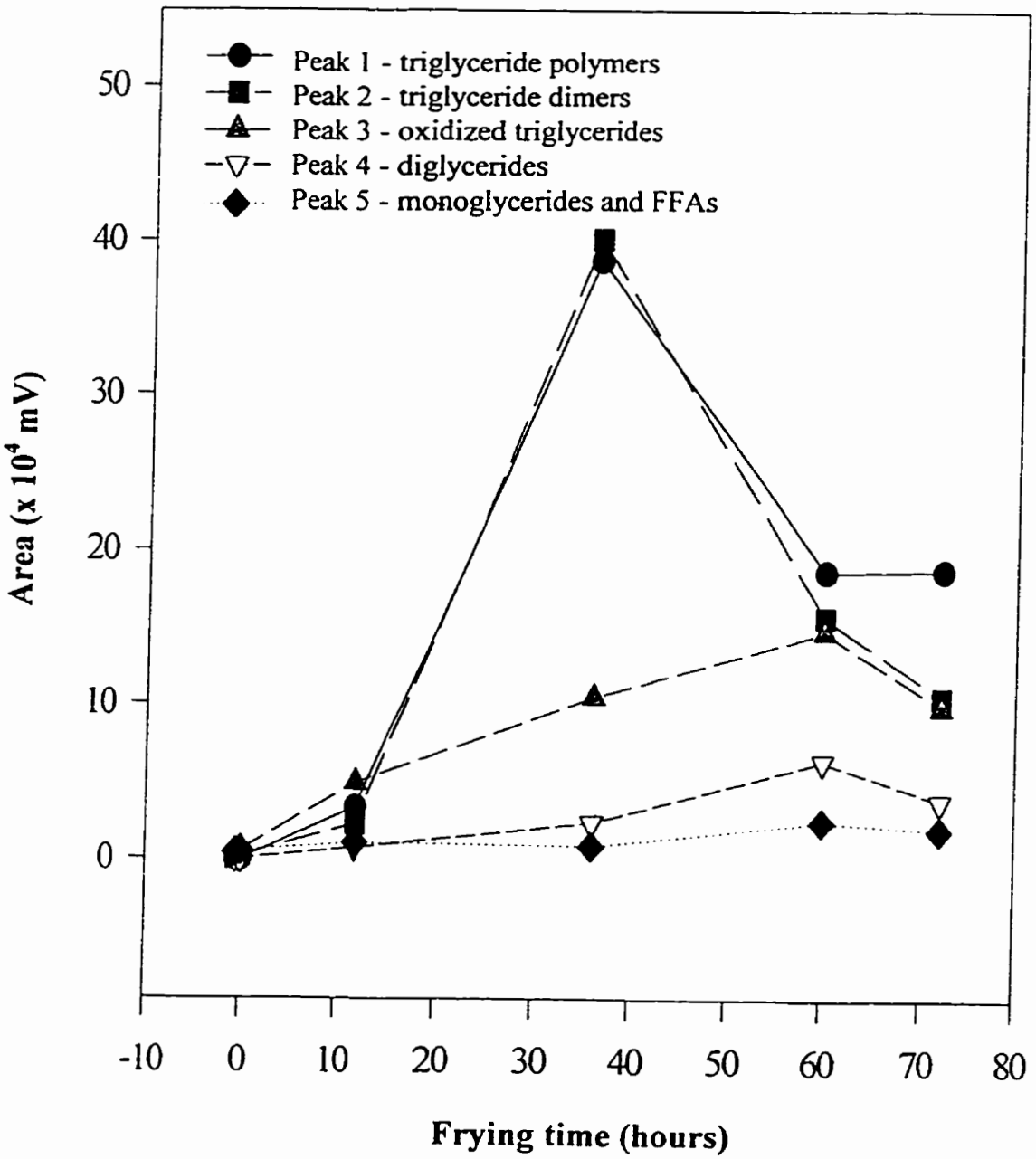


Figure 4.18. Separation of polar components of LLSY over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

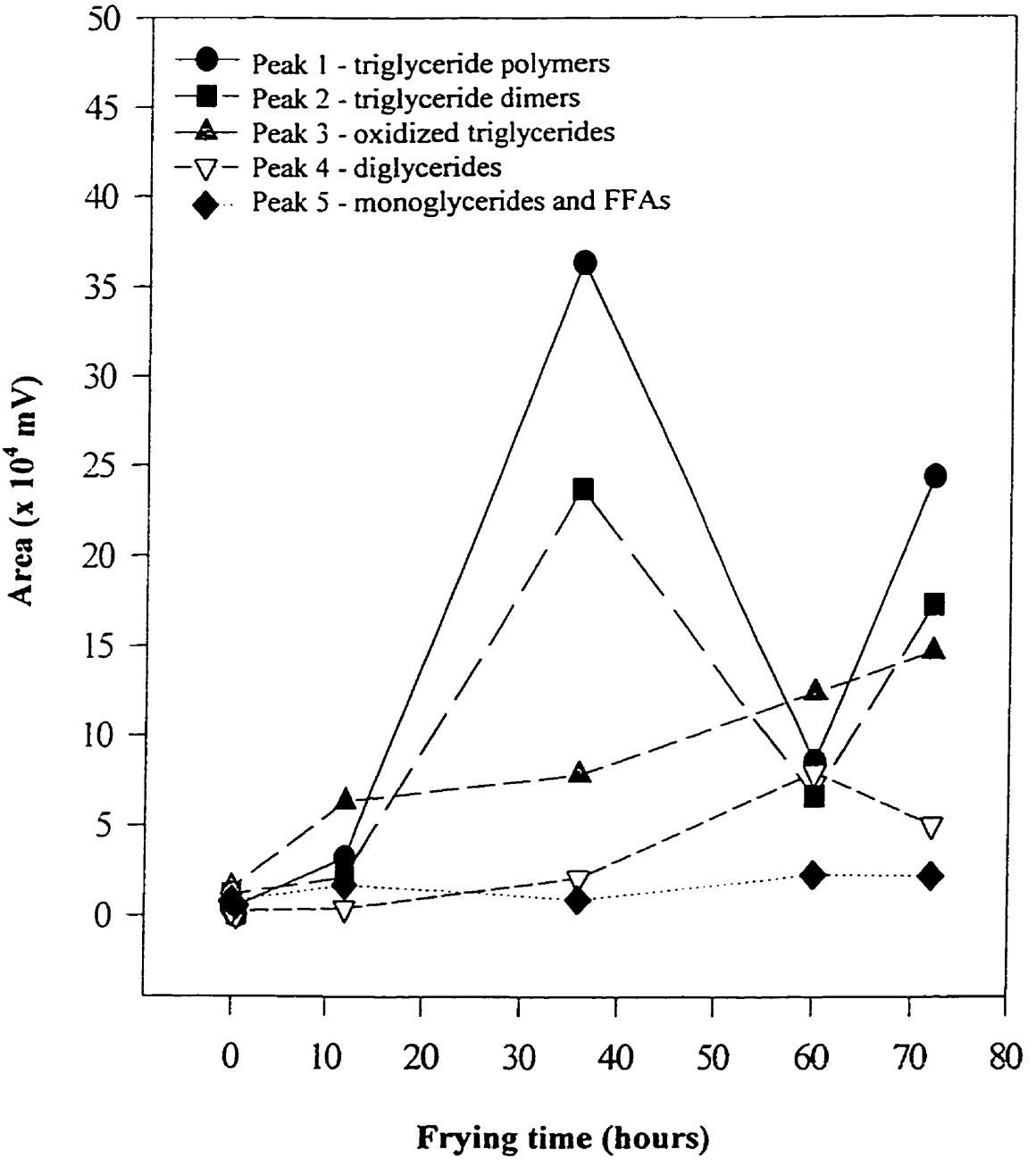


Figure 4.19. Separation of polar components of RSO over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

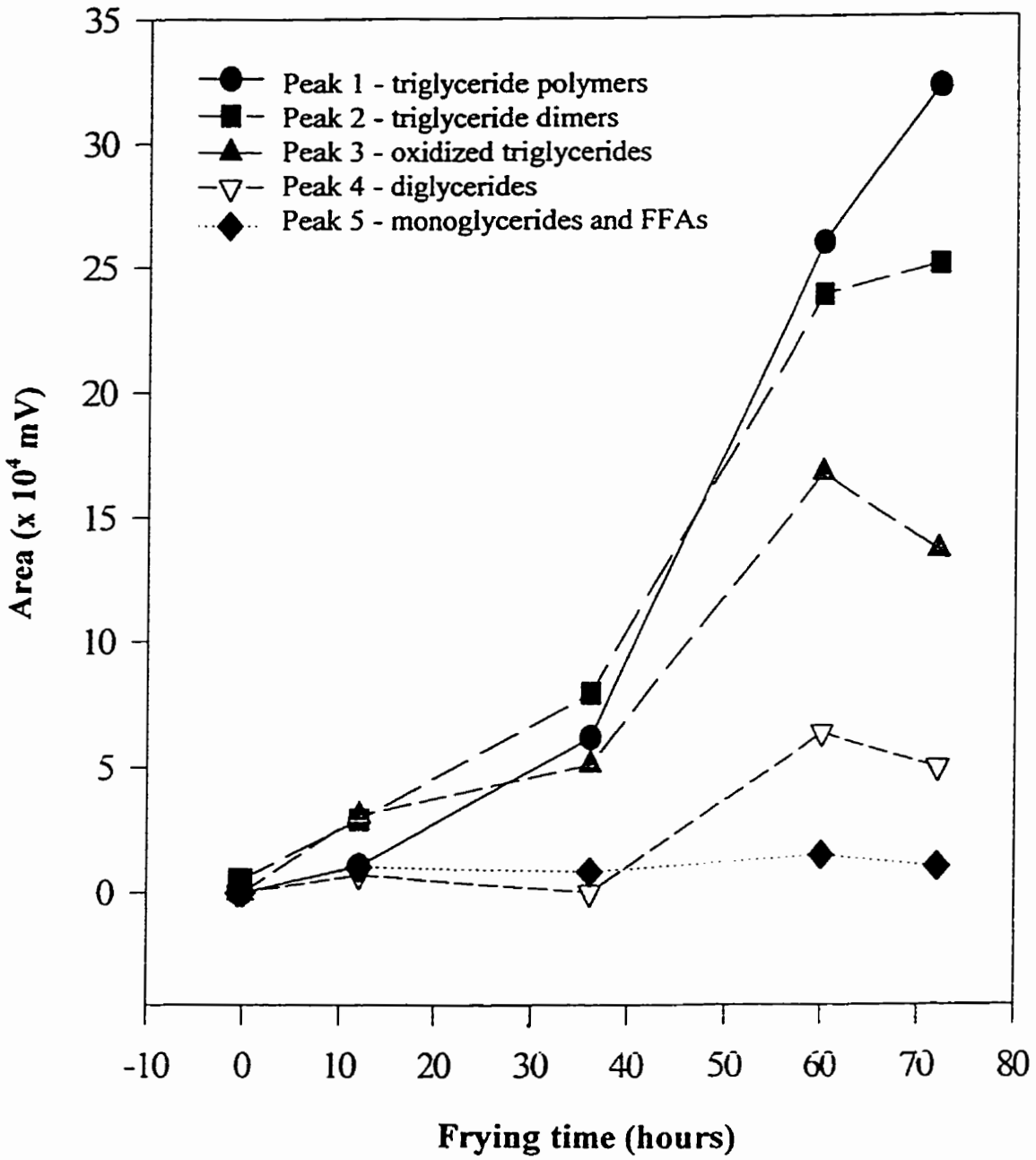


Figure 4.20. Separation of polar components of HOSO over frying time by high performance size exclusion chromatography (HPSEC) of polar fraction

