A COMPARATIVE STUDY OF THE THERMAL OXIDATIVE STABILITY OF HIGH OLEIC ACID SUNFLOWER AND POLYUNSATURATED SOYBEAN OIL BLENDS.

BY

Troy A. Wilkerson

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APPROVED:

Frank D. Conforti

Frank, D. Conforti, Chairman

William E. Barbeau

William E. Barbeau

Roderick W. Young

Roderick W. Young

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(ABSTRACT)

The thermal oxidative stability was evaluated for 7 high oleic acid sunflower (HOSO) and polyunsaturated soybean oil (PUSB) blends ranging from 0.0% to 100.0% of each vegetable oil. Each blend was evaluated in quadruplicate over an eleven day period with 300 minutes of heating at 180°C per day. Thermal oxidative stability was determined by changes in peroxide values (PV), fatty acid and triglyceride composition, and percentage of major volatile products (%MVP). A 2 Way ANOVA, simultaneous confidence intervals, trend analysis, and Paired t-Test, each with a set p value of (0.01) were used for the statistical analysis.

The triolein (OOO), trilinolein (LLL), and fatty acid contents were significantly different ($p < 0.01$) between each blend before and after heating, with the exception of stearic acid levels, which were not significantly different between each blend. Thermal stability

increased for the triglycerides and fatty acids as the degree of saturation increased: (Saturated > Monoenic > Polyenic). The addition of HOSO to PUSB increased the thermal oxidative stability of all the blends when compared to the 100% PUSB. The PV and %MVP increased as the levels of OOO decreased and the levels of LLL increased in the blends. The blends with a (1:1) ratio of each vegetable oil experienced a unique breakdown pattern. The monoenic fatty acid levels decreased and the polyenic fatty acid levels increased for this blend only. Overall, blends with 10% to 75% HOSO addition performed equally as well in the thermal oxidative stability testing.

Acknowledgements

Preparing a thesis takes the efforts and support of many people. Throughout the pages contained in this thesis the influence and expertise of these people are shown. I would like to begin by extending my deepest gratitude to my advisor and mentor Dr. Frank D. Conforti for sparking my interest in lipid research, for providing me with continual support and guidance, and for encouraging the success of this study. I would like to thank Dr. William E. Barbeau for serving on my committee, sharing with me some of his current research on the extraction of leaf proteins, and for providing me with essential background knowledge in food science. I would like to thank Dr. Roderick W. Young for serving on my committee and for allowing me to use his pesticide laboratory to conduct the gas chromatography mass spectrometry on the volatile components of the vegetable oil blend samples. I would also like to extend a special thanks to Sandra Gabbert, Joy Burroughs, Jean McCarthy, and David Ruggio for making me feel welcome at the pesticide laboratory, and for providing me with assistance in using the computer software.

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CHAPTER I.
INTRODUCTION

Vegetable oils are derived from the seeds of plant crops such as sunflower, soybean, corn, canola, peanut, and cotton seed (McGinely, 1991). Vegetable oils are composed largely of triglycerides (triacylglycerol), which are composed of three fatty acids esterified to a molecule of glycerol. The fatty acid composition and arrangement on the triglycerides ultimately determines the stability of a vegetable oil (Neff, et. al, 1993). Research (Adachi, et. al, 1995) has shown that there is a positive correlation between vegetable oil stability and the level of saturation of constituent fatty acids. Hence, fatty acids with no double bonds (saturated) are more stable than fatty acids with one double bond (monoenic/monounsaturated), which are more stable than fatty acids with 2 or more double bonds (polyenic/polyunsaturated). The term stability is used to indicate the resistance of a fatty acid or triglyceride to degradation under conditions such as oxidation and polymerization. Research (Neff, et. al, 1993; Raghuveer and Hammond, 1966) has also shown that the positioning of the fatty acid on the triglyceride can lead to changes in the triglyceride's stability.

Vegetable oils are currently used on a wide scale, ranging from deep-frying in the restaurant and fast food industries, production of

snack food items such as corn and potato chips, precooked frozen food items, such as french fries and chicken strips, and consumer home use. The stability of the vegetable oil is of extreme importance, particularly in the fast food and snack food industries. A quicker rate of vegetable oil breakdown leads to greater costs and a decrease in the quality of the fried food and snack food items (Warner, et. al 1994). At present, many attempts are being employed to increase the vegetable oil's stability by altering the fatty acid content and triglyceride composition of oils. However, hydrogenation (also partial hydrogenation or saturation) is the number one method for increasing the vegetable oil's stability.

Hydrogenation increases the stability of the vegetable oil by randomly saturating the monoenic and polyenic fatty acid contents, often using a nickel catalyst and hydrogen gas. The hydrogenated vegetable oils experience a greater degree of stability and an increased order of triglyceride crystal packing (Gunstone, 1986b). The improved vegetable oil qualities are related to the production of *trans*-fatty acids such as elaidic acid (*trans*-9-oleic acid), (Small, 1986). Unfortunately, the presence of *trans*-fatty acids have been associated with coronary artery disease by inducing an increase in low density lipoprotein levels (LDL) and a decrease in high density lipoprotein levels (HDL) when consumed in the diet (Zock and Katan, 1992).

Hydrogenated vegetable oils are currently the number one

vegetable oils used in the fast food and snack food industries, regardless of the potential health problems they pose. Pick up any snack food item that has been prepared using a vegetable oil, and chances are 90.0% or greater that the term partially hydrogenated vegetable oil will appear on the label.

A number of concerned investigators (Neff, et. al, 1994a; Frankel and Huang, 1994; Dobarganes, et. al, 1993; O'Keefe, et. al, 1993; Warner and Mounts, 1993) are currently seeking alternatives to hydrogenation which will produce vegetable oils of equal or greater stability without the production of *trans*-fatty acids. These alternatives are all based on increasing the vegetable oil's stability by decreasing the level of polyunsaturation and by rearrangement of the fatty acids on the triglyceride. Interesterification has been used by Neff, et. al (1994a) to randomly rearrange the linoleic acid (C18:2) content of polyunsaturated canola oils. Unfortunately, the interesterified canola blends experienced an overall decrease in stability when compared to the unaltered canola oils. Genetic modification of polyunsaturated seed oils has been used successfully by Kinney (1994) to produce oil-bearing seeds with an increased level of monoenic fatty acids. Research (Dobarganes, et. al, 1993) has shown that genetically modified vegetable oils such as high oleic acid sunflower oil experienced an increase in stability when compared to unaltered sunflower oils. Unfortunately, genetic modification of seed oils, which alter the expression of fatty acids in the developing seed by enzymatic regulation, have their

limitations as well. Research (Langstraat, 1976) has shown that developing seed oils, such as soybean and sunflower oil are prone to changes induced by temperature and climate. Increased levels of polyenic fatty acids are associated with seed oil crops grown in temperate and cooler climates (McCormik, et. al, 1992; Wilcox and Cavins, 1992). The genetically modified vegetable oils are prone to the same changes in fatty acid composition in the developing seed oils resulting from climatic change (Wilcox and Cavins, 1992). Therefore, the use of genetic modification is less suitable for seed oil crops grown in cooler climates. Research (Knutzon, et. al, 1992) has shown that a decreased level of polyenic fatty acids lead to a decrease in seed oil yield for temperate climate seed oil-bearing crops.

The simplest and most effective alternative to vegetable oil hydrogenation is vegetable oil blending using high oleic acid and polyenic vegetable oils. Vegetable oil blending using high oleic acid sunflower and polyenic soybean oil has been documented to increase the stability of polyenic vegetable oils (Frankel and Huang, 1994). The vegetable oil blends experienced a positive correlation with an increased level of high oleic acid sunflower oil content and an increase in stability. Unfortunately, only basic trends were identified, such as the correlation of hexanal peak areas to peroxide values. However, the major fatty acids and triglycerides contributing to the breakdown of the vegetable oil blends were not accounted for by the use of hexanal as an indicator.

The complete picture of vegetable oil breakdown is often unclear. Generally, quality indicators such as acid values, peroxide values, or simply changes in color and texture are used. These methods are adequate for on the spot assessment of the vegetable oil or frying oil. However, these methods reveal very little information about what leads to the deterioration of the vegetable oil. In order to predict or estimate the performance of a vegetable oil under such conditions the individual fatty acids and triglycerides must be taken into account. Furthermore, analyses which monitor the production of intermediate hydroperoxides, the liberation of polar components and polymers, and the production of end volatile products must be used. Otherwise, the performance of the vegetable oils and blends can not be compared and contrasted based on the differences in composition.