(Figure 4.13). A second deviation from this trend occurred for RSY and LLSY, where these compounds increased until 36 hours, and then decreased until 72 hours (Figures 4.17 and 4.18). RSO displayed a unique pattern of change in polymers and dimers. In RSO, peaks 1 and 2 increased dramatically up to 36 hours, then dropped by 60 hours, and again increased at 72 hours (Figure 4.19).

In most oils, the levels of oxidized triglycerides (peak 3) increased steadily over frying time, sometimes plateauing or dropping slightly. RSY deviated from this pattern as levels of oxidized triglycerides in this oil reached a maximum at 36 hours, decreased, and then started to increase after 60 hours (Figure 4.17). The levels of oxidized triglycerides at 72 hours surpassed the levels of dimers and higher polymers, in terms of peak areas, for RSY.

Diglycerides (peak 4) are products of hydrolysis reactions which are less volatile than FFAs. Therefore, they remain in an oil and can be monitored as an indication of the extent of hydrolytic reactions which have occurred (Dobarganes et al., 1988). For all eight oils, levels of diglycerides as measured by peak area remained fairly stable over frying time, suggesting that hydrolysis was not the main type of reaction responsible for the frying degradation of the oils.

Peak areas for monoglycerides and FFAs (peak 5) remained fairly stable over time for all oils excluding RSY (Figure 4.17), where a slight increase occurred at 72 hours. The stability rather than increase of FFA levels could be anticipated as these components often leave the oils as volatile components (Dobarganes et al., 1988). Dobarganes et al. (1988) investigated the polar fractions of cottonseed oil. They saw high ratios of diglyceride to FFA peaks, providing evidence of the low stability of FFAs.

Overall, the changes observed for HPSEC analysis of specific polar compounds in this study were similar to those found by other researchers in that FFAs, monoglycerides and diglycerides increased only slightly while oxidized triglycerides, dimers and higher polymers increased steadily over the course of frying (White and Wang, 1986; Dobarganes et al., 1988; Arroyo et al., 1992; Hopia, 1993a; Sanchez-Muniz et al., 1993; Arroyo et al., 1995; Lopez-Varela et al., 1995; Marquez-Ruiz et al., 1995; Romero et al., 1995a). Deviations from this pattern in the current study could not be explained. Lopez-Varela et al. (1995) fried potato chips in sunflower oil over a period of about 25 hours and found that TPCs, polymers and dimers content increased substantially while oxidized triglycerides increased initially and then remained fairly constant. Diglycerides increased only slightly while the FFA levels remained constant. Arroyo et al. (1992) reported similar trends when they evaluated sunflower oil used to fry potato chips intermittently over a total heating time of about 20 hours. A third study which involved frying of potato chips in sunflower oils for a total of about 5 hours and 50 minutes also showed similar results (Sanchez-Muniz et al., 1993). One difference in this study was that diglycerides remained constant while FFAs increased slightly.

4.2.4 Fatty Acid Composition

Only small changes in fatty acid composition occurred over the 72-hour frying period (Table 4.9). In general, levels of linolenic and linoleic acid decreased over frying time. The reduction in linolenic acid content ranged from 0.1% in RSO and HOSO to 2.7% in HOCO. Losses of linoleic acid were slightly higher and ranged from 1.2% in HOLLCO to 6.7% in LLSY. Nichols et al. (1992) reported losses of 0.7% for linoleic acid and 0.1% for linolenic acid in sunflower oil deep fat fried for just over two and a half

Table 4.9. Changes in fatty acid composition of oils after 72 hours of frying[a]

Oil	Δ 18:1[b] (%)	Δ 18:2[c] (%)	Δ 18:3[d] (%)
Canola:			
RCO	+ 2.5	- 1.5	- 1.5
HOCO	+ 3.4	- 2.5	- 2.7
HOLLCO	+ 0.8	-1.2	- 0.7
LLCO	+ 3.6	- 5.4	- 0.8
Soybean:			
RSY	+ 2.4	- 3.4	- 1.8
LLSY	+ 3.0	- 6.7	- 1.0
Sunflower:			
RSO	+ 2.2	- 5.3	- 0.1
HOSO	- 3.2	- 1.7	- 0.1

[a] all values are average of duplicate analysis
[b] change in oleic acid
[c] change in linoleic acid
[d] change in linolenic acid

hours. Losses of these two fatty acids could be anticipated as they were present in abundant amounts in most of the oils, and were also much less stable to oxidative change than any of the other major fatty acids present in vegetable oils (Labuza, 1971). Petukhov (1996) monitored changes in the fatty acid compositions of regular, hydrogenated, low linolenic and high oleic canola oils after frying for 40 hours. Results were reported as ratios of the percentage contribution of specific fatty acids to the contribution of stearic acid in each oil. Greater decreases in linoleic and linolenic acid ratios were found as compared to very small decreases in oleic acid ratios. Percentage contribution of oleic acid to total fatty acid composition increased by 72 hours for most oils, with increases ranging from 0.8% for HOLLCO to 3.6% for LLCO. The exception was HOSO, where oleic acid contribution dropped from 88.9% initially to 85.7% at 72 hours. Liu and White (1992) reported greater increases in oleic acid (up to 12.3%) and decreases in linoleic (up to 24.1%) and linolenic (up to 5.2%) acids among two regular and four low linolenic soybean oils. Even though these oils were heated to frying temperatures for a shorter period of time (~40 hours), the greater changes in fatty acid composition as compared with the current study may be partially explained by the smaller oil volume (i.e. 300 mL) used and lack of oil replenishment in their study. Miller and White (1988) examined changes in the fatty acid composition of two regular, one low linolenic and one high stearate soybean oil after 40 hours of heating to frying temperature. They reported increases in oleic acid content of all oils ranging from 1.7% in the high stearate oil to 4.9% in one of the regular soybean oils. Losses of linoleic acid ranged from 3.7% in one of the regular to 6.2% in the other regular oil. Linolenic acid content decreased in all oils, ranging from a decline of 1.6% in both modified oils to 3.2% in one of the regular oils.

However, the researchers heated the oils for a shorter period of time, used a smaller amount of oil (i.e. 450 g), and did not replenish the oils. After 80 hours of heating between 189 and 200° C, Dobarganes and Perez-Camino (1988) reported an increase of 7.6% in oleic and a decrease of 12.6% in linoleic acid in regular sunflower oil. Under the same conditions, regular soybean oil experienced a 10.4% increase in oleic acid and decreases of 15 and 5.3% in linoleic and linolenic acids, respectively. Dobarganes and Perez-Camino (1988) reported that losses of oleic acid during heating to frying temperature were much greater when an oil contained low levels of PUFAs. This finding is consistent with results from the current study, where the contribution of oleic acid to total fatty acid composition decreased after frying only in HOSO, which had the lowest PUFA content of all the oils. Correspondingly, the researchers reported that the percentage contribution of oleic acid tended to increase in regular soybean and sunflower oils, which were higher in unsaturation than the other oils examined (Dobarganes and Perez-Camino, 1988). This tendency for oleic acid contribution to increase was also apparent in the current investigation for all oils except the previously mentioned HOSO. Specific changes in fatty acid composition in all of these studies appeared to depend on the particular frying conditions. However, a general trend for increases in oleic acid and decreases in linoleic and linolenic acids was common to all of these studies, including the current investigation.

4.2.5 Tocopherols

Rates of tocopherol degradation varied greatly between the oils investigated (Table 4.10, Figures 4.21 to 4.23). Tocopherol degradation rates are reported as the time required in hours for the concentration of tocopherols in the fresh oil to be reduced by

Table 4.10. Total and individual tocopherol degradation rates

Oil	Time (h) to reduce original concentration by 50%		
	Total tocopherols	α-Tocopherol	γ-Tocopherol
Canola:			
RCO	> 72	> 72	60 - 72
HOCO	3 - 6	3 - 6	3 - 6
HOLLCO	48 - 60	> 72	36 - 48
LLCO	3 - 6	3 - 6	3 - 6
Soybean:			
RSY	12 - 24	12 - 24	12 - 24
LLSY	9 - 12	9 - 12	9 - 12
Sunflower:			
RSO	12 - 24	12 - 24	12 - 24
HOSO	0 - 3	0 - 3	0 - 3