Once the relative thermal oxidative stability is determined for each of the vegetable oil blends, the blends with the greatest thermal oxidative stability can be compared against the hydrogenated vegetable oils which are currently in wide use in the fast food and snack food industries. The blend analysis should also clarify what levels of high oleic acid sunflower oil can be added to polyunsaturated vegetable oils such as soybean oil to improve the frying performance of the polyunsaturated soybean oil while maximizing the usage of the high oleic acid sunflower oil. In certain cases the availability of high oleic acid sunflower oil or the polyunsaturated vegetable oil may become a limiting factor in

formulation of vegetable oil blends. In such cases, it would be advantageous to know how the various blends perform, especially when the quality of a fried food or snack food is at stake.

The objectives of this study were as follows:

1. To evaluate the thermal oxidative stability of high oleic acid and polyunsaturated soybean oil blends ranging from 0.0% to 100.0% of each vegetable oil without the addition of food.

2. To determine if these blends exhibit certain patterns in thermal oxidation which can be directly attributed to the blend composition.

3. To establish a ranking system for the thermal stability of fatty acids and the triglycerides triolein and trilinolein occurring in the vegetable oil blends.

4. To establish an overall thermal oxidative stability ranking system for the vegetable oil blends used in this study which clearly indicates the impact of adding high oleic acid sunflower oil to polyunsaturated soybean oil at the following levels: (1) 100%, (2) 90%, (3) 75%, (4) 50%, (5) 25%, (6) 10%, and (7) 0%.

CHAPTER II.
LITERATURE REVIEW

A. Vegetable Oil Composition and Characteristics

A. 1. An Overview of Vegetable Oils and Their Composition

Vegetable oils are derived from the seeds of plant crops such as sunflower, soybean, corn, peanut, rapeseed, and cottonseed (McGinley, 1991). In some cases, the oil is derived from the fruit, such as palm oil (Van Ness, 1981). Each of the oils mentioned are very similar in that they are composed largely of triglycerides (triacylglycerol) after refinement, yet each of the oils have their own unique properties. The differences and similarities arise from the fatty acid composition of the triglycerides (Frankel and Huang, 1994; Neff, et al., 1993), and the crystalline structures of the triglycerides (Raghuveer and Hammond, 1966; Gunstone, 1986b). Factors such as the thermal oxidative stability of a frying oil are ultimately determined by the triglyceride composition and degree of unsaturation (Neff, et al., 1994b; Warner, et al., 1994). Palm oil due to its high levels of palmitic acid (C16:0) and low levels of polyunsaturated fatty acids, exhibits a higher melting point with a narrower melting point range (Langstraat, 1976). It is obvious that subtle differences in the triglyceride composition and structure can greatly influence a vegetable or fruit oil's usability.

A. 2. Fatty Acid Composition of Vegetable Oils

Fatty acids (alkanoic acids) are aliphatic structures that range from 2 to 30 carbons in chain length, containing a methyl ($-CH_3$) terminal end and a carboxylic acid (-COOH) primary end (Gunstone, 1986a). The average carbon chain length for the fatty acids common to vegetables oils are between 12 and 24 carbons in length. Aliphatic structures are defined as compounds consisting largely of hydrocarbons or being alkane-like (Solomons, 1992a). Most naturally occurring fatty acids have even numbers of carbons in the carbon chain and are unbranched (Gunstone, 1986a). Table 1. lists some of the more common fatty acids found in vegetable oils.

Fatty acids generally are divided into two broad classes, saturated and unsaturated. Compounds which contain only single bonds (sigma bonds) in the polymethylene region are classified as saturated, where compounds with one or more double bonds (pi bonds) are classified as unsaturated (Solomons, 1992d). Hence, the monousaturated (monoenic) refers to a fatty acid with only one double bond, and polyunsaturated (dienic and trienic) refers to a compound with two or more double bonds. The presence of double bonds in a fatty acid greatly influences the rate at which the fatty acid decomposes. Fatty acids with increased numbers of double bonds have been shown (Adachi, et al., 1995) to decompose more rapidly than saturated fatty acids. A great deal of research

Table 1. Fatty acid composition of common vegetable oils, expressed as % of total weight per 100.0 milligrams.

Vegetable Oil	C16:0	C18:0	C18:1	C18:2	C18:3
Corn	13	3	28	53	1
Olive	13	3	73	9	1
Peanut	12	3	53	26	2
Soybean	12	4	24	51	7
Sunflow-er	6	5	20	69	0
[H]Sunflow er	4	5	81	6	0

(Mead, et. al, 1986)

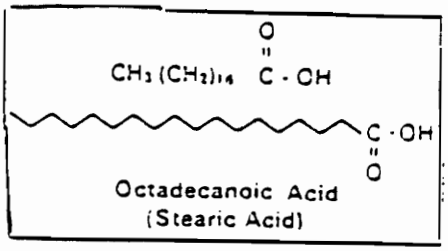
Notes:

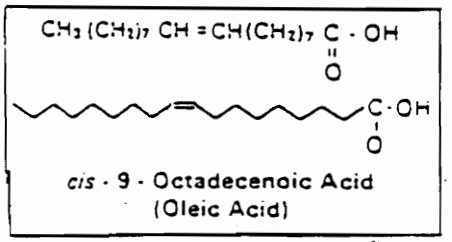
1. C16:0 = Palmitic acid
2. C18:0 = Stearic acid
3. C18:1 = Oleic acid
4. C18:2 = Linoleic acid
5. C18:3 = Linolenic acid
6. H = High oleic acid sunflower oil

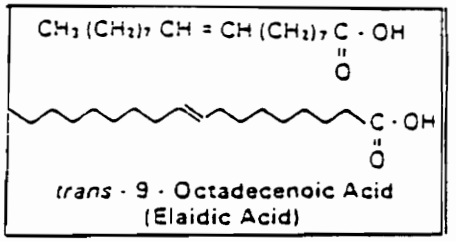
(Dobarganes, et al., 1993; Frankel and Haung, 1994; Neff, et al., 1994a; Neff et al., 1994b) has focused on the alteration of the fatty acid composition to improve the stability of vegetable oils. Figure 1. illustrates the structures of some common saturated and unsaturated fatty acids.

The double bonds found in fatty acid chains can occur at different locations, and can exist in different geometric states. The first consideration is the proximity or the location of the double bonds in a fatty acid chain. Most naturally occurring double bonds in a fatty acid chain are methylene interrupted ($-CH_2-$) (Gunstone, 1986a). The methylene carbons adjacent to the double bonds are the reactive sites for fatty acid autoxidation (Neff and Selke, 1993). Therefore, an increase in the number of double bonds in a fatty acid chain increases the likelihood that autoxidation will occur. Figure 2. illustrates the presence of methylene carbons on a polyunsaturated fatty acid.

The second consideration of the double bonds occurring in the fatty acid chains is the *cis* and *trans* isomerization. The *cis* and *trans* isomers are collectively known as diastereomers. Diastereomers are stereoisomers that differ only in their arrangement of atoms in space and are not superimposable structures (mirror images) of one another (Solomons, 1992c). A common system for identification of more complex *cis* and *trans* isomers is the E,Z system, *cis* (Z, zusammen) and *trans* (E, entgegen),







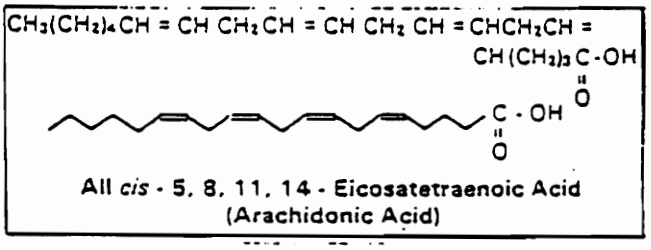
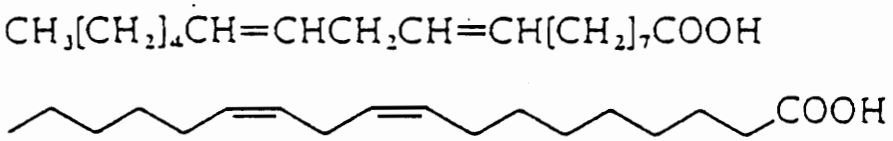


Figure 1. Fatty acid structures common to vegetable oils and hydrogenated vegetable oils. (Small, 1986)



Linoleic acid (18 : 2 9*c*12*c* or 9*Z* 12*Z* or *n* − 6)

Figure 2. Representation of methylene interrupted carbons in linoleic acid. (Gunstone, 1986a)

(Mead, et al., 1986). The E,Z system becomes highly useful when identifying the positions of multiple double bonds in fatty acids and their breakdown products.