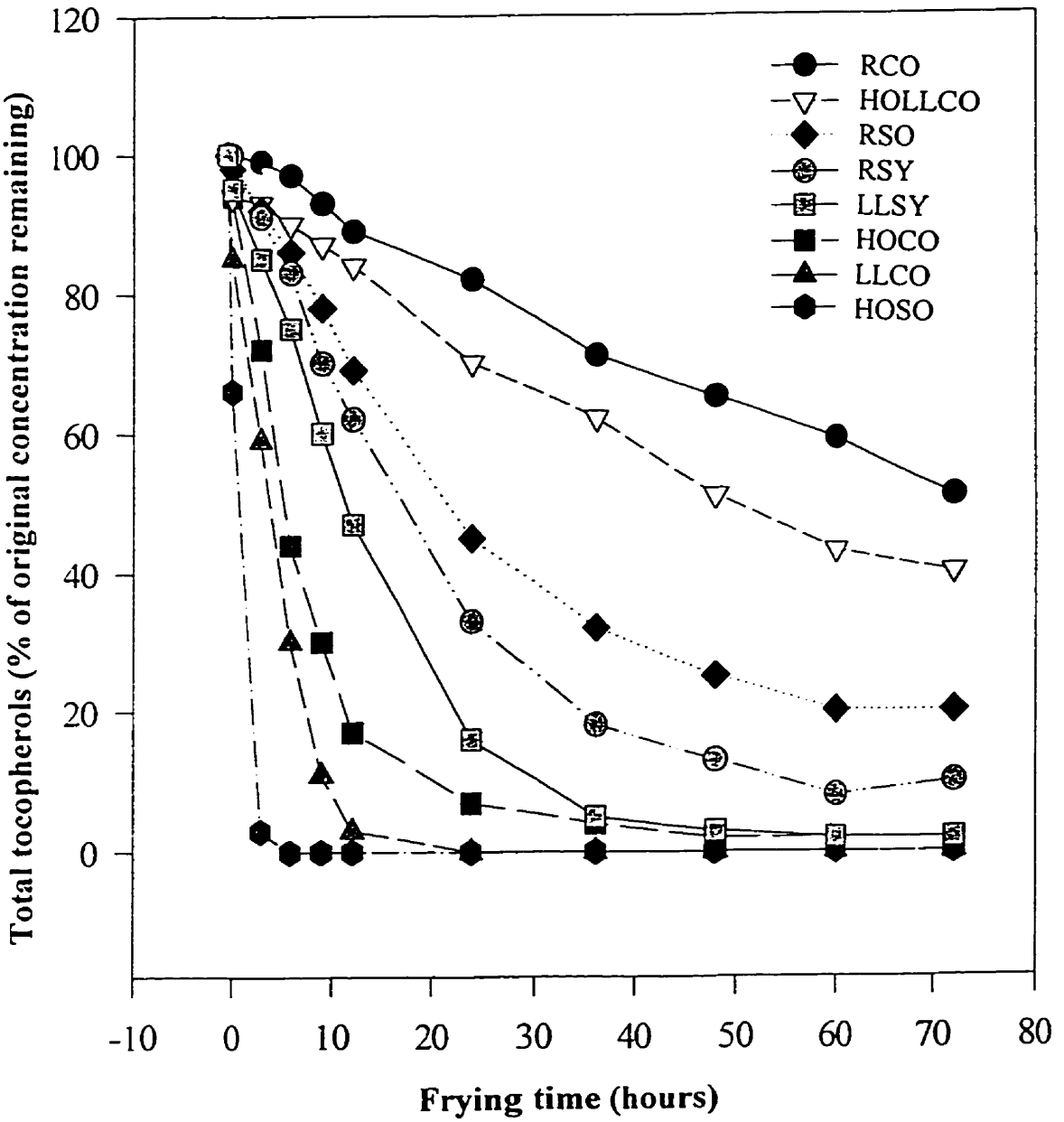


Figure 4.21. Total tocopherols remaining (% of original concentration) over frying time for all oils

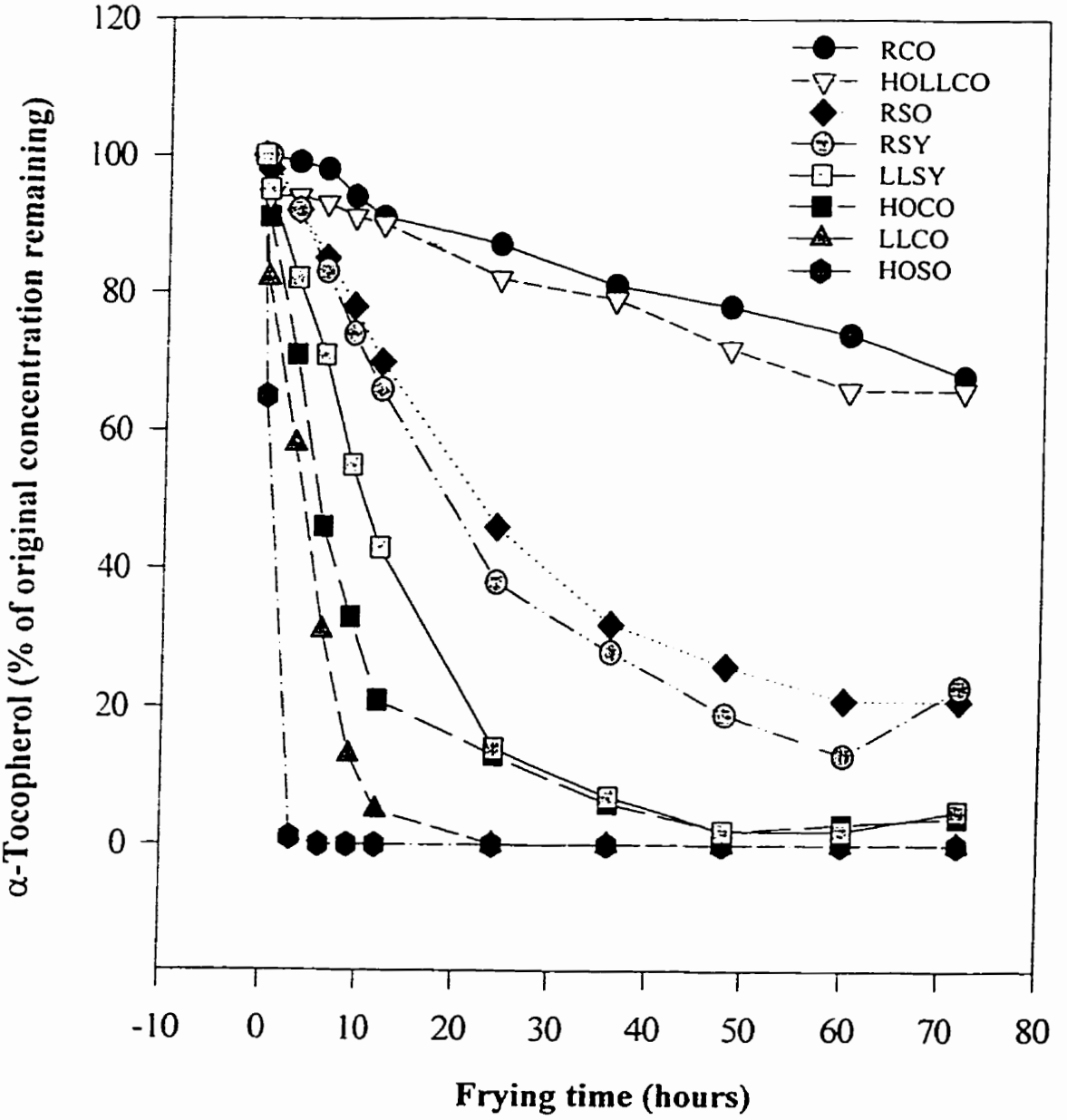


Figure 4.22. Total α-tocopherol remaining (% of original concentration) over frying time for all oils

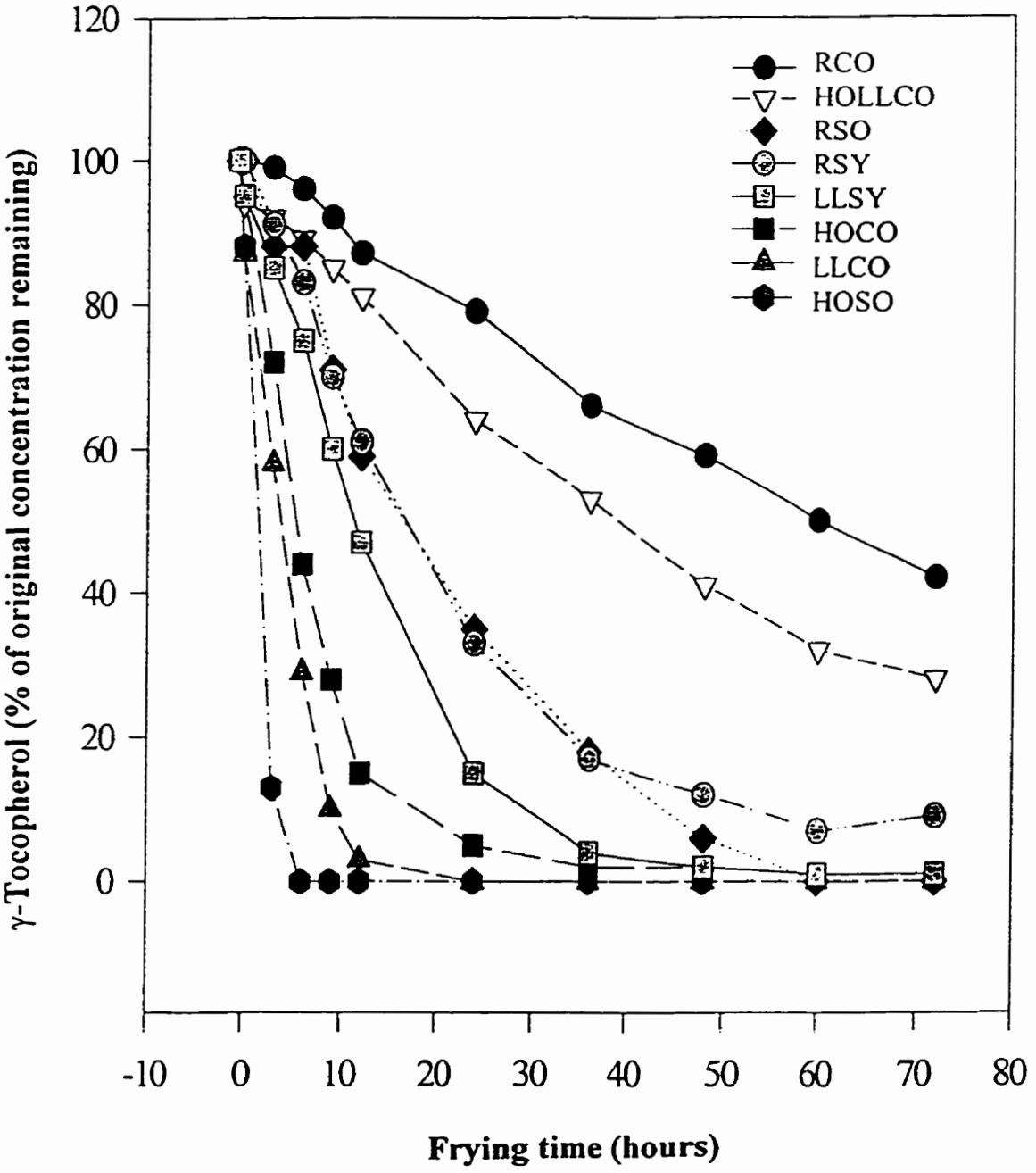


Figure 4.23. Total γ-tocopherol remaining (% of original concentration) over frying time for all oils

half. This method of measuring tocopherol degradation was chosen as it is equivalent to the half-life of the tocopherols under the conditions of the current study. It was also chosen to describe the rate of tocopherol degradation as tocopherol losses follow an approximately linear pattern within this range (refer to Figures 4.21 to 4.23) for all oils. Among the canola oils, total tocopherols in HOCO and LLCO degraded at substantially faster rates. Somewhere between 3 and 6 hours, the initial concentrations of tocopherols were reduced by half in these two oils. The same level of tocopherol degradation was seen between 48 and 60 hours for HOLLCO and, by the end of the 72 hour frying period, had not been reached for RCO. Both α- and γ-tocopherol degraded at the same rate for HOCO and LLCO, taking 3 to 6 hours to reach half of their original concentrations. However, in the case of RCO and HOLLCO, γ-tocopherol degraded at a faster rate than did α-tocopherol. In a frying study involving four types of regular and modified canola oils, Li (1996) also found that γ-tocopherol degraded at a faster rate than α-tocopherol in all four oils. Miyagawa et al. (1991) reported that γ-tocopherol degraded at a faster rate than α-tocopherol during deep frying of french fries; or potatoes coated with wheat flour, egg and water; in a mixture of soybean and rapeseed oils. Gordon and Kourimska (1995a) followed losses of tocopherols during deep fat frying of potato chips in low erucic acid rapeseed oils. They reported that α-tocopherol degraded at a much faster rate than β- or γ-tocopherol.

Only slight differences were observed between RSY and LLSY in their rates of tocopherol degradation. The rates for total, α- and γ-tocopherol degradation were 12 to 24 hours for RSY and 9 to 12 hours for LLSY. No differences occurred in the rates of

individual tocopherol degradation, however soybean oils only contained 5 to 6% of their total tocopherol content as α-tocopherol.

RSO displayed much slower rates of total, α- and γ-tocopherol degradation (12 to 24 hours) compared to HOSO (0 to 3 hours). Within each sunflower oil, α- and γ-tocopherol degraded at approximately the same rate. It should be pointed out, however, that γ-tocopherol only contributed 2 to 3% of the total tocopherol content of the sunflower oils. Melton et al. (1998) studied the degradation of α-tocopherol in high oleic and regular sunflower oils during frying of doughnuts for two 5 day periods. The researchers reported that α-tocopherol was destroyed much faster in high oleic as compared to regular sunflower oil; consistent with the results of the current study. By day three, no α-tocopherol remained in the high oleic sunflower oil, whereas regular sunflower oil still contained between 61 to 68% of its original α-tocopherol content on day five.

Since tocopherols can act as antioxidants, oils in which tocopherols degrade quickly would be expected to show lower stability. A less stable oil is defined in this study as one having significantly faster rates of either TPCs formation or FFAs accumulation. Among the canola oils, HOCO and LLCO exhibited faster rates of tocopherol degradation and were also found to have significantly faster rates of TPCs formation. No significant differences existed among the canola oils with respect to rates of FFAs accumulation. LLSY was found to have a faster rate of tocopherol degradation and TPCs formation than RSY. The soybean oils also did not differ significantly in rates of FFAs accumulation. Although no significant differences were found between RSO and HOSO in rates of TPCs formation, HOSO had a significantly faster rate of FFAs accumulation and a corresponding faster rate of tocopherol degradation than RSO. Oils

with higher tocopherol contents also received greater levels of added tocopherols in the replenishment volume. This greater addition of tocopherols every 12 hours may help explain why oils with higher levels of tocopherols displayed greater frying stability.

The relationship between rates of tocopherol degradation and rates of TPCs formation is shown in Figure 4.24. Oils with faster rates of tocopherol degradation also tended to have faster rates of TPCs formation. Since tocopherols provide a protective effect by acting as antioxidants during oxidation reactions, a relationship between these two rates can be expected. However, the graph also shows two distinct clusters, indicating that a third factor may explain the differences between the oils. The relationship between rates of FFAs accumulation and tocopherol degradation is shown in Figure 4.25. The lack of a clear relationship between these two rates may be due to: 1) relatively small changes in FFAs as compared to tocopherols, and/or 2) the absence of a role for tocopherols in hydrolysis reactions.

4.2.6 Phytosterols

Variations in rates of total and individual phytosterol degradation occurred among canola oils, but not among soybean or sunflower oils (Figures 4.26 to 4.30; Table 4.11). One limitation of this finding is that rates were calculated using only three points or measurements to develop the line. Therefore the results appear to approximate a linear relationship, although further measurements may prove this assumption to be false. Overall, however, changes in phytosterol content were relatively small. LLCO and HOCO had significantly ($p<0.05$) faster rates of total phytosterol degradation (17.9 and 13.9 mg/kg lost/hour, respectively) than did RCO (4.6 mg/kg lost/hour). This same trend was observed for β-sitosterol degradation among the canola oils. Campesterol

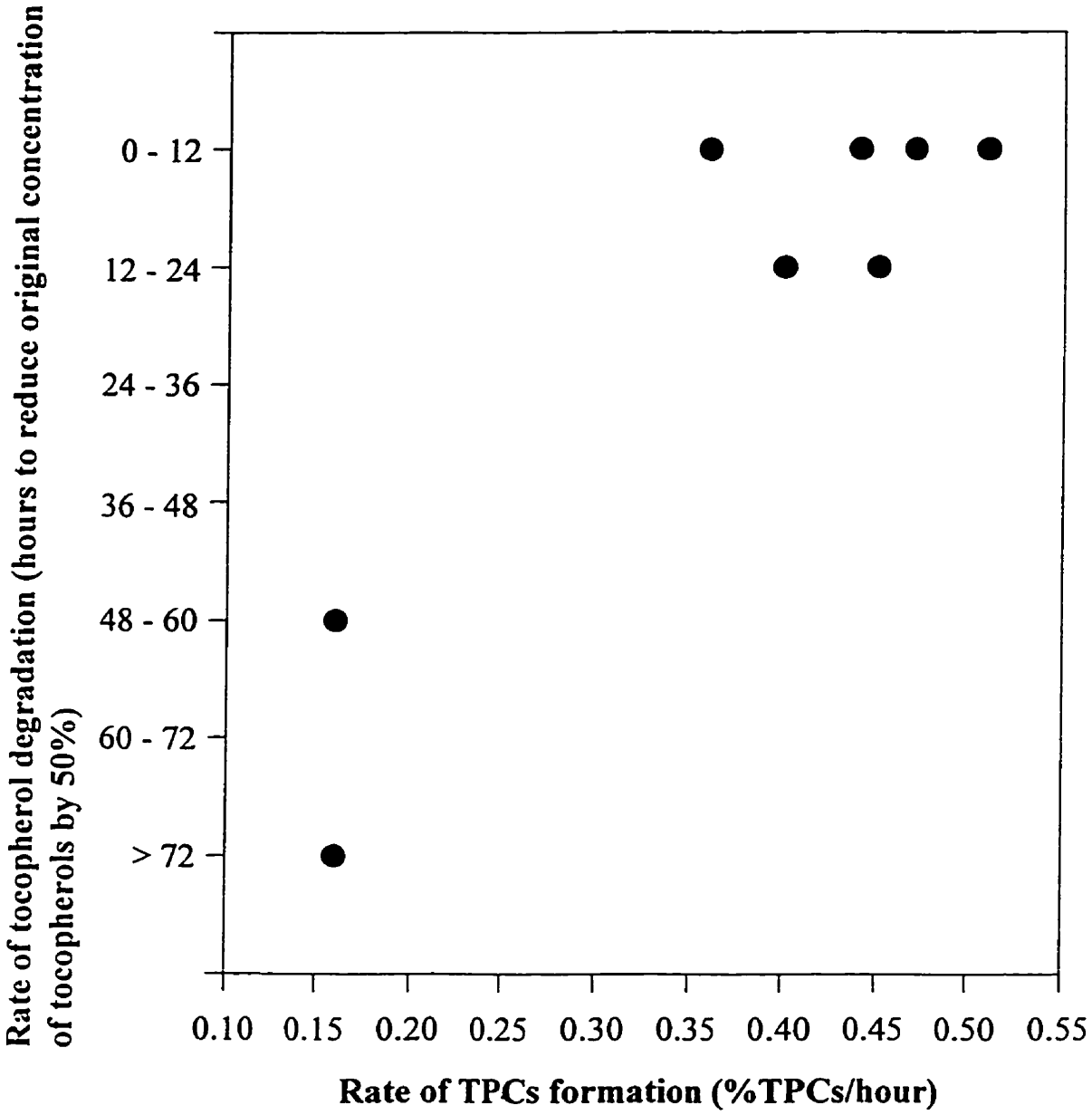


Figure 4.24. Relationship between rates of total polar components (TPCs) formation and tocopherol degradation for all oils