The *cis* isomer is the most naturally occurring type of diastereomer found in fatty acids (Mead, et al., 1986). The *trans* isomer generally results from processing, such as vegetable oil hydrogenation (Gunstone, 1986d, Neff, et al., 1994a). The activation energy required to convert between the *cis* and *trans* isomers is reported to be 30 kcal/mole (Mead, et al., 1986) indicating that these isomers are thermally stable and conversion does not typically occur. Figure 3. shows the difference between *cis* and *trans* isomers.

The orientation of the double bond influences the crystalline structure of the fatty acids, especially when incorporated into a triglyceride. The crystal packing of the triglycerides ultimately influences the melting point of the fat or oil (Mead, 1986; Gunstone, 1986c). Gunstone (1986c) suggests that double bonds in the *trans* configuration form the typical bilayer packing of the fatty acid chains common to methyl stearate. For the *cis* configuration however, there must be a change in the fatty acid chain tilt and the packing is not as tight. Therefore, the fatty acid composition of a vegetable oil will greatly influence its physical properties. Figure 4a. and 4b. illustrates the differences in fatty acid chain packing.

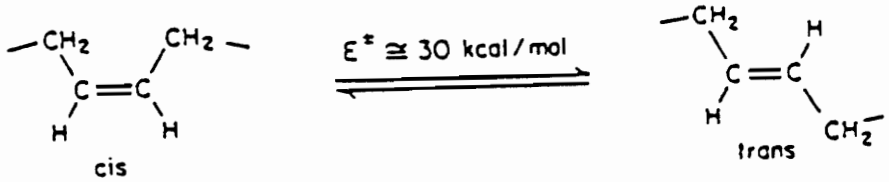


Figure 3. Representation of fatty acid *cis* and *trans* isomerization. (Mead, et. al, 1986)

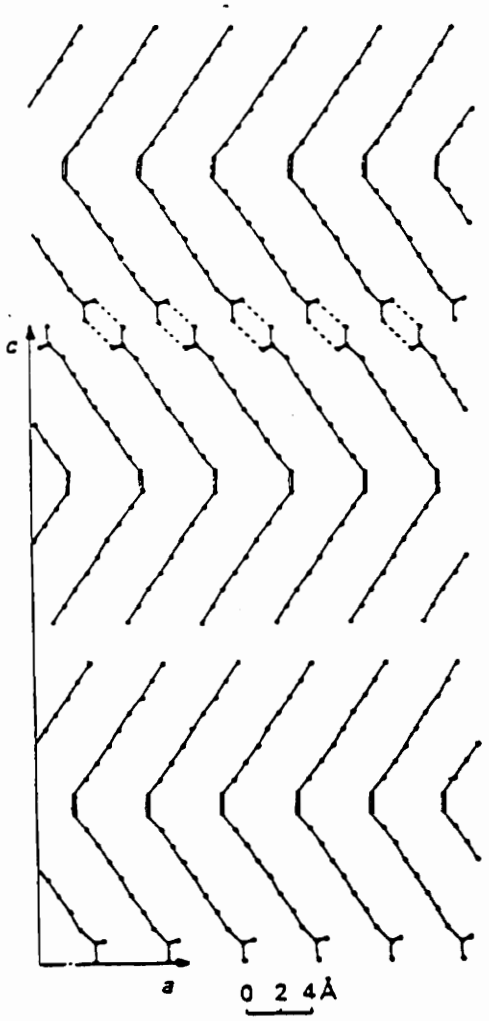


Figure 4. a. Chain tilt occurring in oleic acid as a result of *cis*-isomerization. (Gunstone, 1986b)

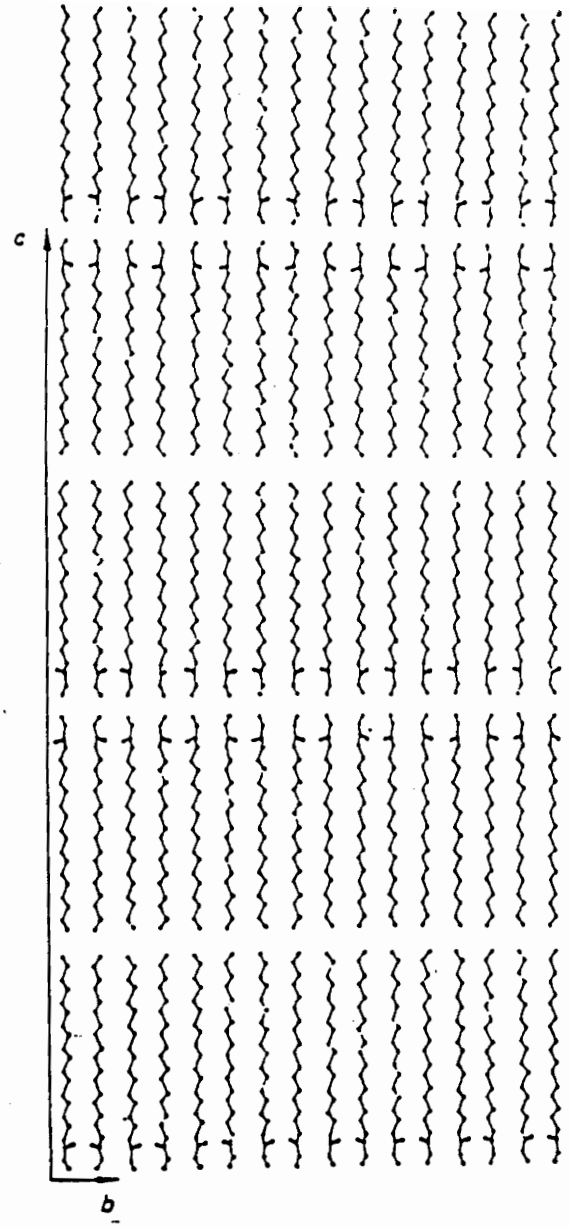


Figure 4. b. Bilayer packing of stearic acid. (Gunstone, 1986b)

The final consideration to take into account is the conformation of the polymethylene carbons in the fatty acid chain. Research (Mead, et al., 1986) has shown that at 25 degrees Celsius the antiplanar and gauche conformations are the predominant conformations, 66% and 34% respectively. The conformation refers to the free rotation of the methylene carbon sigma bonds with the limitation of torsional strain resulting from the eclipsed conformations (Solomons, 1992c). The basic concept is that the alkyl regions bordering any two adjoining methylene carbons can assume conformations ranging from zero to one hundred and eighty degrees apart. Figure 5. represents the most common antiplanar conformation and Figure 6. represents all of the possible conformations of methylene carbons.

The presence of one or more *cis* double bonds limits the degree of rotation, in effect locking the region into the eclipse conformation (Mead, et al., 1986). The eclipse conformation is the underlying factor that accounts for the chain tilt in oleic acid crystalline structures. This of course also accounts for the lower melting points of the unsaturated fatty acids. Therefore, the vegetable oils will ultimately be determined by the properties of the constituent fatty acids.

A. 3. Triglyceride Composition and Characteristics

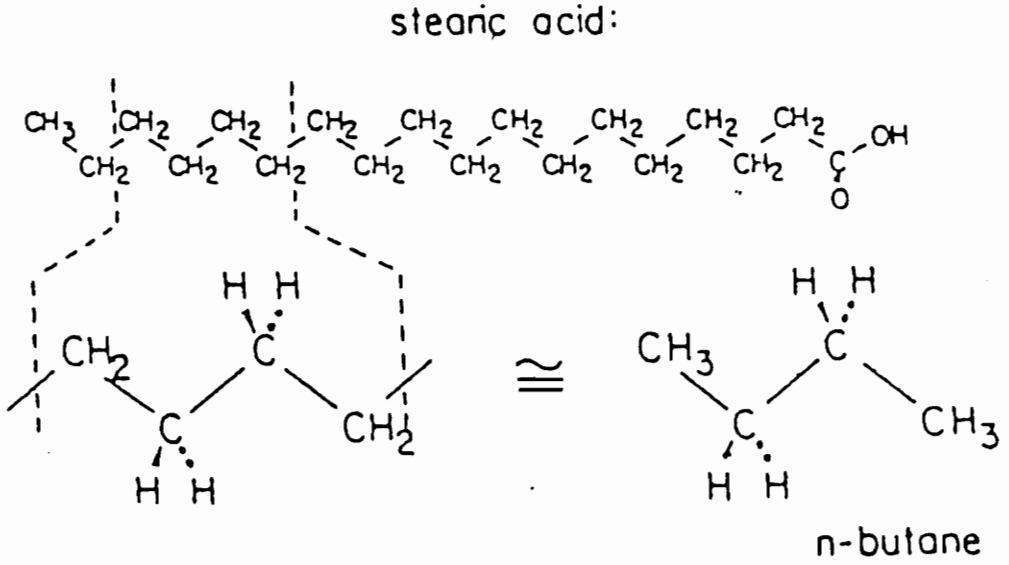


Figure 5. Confirmation of stearic acid with free rotation about the sigma polymethylene bonds. (Mead, et. al, 1986)