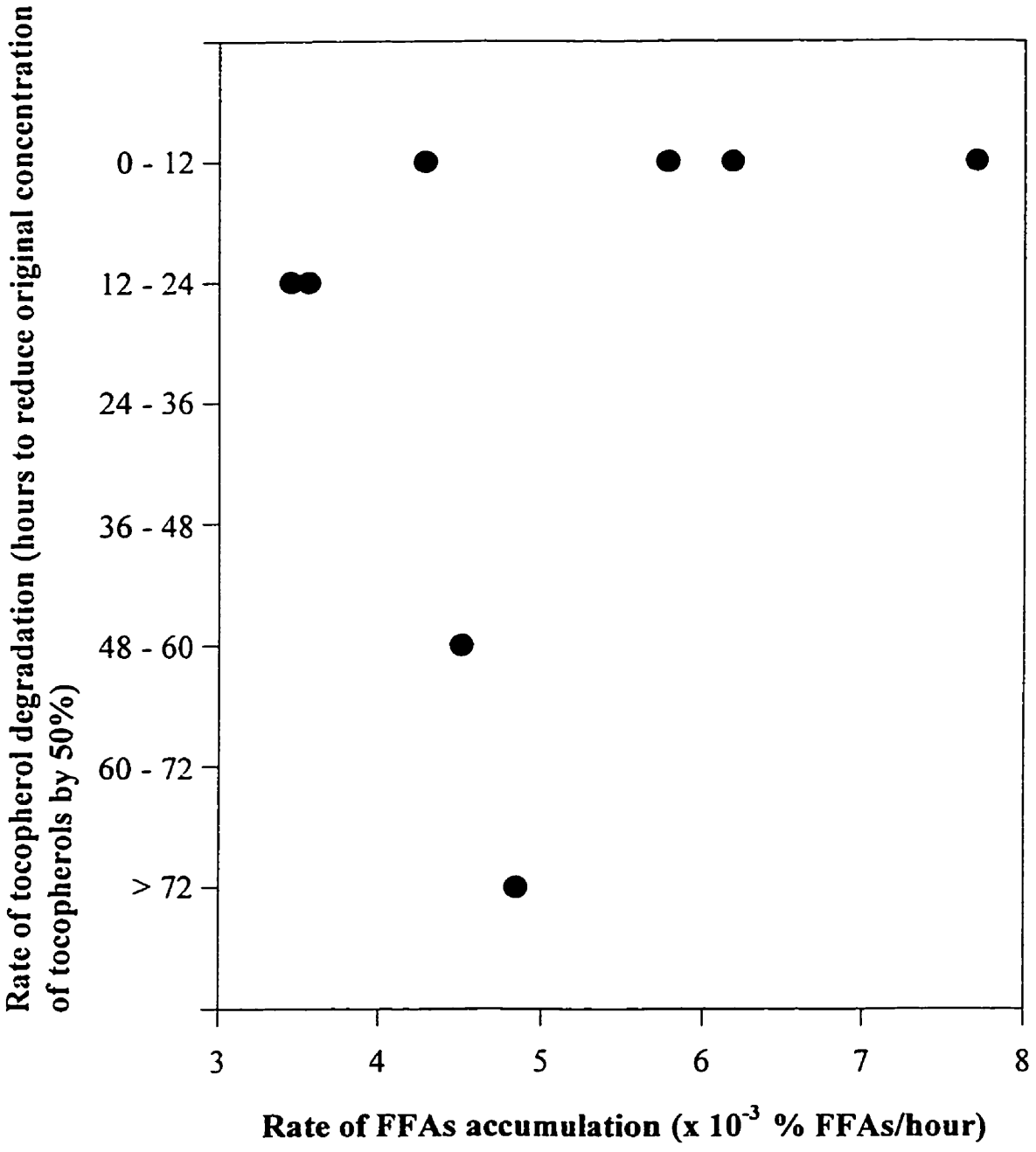


Figure 4.25. Relationship between rates of free fatty acids (FFAs) accumulation and tocopherol degradation for all oils

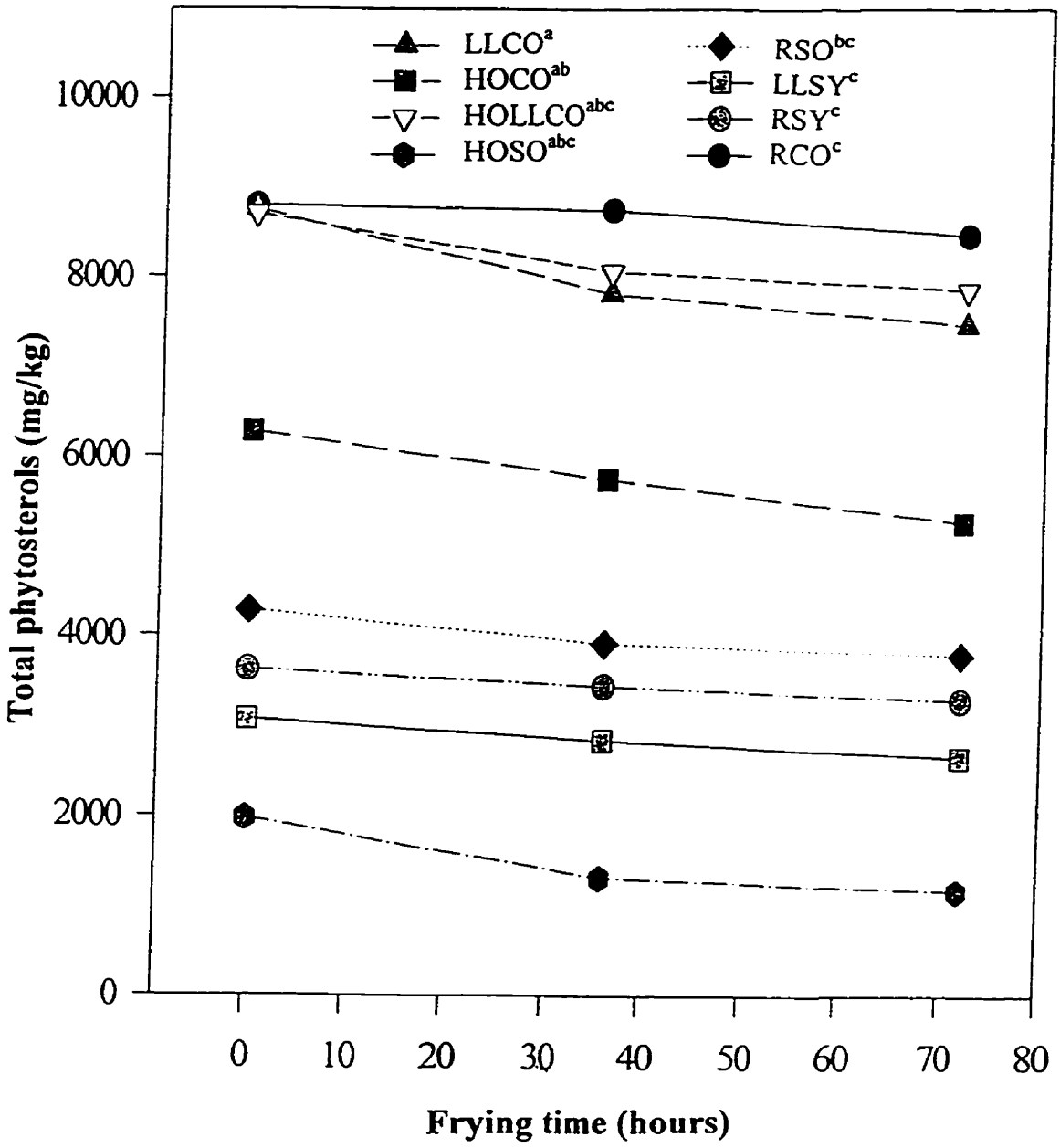


Figure 4.26. Total phytosterols (mg/kg) over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of phytosterol degradation

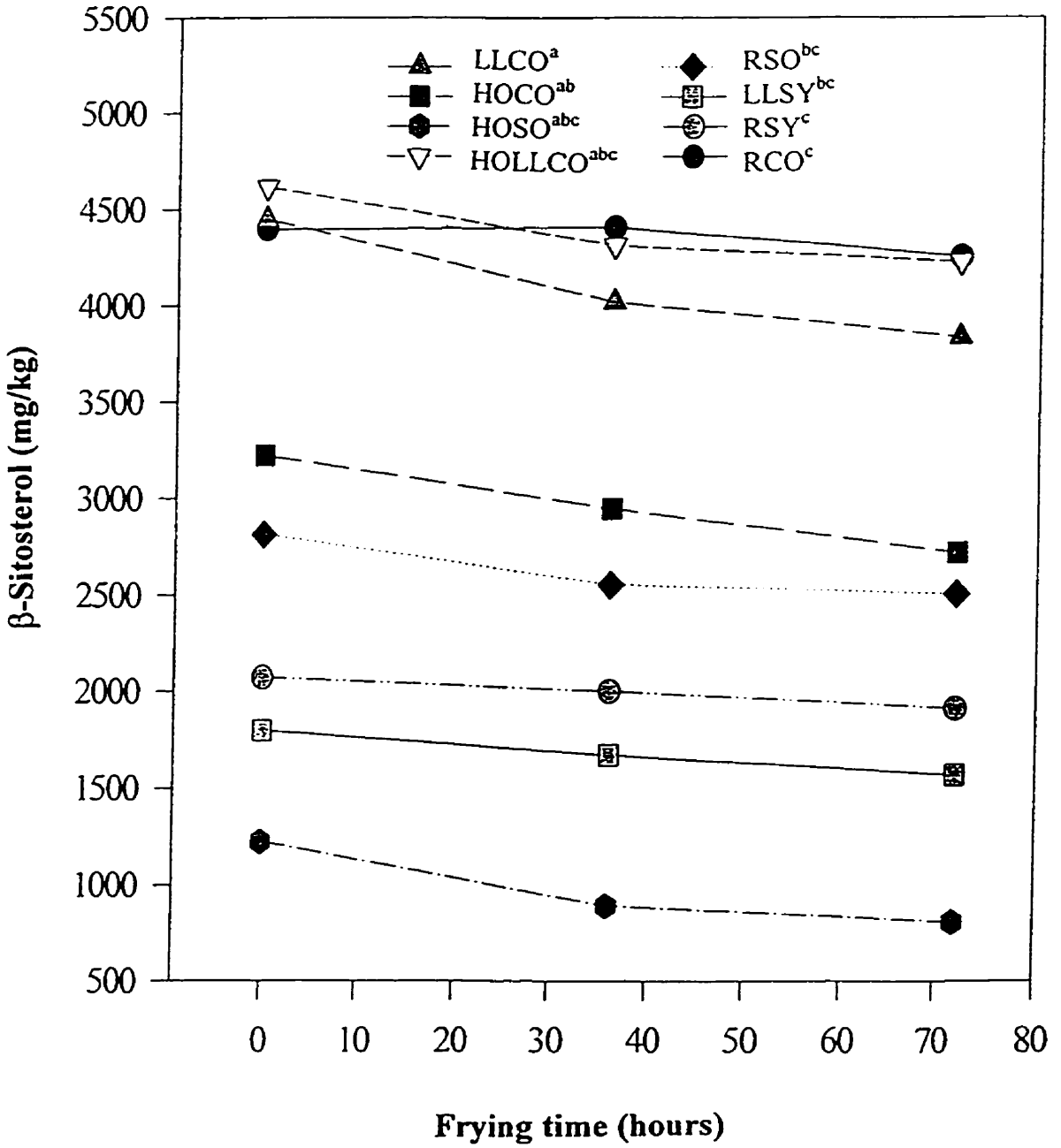


Figure 4.27. β-Sitosterol (mg/kg) over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of β-sitosterol degradation

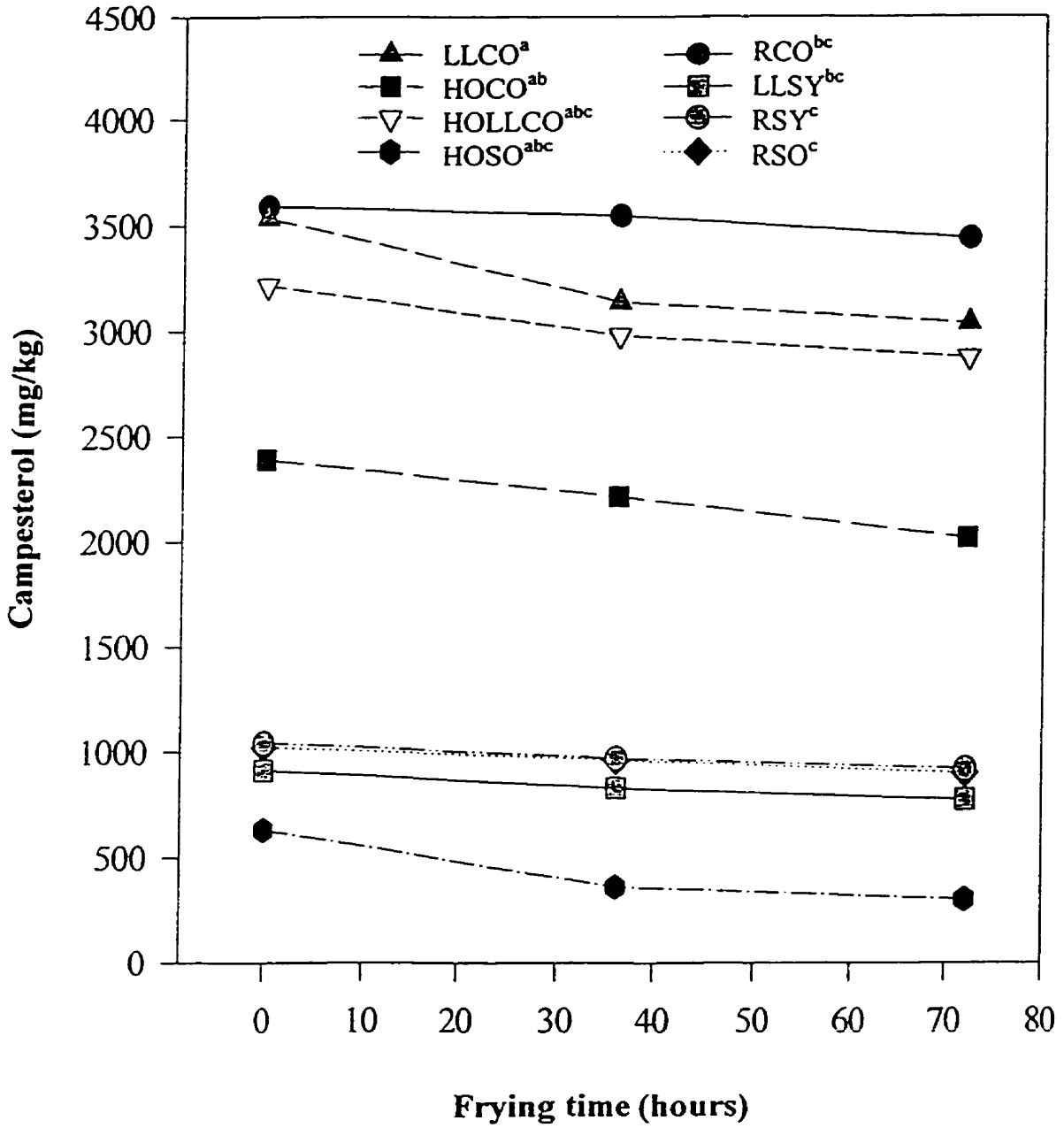


Figure 4.28. Campesterol (mg/kg) over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of campesterol degradation

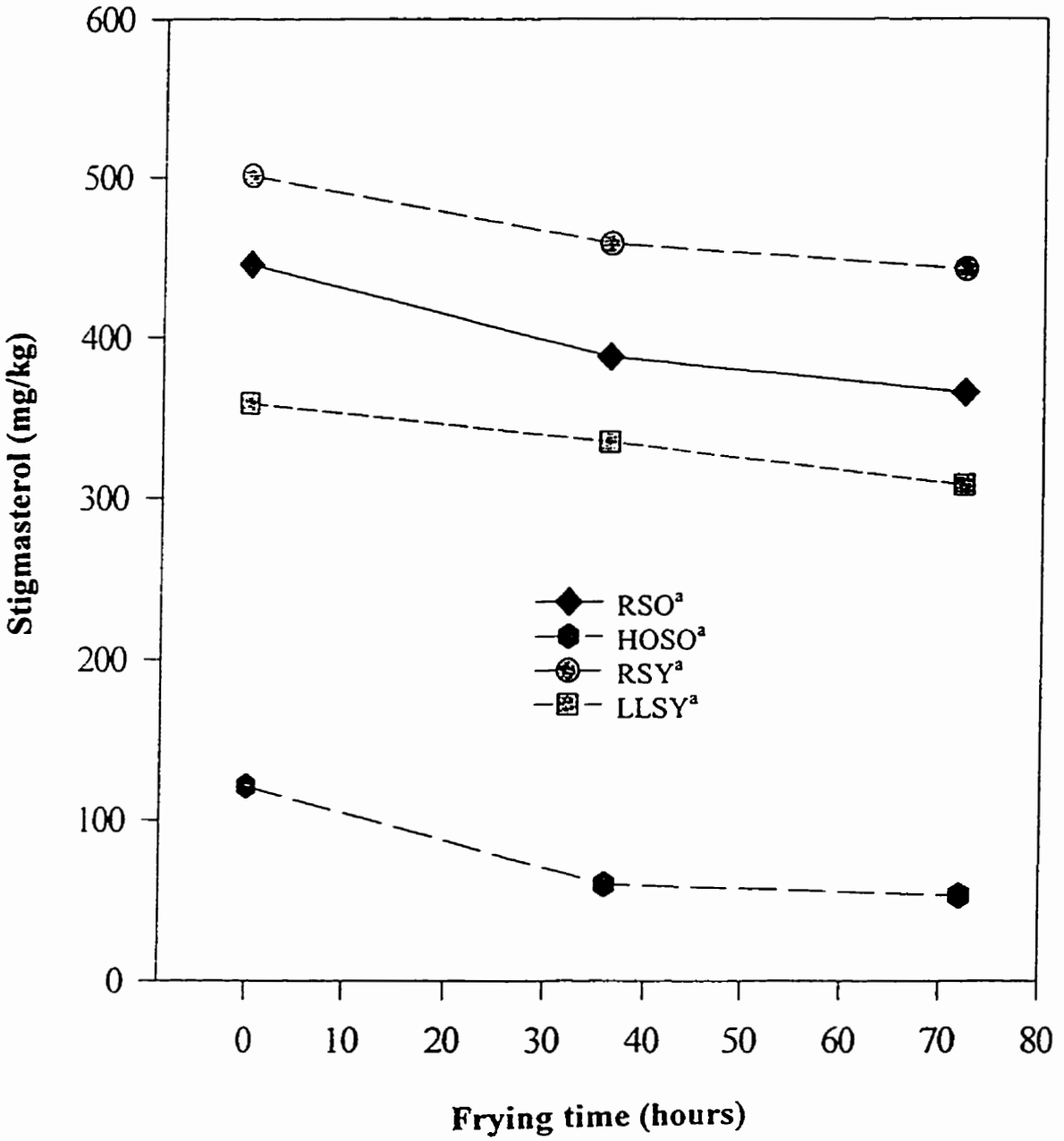


Figure 4.29. Stigmasterol (mg/kg) over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of stigmasterol degradation