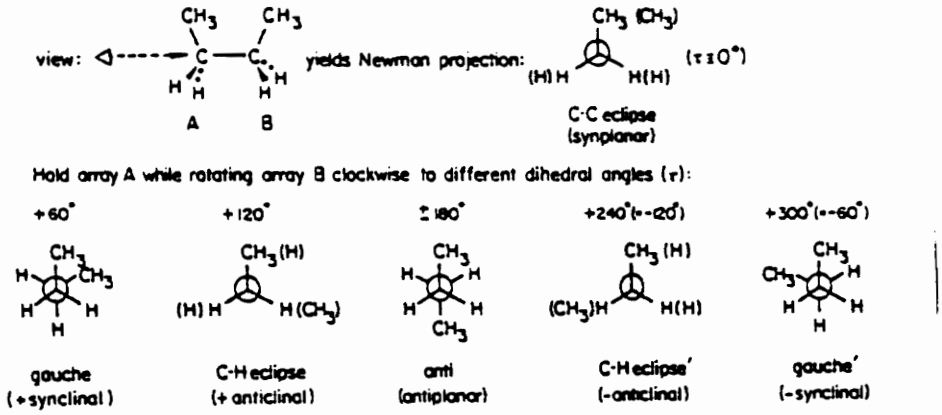


Figure 6. Newman projects: Possible arrays of fatty acid hydrocarbon chains. (Mead, et. al, 1986)

The properties of triglycerides are largely determined by the fatty acid composition of the triglycerides. Factors such as the degree of saturation, diastereomeric configuration, and alkyl chain conformations are responsible for the properties of the triglycerides. Triglycerides are the primary component of refined vegetable oils, constituting 98% or greater of the vegetable oil (Langstraat, 1976). The composition of the triglycerides found in vegetable oils varies greatly between different vegetable oil types (Van Ness, 1981) and depend on climate, region, and time of harvesting (McCormik, et al., 1992; Wilcox and Cavins, 1992). Furthermore, genetic modification of seed oils produces vegetable oils of different triglyceride composition (Kinney, 1994; Knutzon, et al., 1992). Triglycerides (triacylglycerols) are derived from the esterification (condensation) of 3 fatty acids to a single glycerol (Gunstone, 1986b). In this process one mole of water is liberated for every fatty acid esterified to the glycerol. A total of 3 moles of water are liberated in the synthesis of a triglyceride and the result is a glycerol (triacylglycerol) containing 3 fatty acids. Hence, esterification of monoglycerides (monoacylglycerols) liberate one mole of water and contain one fatty acid and diglycerides (diacylglycerols) liberate two moles of water and contain two fatty acids. Glycerol, the backbone of acylglycerols exhibits enantiomeric properties (Small, 1996) leading to different possible glycerol-based structures.

Glycerol is an asymmetric molecule, with the functional group

present (hydroxy or acyl) at the second carbon facing in an opposite direction than those found on the first and third carbon. The most common terminology used to indicate the position of the fatty acids on a glycerol for the first, second, and third position are the alpha, beta, and, alpha' or the *sn* 1, 2, and 3 (Gunstone, 1986b). The *sn* nomenclature is more commonly used to avoid confusion with the crystalline structures of triglycerides.

The enantiomeric properties of the glycerol together with the fatty acid chain associations determines the types of crystals that are formed from triglycerides. Three basic crystalline forms of triglycerides are documented (Small, 1986; Nawar, 1985; Gunstone, 1986d). The crystalline forms are characterized as hexagonal (*A*), orthorhombic (*B'*), and triclinic (*B*). The crystal packing increases in density from the hexagonal to the triclinic crystal lattice. The hexagonal is characterized as being random and having the lowest melting point (Small, 1986). The triglycerides exhibit polymorphism, where any of the 3 major crystalline states can occur, depending on temperature conditions, and especially the rate of cooling (Gunstone, 1986d).

When the fatty acid chains are similar, the triglycerides most commonly pack in a bilayer structure (Nawar, 1985). In the bilayer system the *sn* 1 and 2 positions point in opposite directions, maximizing the hydrophobic Van der Waals interactions between the alkyl regions of the fatty acid side chains (Gunstone, 1986c).

However, when the fatty acid side chains are significantly different the less dense trilayer packing of the triglycerides occur (Small, 1986). Figure 7. represents the bilayer and trilayer triglyceride packing structures.

It should be apparent based on the current knowledge of triglyceride composition and polymorphism that alteration of vegetable oils to improve stability and frying performance is a definite possibility. In fact, a number of researchers (Cowan, et al., 1971; Dobarganes, et al., 1993; Frankel and Huang, 1994; Knutzon, et al., 1992; Neff, et al., 1994a) have taken advantage of the properties of triglycerides previously mentioned, to produce high quality vegetable oils. Furthermore, it should be apparent that differences in the crystalline state due to triglyceride composition will have an impact on the rate of thermal breakdown and autoxidation (Raghuveer and Hammond, 1966).

A. 4. Trace Components of Vegetable Oils

A majority of the trace components are removed from the vegetable oils during the refinement process (Langstraat, 1976; Van Ness, 1981; Ackman, 1983;McCormik, et al., 1992; McGinley, 1991). The trace components or minor components in vegetable oils fall into several different classes: polar components, transition metals, phosphatides, sterols, tocopherols, and pigments. Polar components

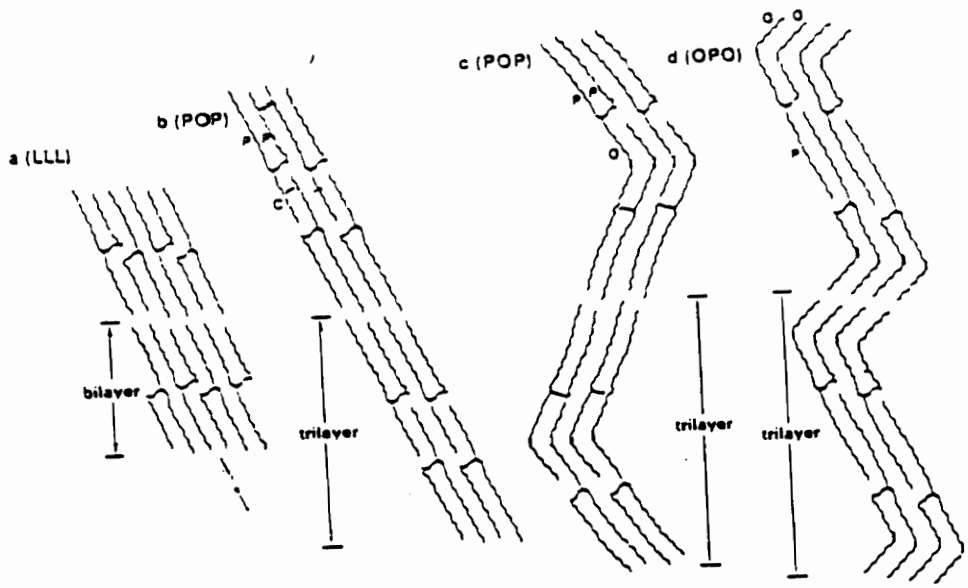


Figure 7. Triglyceride bilayer and trilayer packing structures. (Small, 1986)

largely consist of free fatty acids, monoglycerides, and diglycerides. Free fatty acids, monoglycerides, and diglycerides are generally present as a result of improper handling and storage (Van Ness, 1981) and due to increased levels of lipase activity found in the seed or fruit oil (Langstraat, 1976). The presence of free fatty acids in a vegetable oil leads to undesirable breakdown products, and thus, production of off flavors (Vercellotti, et al., 1992). Free fatty acids undergo autoxidation at a more rapid rate when compared to intact triglyceride fatty acids (Adachi, et al., 1995; Robards, et al., 1988). The presence of monoglycerides and diglycerides interrupt the crystalline structure of the triglycerides, which decreases the temperature at which the oil melts (Small, 1986). Vegetable oils with high levels of monoglycerides and diglycerides have an effect on lowering the smoke point (Fritsch, 1981) when used as a frying oil.