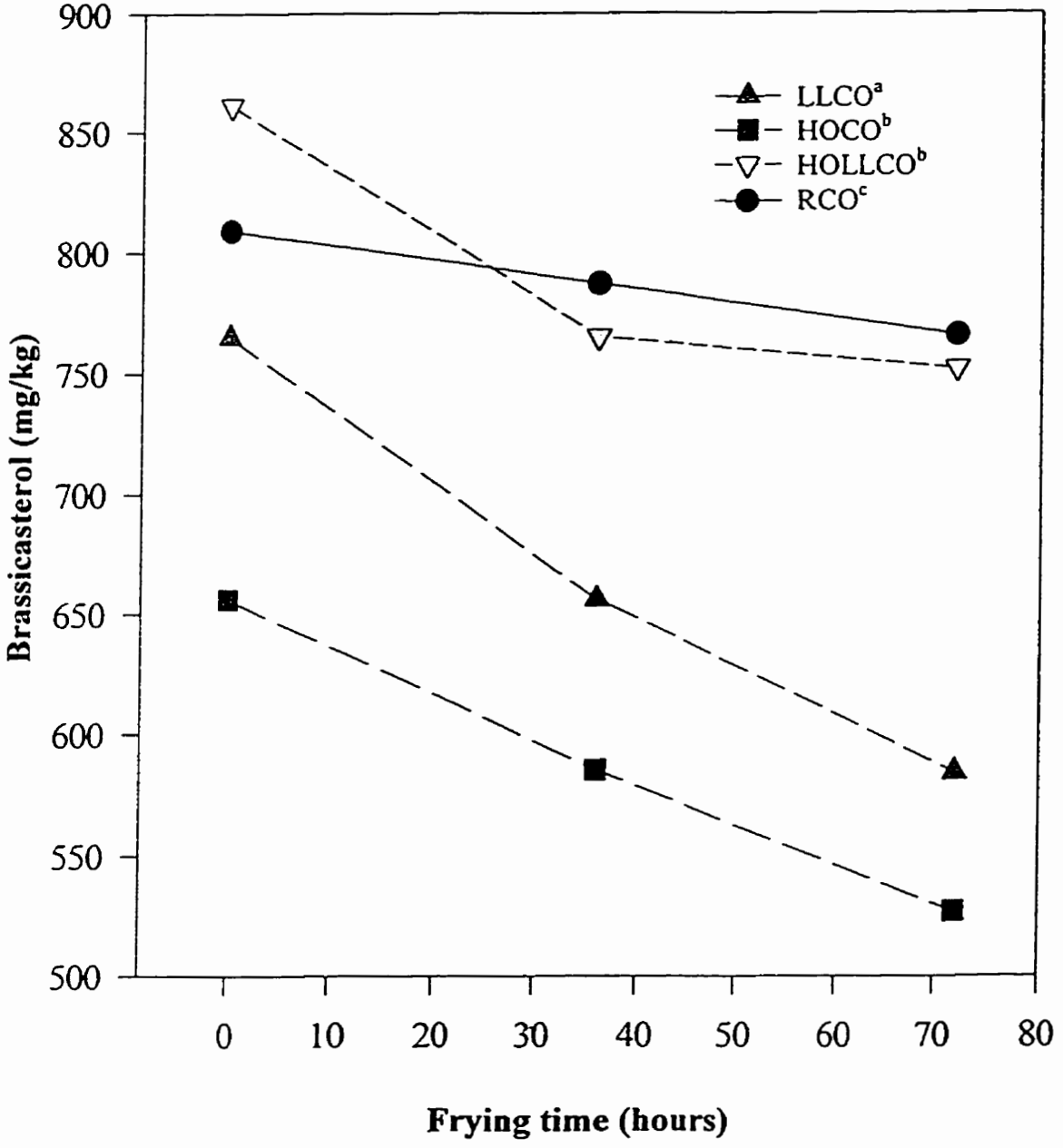


Figure 4.30. Brassicasterol (mg/kg) over frying time for all oils
N.B. oils with the same letter as a superscript displayed no significant differences ($p<0.05$) in rates of brassicasterol degradation

Table 4.11. Statistical comparison of total and individual phytosterol degradation rates among all oils

Oil	Rate of phytosterol degradation (mg/kg lost/hour)				
	Total	β-Sitosterol	Campesterol	Stigmasterol	Brassicasterol
LLCO	17.91^{a}	8.49^{a}	6.92^{a}	-	2.50^{a}
HOCO	13.94^{ab}	6.97^{ab}	5.18^{ab}	-	1.79^{b}
HOLLCO	11.65^{abc}	5.40^{abc}	4.73^{abc}	-	1.52^{b}
HOSO	11.29^{abc}	5.76^{abc}	4.58^{abc}	0.95^{a}	-
RSO	7.05^{bc}	4.27^{bc}	1.70^{c}	1.09^{a}	-
LLSY	5.79^{c}	3.17^{bc}	1.92^{bc}	0.71^{a}	-
RSY	4.77^{c}	2.23^{c}	1.71^{c}	0.82^{a}	-
RCO	4.63^{c}	1.94^{c}	2.09^{bc}	-	0.60^{c}

N.B. oils with the same letter as a superscript within a column displayed no significant differences ($p<0.05$) in rates of degradation of that particular phytosterol

degradation occurred at a significantly faster rate in LLCO (6.92 mg/kg lost/hour) than in RCO (2.09 mg/kg lost/hour). Brassicasterol, which has been shown to be the least stable to frying among the four phytosterols identified in this study (Li, 1996), degraded at a significantly faster rate (2.5 mg/kg lost/hour) in LLCO as compared to any of the other canola oils. In addition, HOCO and HOLLCO displayed significantly faster rates (1.8 and 1.5 mg/kg lost/hour, respectively) of brassicasterol degradation than did RCO (0.60 mg/kg lost/hour). However, brassicasterol only accounted for 9 to 10% of total phytosterols in canola oils.

Of all the identified phytosterols in this study, stigmasterol was the most stable with the lowest rates of degradation during frying (Table 4.11). This finding is in agreement with Li (1996), who also found stigmasterol to be the most stable of the four phytosterols.

The two canola oils, LLCO and HOCO, with significantly faster rates of total phytosterol degradation than RCO also had significantly faster rates of TPCs formation than did HOLLCO and RCO. LLCO also had significantly faster rates of β-sitosterol, campesterol and brassicasterol degradation than RCO. However, research on the influence of these particular phytosterols on oxidative stability during frying is very limited. β-Sitosterol and stigmasterol have been shown to have no effect or to be slightly prooxidative towards safflower oil at frying temperature (Sims et al., 1972). Gordon and Magos (1983) also found stigmasterol had essentially no effect on oxidation of triglyceride mixtures heated to 180°C for 72 hours. In addition, β-sitosterol was shown to be incapable of slowing down the oxidative deterioration of soybean oil heated to 180°C for 28 hours (White and Armstrong, 1986). The influence of brassicasterol and

campesterol on the stability of oil at frying temperatures has not been investigated. Therefore, based on current knowledge, the differences in total and individual phytosterol degradation rates for the particular phytosterols identified in this study do not provide explanations for the differences in oxidative stability of the canola oils. Rates of total and individual phytosterol degradation did not differ significantly among the soybean or the sunflower oils. Therefore, they provide no possible explanations for the stability differences seen among these oils.

4.3 Summary of Results

4.3.1 Canola Oils

4.3.1.1 Initial Quality

The following is a summary of the results for the initial quality of the canola oils:

- initial FFA levels and PVs were low, indicating good initial quality of the oils
- levels of TPCs among the fresh oils were acceptable
- HPSEC revealed no extensive polymerization in the fresh oils
- PUFA content was lower in modified oils (15.1% in HOLLCO, 16.0% in HOCO and 27.7% in LLCO) as compared to the corresponding regular oil (31.5% in RCO)
- HOLLCO displayed higher initial levels of tocopherols than RCO, HOCO and LLCO
- the major tocopherol present in the canola oils was γ-tocopherol (65 to 70%), with 30 to 35% α-tocopherol present
- initial phytosterol content was lower in HOCO as compared to the other three canola oils, which displayed similar levels of phytosterols
- β-sitosterol and campesterol were the major phytosterols present in the oils
- very low levels of copper were detected in RCO and HOCO. LLCO contained a very small amount of nickel

4.3.1.2 Frying Performance

The following is a summary of the results for the frying performance of the canola oils:

- canola oils did not differ significantly in rates of FFA accumulation
- LLCO and HOCO had significantly faster rates of polars formation than RCO and HOLLCO
- canola oils displayed greater levels of thermoxidative as opposed to hydrolytic products as measured by HPSEC
- levels of linoleic and linolenic acid decreased, and levels of oleic acid increased after 72 hours of frying in all oils
- LLCO and HOCO had much faster rates of total and individual tocopherol degradation than RCO and HOLLCO. In RCO and HOLLCO, γ-tocopherol degraded at a faster rate than α-tocopherol
- LLCO displayed significantly faster rates of total and individual phytosterol degradation than RCO

4.3.2 Soybean Oils

4.3.2.1 Initial Quality

The following is a summary of the results for the initial quality of the soybean oils:

- initial FFA levels and PVs were low, indicating good initial quality of the oils
- levels of TPCs among the fresh oils were acceptable
- HPSEC revealed no extensive polymerization in the fresh oils
- PUFA content was lower in the modified oil as compared to the regular oil (59.0% in LLSY as compared to 63.0% in RSY)
- RSY displayed higher initial levels of tocopherols than LLSY
- the major tocopherol present in the canola oils was γ-tocopherol (92 to 94%)
- initial phytosterol content was lower in LLSY as compared to RSY
- β-sitosterol and campesterol were the major phytosterols present in the oils

- very low levels of copper were detected in both soybean oils

4.3.2.2 Frying Performance

The following is a summary of the results for the frying performance of the soybean oils:

- soybean oils did not differ significantly in rates of FFA accumulation

- LLSY had a significantly faster rate of polars formation than RSY

- soybean oils displayed greater levels of thermoxidative as opposed to hydrolytic products as measured by HPSEC

- levels of linoleic and linolenic acid decreased, and levels of oleic acid increased after 72 hours of frying in both oils

- LLSY had slightly faster rates of total and individual tocopherol degradation than RSY

- RSY and LLSY displayed no significant differences in rates of total and individual phytosterol degradation

4.3.3 Sunflower Oils

4.3.3.1 Initial Quality

The following is a summary of the results for the initial quality of the sunflower oils:

- initial FFA levels and PVs were low, indicating good initial quality of the oils

- levels of TPCs among the fresh oils were acceptable

- HPSEC revealed no extensive polymerization in the fresh oils

- PUFA content was lower in the modified oil as compared to the regular oil (3.5% in HOSO as compared to 69.5% in RSO)

- RSO displayed higher initial levels of tocopherols than HOSO

- the major tocopherol present in the sunflower oils was α-tocopherol (94 to 96%)

- initial phytosterol content was lower in HOSO as compared RSO

- β-sitosterol and campesterol were the major phytosterols present in the oils

• copper, iron and nickel were not detected in the sunflower oils

4.3.3.2 Frying Performance

The following is a summary of the results for the frying performance of the sunflower oils:

• HOSO had a significantly faster rate of FFA accumulation than RSO

• RSO and HOSO displayed no significant differences in rates of polars formation

• sunflower oils displayed greater levels of thermoxidative as opposed to hydrolytic products as measured by HPSEC

• levels of linoleic and linolenic acid decreased in both oils, levels of oleic acid increased for RSO and levels of oleic acid decreased for HOSO by 72 hours of frying

• HOSO had much faster rates of total and individual tocopherol degradation than RSO

• RSO and HOSO displayed no significant differences in rates of total and individual phytosterol degradation

4.4 Summary of Discussion

The eight oils used in this experiment were of similar initial quality as indicated by PVs, FFAs, TPCs and HPSEC. The modified oils contained lower levels of PUFAs than the corresponding regular oil and were expected to show greater stability. Based on rates of TPCs formation and FFAs accumulation, the modified oils either showed no improvement in stability or were significantly less stable than their regular counterpart. Thus it can be concluded that fatty acid composition was not the sole determinant of frying stability in these oils.