The presence of transition metals (copper, iron, manganese, cobalt, and nickel) ranging from 1 ppm to 10 ppm and must be removed from the vegetable oil during refinement to prevent spoilage during storage (Van Ness, 1981). Transition metals found in vegetable oils liberate free radicals, which is the first step of autoxidation (Pokorny, 1987). The color of the vegetable oil is largely due to the presence of carotenoids and chlorophyll and its derivatives in some cases (Van Ness, 1981). Pigments are removed during the bleaching process of vegetable oil refinement (Van Ness, 1981).

Other components such as sterols, phosphatides, and tocopherols (vitamin E) occur at varied levels, and are also generally removed during refinement (Van Ness, 1981). The most important contributor to the vegetable oil's stability is the level of naturally occurring antioxidants, largely consisting of the tocopherols, phenolic compounds, and to some degree other plant pigments. Pokorny (1987) defines antioxidants as substances that actively suppress the concentration of free radicals. The mechanism by which the antioxidant reacts is not completely understood. Two common mechanisms are proposed: a free radical and a free radical/ionic mechanism (Simic, et al., 1992). The free radical mechanism suggests that free radicals, including oxygen radicals, abstract a hydrogen directly from the antioxidant, which has a lower energy barrier than the unsaturated fatty acids present in the vegetable oil. The result is an antioxidant that can internalize the single electron by delocalization. The second mechanism (Simic, et al., 1992) suggests that there is a two step redox reaction. In the first step the antioxidant reacts with the free radical producing a protonated antioxidant radical. In the second step the antioxidant intermediate is deprotonated, forming an antioxidant radical and hydronium ion. In both cases, the antioxidant reacts with the free radical more readily than the unsaturated fatty acids and the end result is the same.

There are many classes of antioxidants such as phenolic compounds, some carotenoids, and tocopherols to name a few. The

phenolic compounds may be naturally occurring or synthetic. Some of the more common phenolic compounds are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary-butylhydroxyquinone (TBHQ) (Hawrysh, et al., 1990). Pokorny (1987) indicates that TBHQ is the most suitable antioxidant to be added to vegetable oils due to its heat stability. Pokorny (1987) also indicates that the TBHQ helps to retain the color of the vegetable oil by preventing the oxidation of carotenoids and tocopherols. Tocopherols are the antioxidants occurring in the highest levels in most vegetable oils. Tocopherols occur in eight basic isomeric forms (Pokorny, 1987). Each of the isomeric forms contain a hydroxylated dihydrochroman ring with a phytol side chain. The isomers differ in the substitution of methyl groups or hydrogens at the 3 R groups designated R^1, R^2, and R^3 on the aromatic ring segment. The 3 most common tocopherol isomers are the alpha, gamma, and delta forms. Pokorny (1987) indicates that the alpha is less active than the gamma, which is less active than the delta isomer. This order of activity also represents the order of heat stability, with the delta isomer having the greatest heat stability. The reported values of the three most common tocopherol isomers for sunflower and soybean oil are shown in Table 2.

B. Current Analysis for the Comparison and Stability of Vegetable Oils and Fats.

Table 2. Tocopherol isomer levels of sunflower and soybean oil, reported as micrograms per gram of vegetable oil.

Vegetable Oil	Alpha isomer	Gamma Isomer	Delta isomer
Soybean Oil	116	737	275
Sunflower Oil	608	0	0

(Ackman, 1983)

B. 1. Lipid Extraction and Isolation

The fats and oils derived from animal and plant tissues often exist as a complex lipid mixture. The lipid mixture contains compounds such as phospholipids, sterols, mono and diglycerides, free fatty acids, triglycerides, and other lipid soluble components. In order to produce a high quality frying oil the triglycerides must be effectively isolated and purified. For this reason, the method used to isolate triglycerides must be practical and result in nearly 100% purification.

The first step in isolating an oil or fat for analysis or intended food use is to isolate the lipid fraction from the tissue source. Vegetable oils are isolated from the endosperm of seeds (McGinely, 1991) and contain high percentages of linoleic (C18:2), oleic (C18:1) and linolenic acids (C18:3), (Kinney, 1994; Langstraat, 1976). Fats that are obtained from the adipose tissue of animals contain higher levels of saturated fatty acids such as stearic acid (C18:0), and palmitic acid (C16:0), (Mead, et. al, 1992). There are exceptions to this general rule, for example palm oil contains high levels of C16:0, (Langstraat, 1976). Bligh and Dyer (1959) established the Bligh and Dyer Method for the extraction and isolation of fats and oils from biological samples. This method is the most widely used and has many adapted applications for plant and animal tissues, as well as, food samples (Kates, 1975).

The Bligh and Dyer Method alleviates the need for multiphase solvent systems and the need for heating or drying which can readily induce oxidation (Kates, 1975) and polymerization (Berman and Loeb, 1975). In the Bligh and Dyer Method chloroform-methanol-water [1:2:0.8 v/v] are used to isolate the lipid fraction, which is then separated into two phases using chloroform-water [1:1]. The result is the solvation of the components into a polar and a nonpolar phase. The methanol and water are used initially to disrupt the hydrogen bonds and Van der Waals hydrophobic associations between hydrocarbon chains, (Solomons, 1992d; Kates, 1975). With the addition of water in the second step of the extraction, the methanol is captured into the polar phase. At this point the triglycerides and other neutral to nonpolar components can be isolated from the chloroform phase. Further purification and separation of triglyceride species can be conducted after the final isolation step, which will be discussed in section B. 3..

Traditionally, seed oils have been isolated and purified in a processing plant on a large scale. The processing plants are designed to obtain vegetable oils from specific seed crops, such as soybean, sunflower, and rapeseed, (Van Ness, 1981; McCormick, et al., 1992). Although the collection, cleaning, and dehulling vary from seed crop to seed crop, the same basic scheme is applied for the isolation of the vegetable oils. Van Ness (1981) describes the process in several generalized steps. After cleaning and dehulling, the crude oil is extracted using hexane, which can be applied in a

number of ways. Most seed oils are extracted using percolation rather than liquid-solid immersion.

The crude oil undergoes a desolventizing step and is then ready for further refining. The vegetable oil refinement involves four basic steps (Van Ness, 1981). The first step, known as degumming removes the phosphatides, which are slightly polar phospholipids. The second step deacidification, separates the oil from the soap stock, which is largely composed of free fatty acids. In the third step the crude oil is bleached, which removes the pigments and other hydrocarbons. The final step is deodorization, which removes volatile components by sparging steam at a temperature of 175 to 270 degrees Celsius batchwise through the oil. The result is a refined vegetable oil, ready for use or modification.

Recent technology (Neff, et al., 1994b) is being used to isolate and purify vegetable oils by Solid Phase Extraction (SE). In this procedure, roughly 15 grams of seeds are ground and soaked in hexane, which is then sonicated and filtered. The filtrate is then passed through a sodium sulfate drying tube to remove moisture, followed by evaporation of the solvent. The result is a crude oil containing a mixture of polar, neutral, and nonpolar lipids. To purify the lipid fractions, the crude oil is mixed with activated carbon and soaked in a small quantity of hexane. The mixture is then placed in a solid phase extraction chamber and pressurized with helium gas. The nonpolar phase is extracted with hexane, followed by the

extraction of the triglycerides using diethyl ether:hexane [1:9], and the polar fraction using methanol.

The importance of the previous steps covered for the SE becomes evident when the procedure is used to purify fats and oils which have already been isolated from seed oil processing plants. The SE chromatography was used by Neff, et al. (1994a) to purify the triglycerides of interesterified blends of soybean oil and palm olein. The SE chromatography was used to produce a purified oil that may not have been obtainable by other conventional techniques. The greatest advantage of SE chromatography is that it can be applied on a small scale in the laboratory or potentially incorporated into vegetable oil processing and refinement.

The purity of the triglyceride fractions can be evaluated using relatively simple and quick High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) techniques. Conforti, et al. (1993) used a gradient HPLC system to separate the lipid fractions obtained from wheat flour. A ternary gradient system consisting of hexane-tetrahydrofuran [99:1], isopropanol, and water was used to separate steryl esters, triglycerides, mono and diglycerides, free fatty acids, phospholipids, and glycolipids. The AOCS Official Method Cd 20-91, **Determination of Polar Compounds in Frying Fats**, (AOCS, 1991) is another method which can be readily adapted for the detection of nontriglyceride components in a purified vegetable oil. TLC, which is generally used

to assess the column chromatography efficiency, can be used solely as a quick means to detect the presence of nontriglyceride components. Column chromatography can be used as well, however, this technique is laborious and time consuming.

B. 2. Fatty Acid Determination of Vegetable Oils

The fatty acid content and arrangement on the triglyceride is perhaps the most important factor in assessing vegetable oil quality and usability. Research (Fatemi and Hammond, 1975) has shown that fatty acid methyl esters (FAMES) of oleate, linoleate, and linolinoleate react differently in their rates of oxidation. In this case, as the number of double bonds increases the rate of oxidation increases. The double bonds represent the degree of unsaturation, and in this case the polyunsaturated FAMES are more prone to oxidation than the monounsaturated FAMES. Another study (Adachi, et al., 1995) has shown similar results, where polyunsaturated fatty acids are oxidized more rapidly than monousaturated fatty acids. The fatty acid content of a vegetable oil not only influences the rate of oxidation but also the color, viscosity, and the sensory characteristics of frying oil and fried foods, (Robards, et al., 1988).

Due to the importance of the fatty acid composition of vegetable oils, analytical techniques which accurately identify and quantify the fatty acids are imperative. A well established method, also recognized as an AOCS Official Method is described by Liu (1994) for

the analysis of fatty acids. In general terms, sodium methoxide is reacted with the triglycerides of the vegetable oil to produce methylated fatty acids and glycerol. The FAMES are isolated by solvent extraction followed by gas chromatography (GC) analysis. Typically, a 30 meter by 5.30 micrometer internal diameter column with a 0.88 micrometer film thickness consisting of 5% phenylmethyl silicone is used, (Hyver, 1989). The temperature programming varies from 60 to 180 degrees Celsius, depending on the fatty acids contained in the sample. The FAME-GC method when used with an internal standard, can be used for quantification purposes (Liu, 1994). However, to determine the exact origin of the fatty acid derivatives, the composition and purity of the vegetable oil must be known. Other methods can be used to determine the free fatty acid content (Robards, et al., 1988) and polar components (Sanchez-Muniz, 1993; Kates, 1975).

Secondary and relatively quick methods (Robards, et al., 1985; Kates, 1975) can be used to obtain basic information about the composition of the fatty acids without positive identification. For example, the iodine value, can be used to determine the number of double bonds present in an oil sample, where lower iodine values indicate a greater degree of unsaturation. The refractive index, (Nawar, 1985) is another method used to indicate the number of double bonds in an oil sample. This method is commonly used to check the degree of saturation in hydrogenated vegetable oil.

B. 3. Triglyceride Determination of Vegetable Oils

Traditionally, triglycerides have been separated as an entire fraction from other lipid components by using methods such as the solid-liquid adsorption chromatography (SLAC), described in the AOCS Recommended Practice Cd 11c-93 **Quantitative Separation of Monglycerides, Diglycerides, and Triglycerides by Silica Gel Column Chromatography** (AOCS, 1993). While this method is good for the quantification of the different lipid fractions, it reveals little about the actual triglyceride composition. Raghuveer and Hammond (1966) demonstrated that triglycerides with more than one polyunsaturated fatty acid were more prone to autoxidation, also demonstrating that the triglyceride composition is of extreme importance. The triglyceride composition and relative autoxidation rates will be covered in greater detail in a later section (C. 1.). Therefore, methods for the determination of the actual triglyceride composition will be covered.

A variety of analytical methods have been used successfully to determine the triglyceride composition of most common fats and vegetable oils. High Performance Liquid Chromatography (HPLC) is generally the method of choice for several reasons. First, the triglycerides contained in an oil sample can be determined without derivitization, (Palmer and Palmer, 1989). Second, highly saturated fats and standards are readily solubilized by adding chloroform to the solubilization solvent acetone, AOCS Official Method Ce 5c-93

Individual Triglycerides in Oils and Fats by HPLC (AOCS, 1993). Third, and of significant importance, the resolution order for most systems using reverse-phase HPLC is determined by the total number of double bonds and carbon number for each triglyceride. In cases where absolute positive identifications can not be made, the total number of double bonds for a given triglyceride peak can be highly useful as a measure of relative oxidative stability. Vegetable oil reference standards and individual triglyceride standards are generally used for positive confirmation, (AOCS Official Method Ce 5b-89; Palmer and Palmer, 1989).

Caboni, et al., (1992) used an isocratic, reverse-phase HPLC system with light scattering detection, also known as mass detection, for the identification of a variety of triglyceride species in vegetable oils. The method was published as a rapid method for triglyceride analysis with a special focus on the monitoring of olive oil. The method varied from the AOCS Official Method Ce 5b-89 **Triglycerides in Vegetable Oils by HPLC** (AOCS, 1989) and AOCS Official Method Ce 5c-93 (AOCS, 1993) in several ways. A 15 centimeter by 4.6 millimeter column with 12% carbon loading and 3.0 micrometer particle size packing was used instead of the 25 centimeter by 4.5 millimeter column with 22-23% carbon loading and a 5.0 micrometer particle size packing. The packing consisted of silica and the carbon load coating consisted of ocatdecylsilane. The column length dimensions determine the number of theoretical plates and the reactive sites determine the degree of partitioning

between the stationary and mobile phases. Therefore, the shorter column used by Caboni, et al., (1992) most likely enhanced coelution of triglyceride species. However, the time was reduced by as much as 70%, when the method is compared to the AOCS Official Methods Ce 5b-89 and Ce 5c-93. Another variation from the official methods used by Caboni, et al., (1992) was that ethyl ether was added to the acetonitrile:acetone mobile phase, producing a mobile phase consisting of 65/25/10 [v/v/v] acetone:acetonitrile:ethyl ether. The typical composition of the mobile phase generally is acetone:acetonitrile 75/25 [v/v]. In effect, the polarity or polarity index of the mobile phase was decreased, thereby increasing the rate at which the triglycerides moved through the column. Stated otherwise, the partitioning between the stationary phase and mobile phase was decreased, whereby the triglycerides had a higher affinity for the mobile phase. The differences in resolution are illustrated in Figure 8.

The resolution order of the triglycerides, monoglycerides, and diglycerides are determined by the carbon number of the fatty acids contained on the glycerol backbone and the number of double bonds of the fatty acids present (Caboni, et al., 1992; Palmer and Palmer, 1989; Neff, et al., 1994a; AOCS Official Methods Ce 5b-89 and 5c-93, AOCS, 1993). With the mobile phase being more polar than the stationary phase in all the methods covered, the triglycerides with the greatest number of double bonds and shortest fatty acid chains resolve first. It should be noted that positional triglyceride isomers