Initial phytosterol levels and rates of total and individual phytosterol degradation did not provide an explanation for stability differences among the oils. Metals (Cu, Fe and Ni) were present at similar levels for oils within each group, such that any influence

on oil oxidation would be similar among the oils and provide no possible explanation for differences in frying stability.

Tocopherol levels and degradation rates appeared to play a role in oil stability. For both soybean and sunflower oils, the regular oil had a higher initial level of tocopherols, a slower rate of tocopherol degradation and displayed either no difference in stability or greater stability than its corresponding modified oil, despite the reduction of PUFAs in the modified oils.

Among the canola oils, HOLLCO displayed higher levels of tocopherols and slower rates of tocopherol degradation than LLCO and HOCO. Correspondingly, HOLLCO was found to be significantly more stable than both of these oils, even though the levels of PUFAs were similar for HOCO and HOLLCO. Finally, HOLLCO showed no significant difference in stability from RCO. This finding was surprising as HOLLCO displayed a lower level of PUFAs and higher initial level of tocopherols than RCO. RCO displayed slightly slower rates of tocopherol degradation than HOLLCO, but the difference was relatively small. The fact that HOLLCO did not show a significant improvement in stability over RCO suggests that factors other than PUFA content, initial tocopherol level and rate of tocopherol degradation influenced oil stability during frying.

Chapter 5. Conclusions, Limitations and Implications for Future Research

5.1 Conclusions

The results obtained in this study allow the following conclusions to be made:

1. Fatty acid composition is not the sole determinant of frying stability of vegetable oils. Oils modified to reduce PUFA content showed no significant improvements in frying stability and were often significantly less stable than the corresponding regular oils. The results of the present study suggest that minor oil components may have an important role in determining oil stability.
2. The results of the current study suggest that tocopherols were significant factors affecting oil stability during frying. Oils with high levels of tocopherols and/or relatively slow rates of tocopherol degradation tended to show greater stability during frying.
3. Phytosterols appeared to have a negligible effect on frying stability.
4. Metal content was similar within each oil group. Therefore, whatever effect metals had on oil oxidation was similar among oils and could not explain differences in frying stability.
5. Other minor oil components; such as pigments, FFAs, PLs, mono- and di-glycerides; and their degradation products may also have contributed to the frying stability of oils. This conclusion arises out of the difficulty in explaining differences in frying stability based solely on those components evaluated in the current study.
6. Thermoxidative rather than hydrolytic reactions predominant during the frying process based on results from HPSEC. Therefore it can be concluded that the majority of oil breakdown during frying is due to oxidation reactions.

5.2 Limitations and Implications for Future Research

The results of this study showed that frying stability of an oil is not solely determined by its fatty acid composition. Tocopherol content and degradation rate could explain, in part, some of the differences in frying stability between oils. Future research should focus on determining what other factors; such as the presence of other minor oil components and their degradation products, environmental factors and the modification procedure itself; may impact on frying stability, and how these actions are exerted.

In this study, comparisons were made between regular and modified oils of the same type - i.e. canola, soybean or sunflower oils. However, the oils were not matched for factors; such as growing location, seed maturity at harvest, seed grade, growing season, and refining conditions and facilities; which may have influenced their stability. The maturity of the seed at harvest is an important factor to control as it will influence the quality of the crude oil, which ultimately affects refined oil quality. Ideally, a frying study needs to be set up in which the modified seed would be derived from the regular seed to which its oil stability will be compared; so that the only differences between the oils would be those produced through the modification procedure itself.

In addition to tocopherols, phytosterols and metals; other minor components in the oil may play a role in frying stability. These could include FFAs, pigments, phospholipids (PLs), mono- and di-glycerides, and phenolic compounds. Although many of these minor components are not stable at frying temperatures, the impact of their oxidation or degradation products on oil stability may be important.

The interactions between minor components may also play a significant role in frying stability. For example, a given component may only impede oil degradation in the

presence of another component. An example is the synergistic relationship between PLs and tocopherols (Hudson and Mahgoub, 1981; Hildebrand et al., 1984), although it has not been established as to whether this antioxidant synergism occurs at frying temperatures.

Some minor components act as antioxidants only at certain concentrations and temperatures. Some of these components may have no effect on oil oxidation or even act as prooxidants at higher and/or lower concentrations and/or temperatures. Therefore, future research should focus on determining at what range of concentrations minor components enhance oil stability. This is particularly important in the case of tocopherols, which are currently used as natural antioxidants.

In many instances, each minor component can occur in a number of slightly different isomeric forms. The interactive effects of different isomers of minor components in different combinations, concentrations and types of frying oils should be explored further. For example, a study could be conducted to determine whether γ-tocopherol alone or in combination with α-tocopherol provides greater protection against oxidation.

Fatty acid triglyceride distribution (i.e. how fatty acids are arranged on the glycerol molecule), which may be altered during modification, could also have an impact on frying stability. Differences in susceptibilities to oxidation within the glycerol molecule warrants further investigation.

The results reported in this study are not directly applicable to large scale, continuous frying operations more typical of industry. In these operations, oil turnover time is shorter and the relative amount of oxygen exposure at the surface is lower.

Therefore, future studies should look at the frying stability of oils under these conditions where the benefits of improved frying oil stability occur on a much larger scale.

Finally, HPSEC is a good method for differentiating between oils with similar levels of TPCs. Oils containing trimers and higher polymers will have undergone more extensive degradation than those with only dimers and oxidized triglycerides, even though TPCs may be similar. The nutritional implications of consuming these different products will vary. Therefore, future research should focus on the development of these methods to set strict specifications regarding alteration of frying oils to minimize health hazards to the consumer.

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IMAGE EVALUATION
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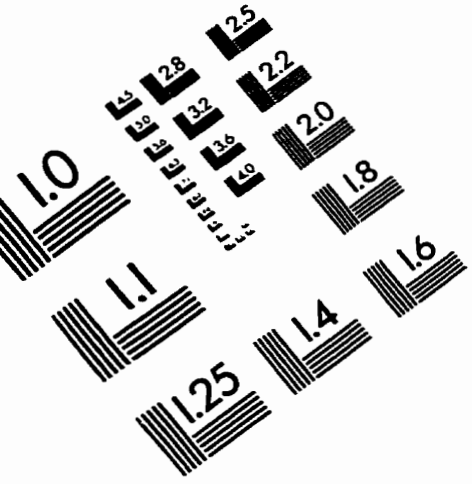

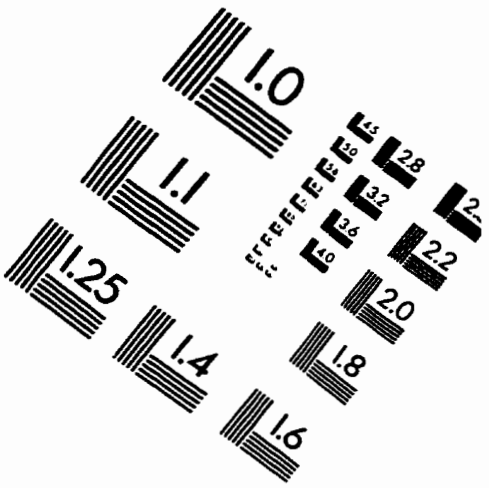

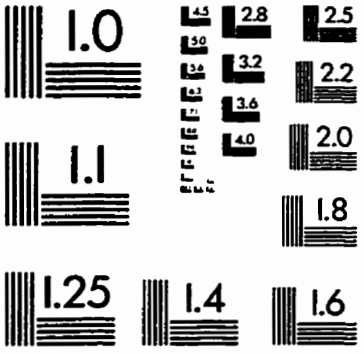

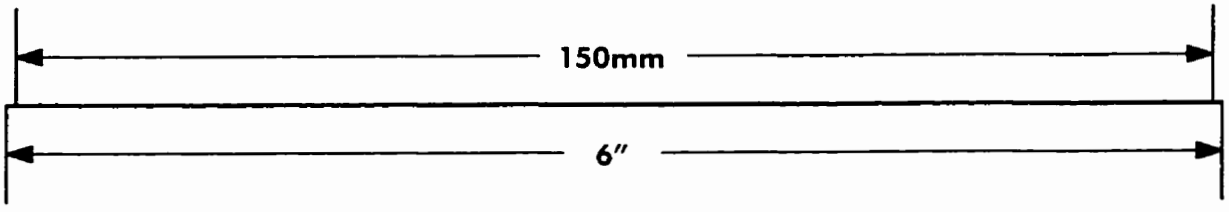

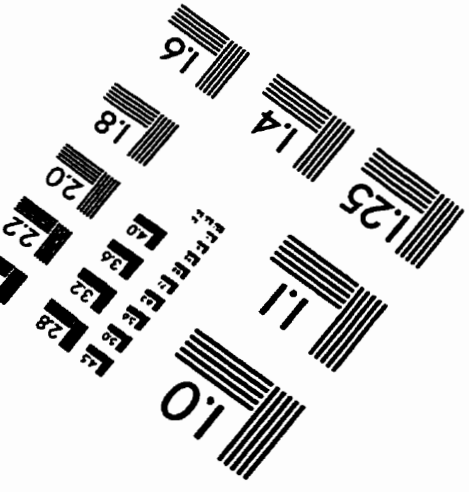

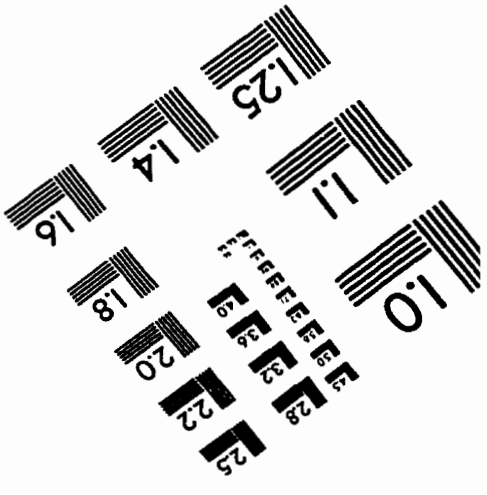

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