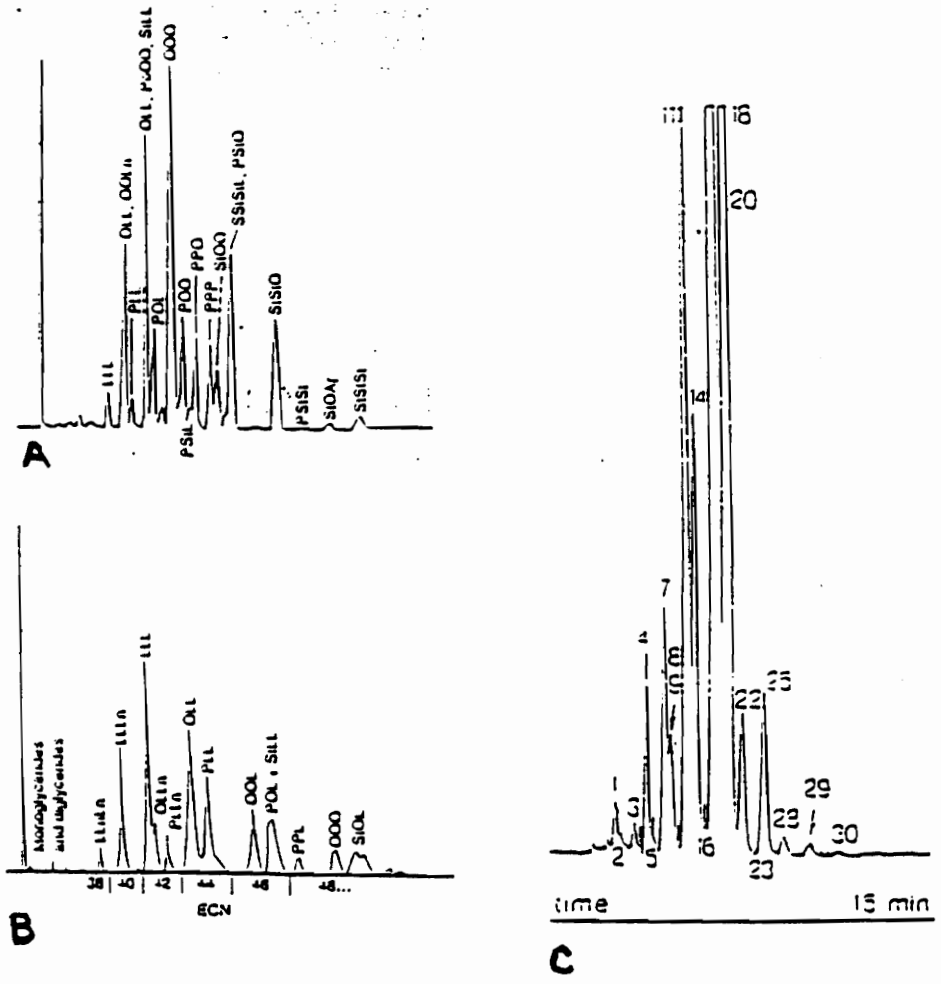


Figure 8. Representative triglyceride chromatograms using isocratic reverse-phased HPLC. A= AOCS Official Method Ce 5c-93, B= AOCS Official Method Ce 5b-89, C= Caboni, et. al, 1992.

are not determined by these methods. The monogylcerides and diglycerides follow a similar pattern but resolve before most of the triglycerides found in the more common vegetable oils such as soybean, sunflower, peanut, canola, and olive oil. The relative resolution orders are demonstrated in Figure 8.

Several methods for the proper identification of the triglycerides are: the use of reference standards (Caboni, et al., 1992; Palmer and Palmer, 1989; Neff, et al., 1994a), the equivalent carbon number (ECN) (AOCS Official Method Ce 5b-89, AOCS, 1989) and the mole to mole comparison of the FAMES to the triglyceride peaks, (AOCS Official Method Ce 5c-93, AOCS, 1993). Reference standards are by far the best way to identify and quantify the triglycerides resolved by reverse-phase HPLC with mass detection. However, many of the more complex triglyceride species are often unavailable, unlike triglyceride species such as triolein, tripalmitin, tristearin, trilinolenin, and trilinolein. This of course makes identification more difficult when these common triglycerides are not the major contributors of the vegetable oils.

The first approach is to calculate the ECN, (AOCS Official Method Ce 5b-89, AOCS, 1989). The ECN is defined as the total carbon number of the fatty acids present minus two times the number of double bonds. The ECN gives a relative resolution order for all the anticipated or possible triglycerides in a vegetable oil sample. Unfortunately, several triglycerides can have the same ECN value,

which again makes identification difficult. The second approach is to determine the mole to mole relationship derived from the FAME content (Liu, 1994) and the triglyceride peak area derived from AOCS Official Method Ce 5c-93 (AOCS, 1993). This procedure involves constructing a balance sheet of the FAMES and then calculating the possible triglyceride content based on the peak areas. The best match should give a 1 to 1 mole value, which can then be validated with available standards, reference chromatograms, and the ECN value. However, if coelution of the triglycerides occur, exact matches will not be possible. By using the balance sheet, available reference standards, reference chromatograms, and the ECN value, a fairly accurate triglyceride profile should be possible. The points to keep in mind when determining the triglyceride content of a vegetable oil sample, is that coelution is possible and that the triglyceride content of similar vegetable oil types can vary, depending on treatment, (Langstraat, 1976) and climatic differences, (Wilcox and Cavins, 1992; McCormik, et al., 1992).

B. 4. Peroxide Detection and Determination

Peroxide formation represents the first major step of lipid oxidation, (Solomons, 1992e; Robards, et. al, 1988). For this reason, the determination of peroxide levels is generally the method of choice to evaluate the quality of vegetable oils and fats. The most widely used and accepted method for peroxide detection is the AOCS Official Method Cd 8-53 **Peroxide Value: Acetic Acid-**

Chloroform Method (AOCS, 1993). The PV is generally reported as milliequivelents of peroxides/1000 grams of sample or iodine formed/1000 grams of fat. In this test the hydroperoxides formed by the oxidation of unsaturated fatty acids react with potassium iodide to form iodine, which in turns is measured by titration with sodium thiosulfate, (Chan and Coxon, 1987). Nawar, (1985) points out that the peroxide values are highly empirical, very sensitive to temperature, and vary depending on the amount of dissolved oxygen in a lipid sample. Chan and Coxon (1987) recommend that the minimum amount of peroxide that can be determined with any accuracy is about one micromole per gram.

Despite the variability in peroxide values within samples and between samples, it is still the most widely used method to evaluate the early stages of autoxidation, (Frankel and Huang, 1994; Hansen, et al., 1994; Hawrysh, et al., 1990; Raghuveer and Hammond, 1966). Researchers have attempted to correlate peroxide values with the oxidation of vegetable oil samples. In some cases, (Frankel and Huang, 1994) the peroxide values correlated with the expected oxidation rates, based on the degree of polyunsaturation of the vegetable oils tested. In a study conducted by Frankel and Huang (1994), high oleic sunflower oil was used to create blends with polyunsaturated vegetable oils with the intention of improving the oxidative stability. The results of this study are illustrated in Figure 9.

The blends with the highest level of oleic acid possessed the lowest peroxide values and hexanal peak area percentage. The hexanal peak area, is another indicator of the early stages of vegetable oil oxidation. In this study the hexanal peak areas showed good correlation with the peroxide values, where an increase in the degree of polyunsaturated fatty acids resulted in an increase in peroxide values and hexanal peak area percentage.

The second major limitation of the peroxide value is that the peroxides are measured collectively and are not identified individually. For this reason, the peroxide values obtained from the testing of vegetable oil samples may or may not have an impact on the rate of autoxidation. Research (Adachi, et. al, 1995) has shown that the rates of autoxidation are rapid after the onset of peroxide formation. Therefore, the actual peroxides formed during the process of autoxidation exists in a very brief state, and are very unstable. Ultimately the type of volatiles produced during autoxidation are excellent indicators of the types of peroxides that are formed as intermediate reactants. This process will be covered in section B. 5..

A study was conducted by Neff, et al., (1992) where triglyceride monohydroperoxides were isolated and used as a measure of oxidative stability. Reverse phase HPLC with ultraviolet detection set at 235 nanometers was used to detect monohydroperoxides of linoleic, linolenic, oleic, and palmitic acid containing triglycerides.

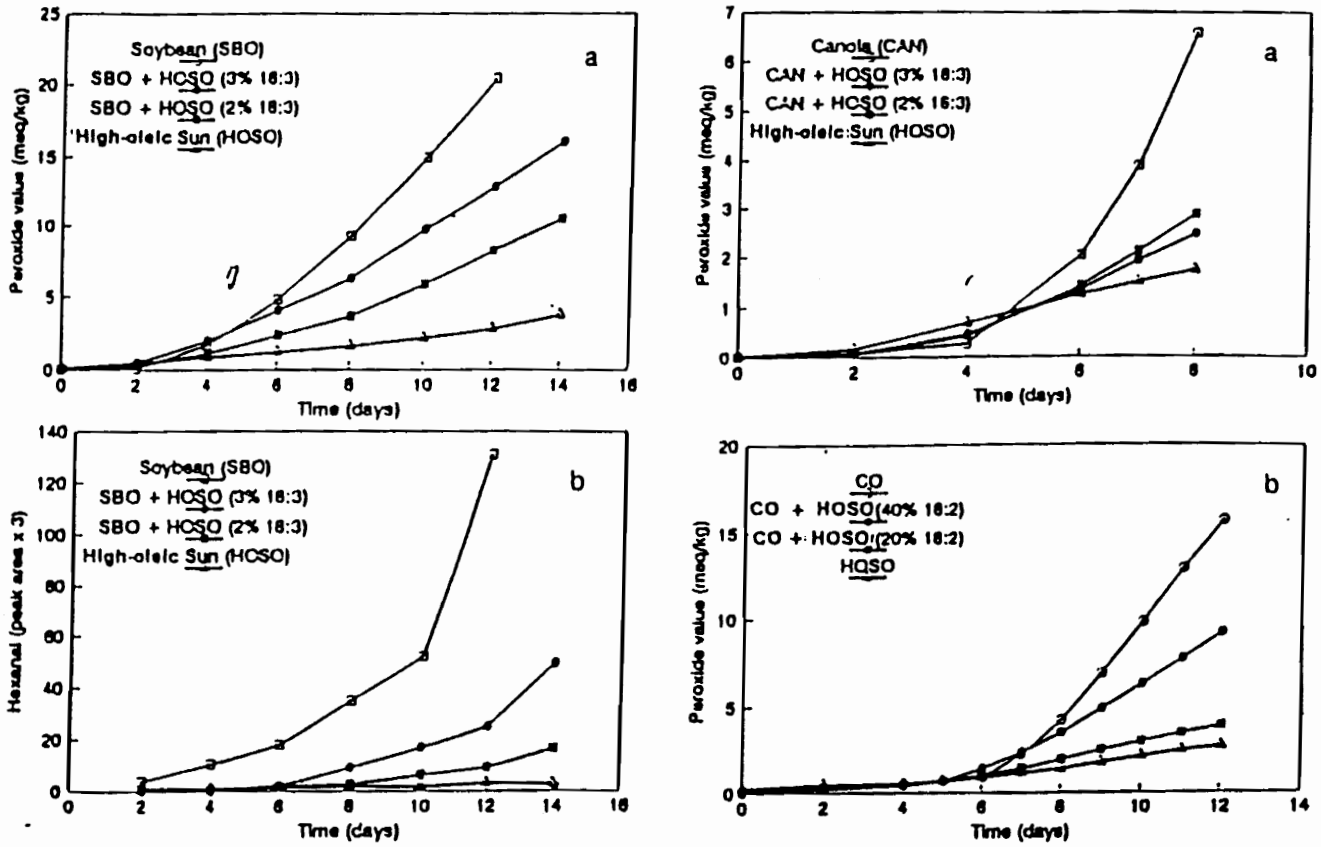


Figure 9. Correlation of peroxide values with hexanal peak areas for high oleic sunflower , canola, and soybean oil blends. (Frankel and Huang, 1994)

The column dimensions were similar to those used in the HPLC triglyceride analysis, 25 by 0.46 centimeters and the mobile phase consisted of chloroform:methanol:acetonitrile, 5/5/90 [v/v/v] run under isocratic conditions. However, the ultra violet detector was incapable of detecting monoene hydroperoxides due to the lack of a chromophore produced by the conjugated diene systems, (Caboni, et al., 1992). To effectively remove residual monoene hydroperoxides from the column, the column was washed with chloroform between injections.

B. 5. Volatile Detection and Identification

Volatile detection and identification by gas chromatography (GC) and mass spectroscopy (MS) in oxidized vegetable oil samples has been one of the most successful and reproducible methods as an indicator of autoxidation (Chang, et al., (1977); Wu and Chen, (1992); Neff and Selke, (1993); Neff, et al., (1994a); Neff, et al., 1994b). Volatiles are the end product of lipid oxidation, and are directly related to the initial fatty acid composition of the vegetable oil (Nawar, 1985). There are numerous methods to collect volatiles and numerous variations to each method. The AOCS Recommended Practice Cg 4-94: **Volatile Organic Compounds (VOC) in Fats and Oils by Gas Chromatography** (AOCS, 1994) describes three basic gas chromatographic (GC) procedures for the analysis of volatile compounds in lipid-containing samples. These three basic procedures are the core methods on which the majority of the other

methods are based upon. Each of the basic methods to be described are similar in that the same GC column and temperature programming can be used. Silica capillary columns with either polar or nonpolar liquid phases are recommended. The recommended temperature programming of the column is from 50 to 250°C at a rate of 5°C per minute. Another similarity is that the volatile compounds are confirmed either by mass spectroscopy (MS), reference standards, or both.

The first method described is the static headspace method, which is selective towards low molecular weight to moderate molecular weight volatiles ranging from pentane (C_5H_{12}) to 2,4-decadienal ($C_{10}H_{16}O$). The static headspace involves heating the samples individually in closed vials, generally 10 to 50 milliliters. The heating conditions most often reflects modified storage temperatures or frying temperatures, 60 and 180°C, respectively. Time exposure to the given temperature varies, depending on the objective of the experiment.

A sample from the headspace of the heated oil is automatically drawn up by a syringe and injected into the GC column. The static headspace method produces chromatograms that lack a solvent peak. Solvents are used in other volatile trapping methods, such as the purge and trap method described by Olafsdottir, et. al (1985). The low boiling volatiles often elute with the solvent peak, making their identification more difficult, therefore, in this respect the static

headspace is advantageous. However, the static headspace analysis is more selective towards the lower molecular weight volatiles, and higher molecular weight volatiles are most often poorly resolved (Reineccius, 1985). Even with this limitation of the static headspace, it has been used successfully for volatile analysis in vegetable oils (Yoon, et al., 1988).

The second GC method described by the AOCS Recommended Practice Cg 4-94 (AOCS, 1994) is the direct injection method. In the direct injection method the glass liner residing in the injector port is plugged with clean and silanized conditioned glass wool. The oil sample is then injected or pipetted directly into the plug of glass wool. The injector temperature is generally reduced from 240 or 250°C to 120 through 180°C to enhance the recovery of the volatiles. The direct injection method generally recovers volatiles ranging from low to high molecular weights. Reineccius (1985) indicates that the direct injection method has good sensitivity, 10 ppb, and good reproducibility. However, the direct injection method is also characterized by poor chromatographic resolution, long analysis time, and samples containing appreciable amounts of water that can not be used.

The third method covered by the AOCS Recommended Practice Cg 4-94 (AOCS, 1994) is the dynamic headspace or purge and trap method. In the dynamic headspace method the oil sample is placed in a tube which is heated and purged with an inert gas such as helium

for a given time period. The heating temperatures range from 60 to 180°C and the purging periods range from 5 to 25 minutes. The volatiles are collected on a porous polymer trap, usually tenax (2,6-diphenylparaphenylene oxide polymer), and then thermally desorbed at a temperature of 150 to 250°C for 5 minutes onto the GC column. A temperature program is set that maximizes the elution and separation of the volatile compounds of interest.

Each of the methods described in the AOCS Recommended Practice Cg 4-94 (AOCS, 1994) are exceptional methods and have their applications depending on the nature of the samples to be tested. Several variations of the volatile analysis covered have been in existence. Olafsdottir, et al., (1985) established a dynamic gas-purging apparatus where beverages or slurries of food were placed in a 250 milliliter round bottom flask and purged onto a tenax-GC collection tube. The volatiles were then eluted from the tenax polymer using diethyl ether, concentrated between 3 and 5 microliters and manually injected onto the GC column. Olafsdottir, et al., (1985) indicated that the percent recoveries of the volatile compounds are low, ranging from 0.4 to 5.7%. This low recovery is attributed to volatile breakthrough, a phenomenon where the volatile compound is purged from the tenax polymer by the purging gas. Reineccius (1985) defined breakthrough as the saturation of the porous polymer trap with a given volatile, where the volatile is no longer trapped but passes through. The low percent recoveries were also attributed to a low purge efficiency and a low affinity of the

volatile compound for the tenax polymer.

The low affinity volatile compounds would include low molecular weight alcohols and compounds with increased polarity. Olafsdottir, et al., (1985) also demonstrated that varied purge times and rates had an impact on volatile recovery. The best recoveries were reported at a purge rate of 50.0 ml per minute for 3 hours, which were selective for the moderate molecular weight volatiles.

A direct capillary gas chromatography method was established by Dupuy and Flick (1987) whereby volatile compounds were recovered from fresh and rancid crude catfish and refined menhaden oil samples. In this method the volatiles were concentrated on the upper portion of a capillary column by cryofocusing after a 3 minute heating period at 200°C in the inlet liner. The chromatograms obtained from rancid catfish oil and crude menhaden oil each contained over 20 major volatiles, which were correlated with a peroxide value, 18.4 and 18.8 respectively, (see Figures 10 and 11 for chromatograms).

Another commonly used method covered by Reineccius (1985) is steam distillation under vacuum. The general process of steam distillation is to add water to the lipid sample, agitate vigorously and distill off a portion of the water under a vacuum. The aqueous distillate is then extracted with a solvent such as diethyl ether and concentrated for GC analysis. Vercellotti, et al. (1988) established

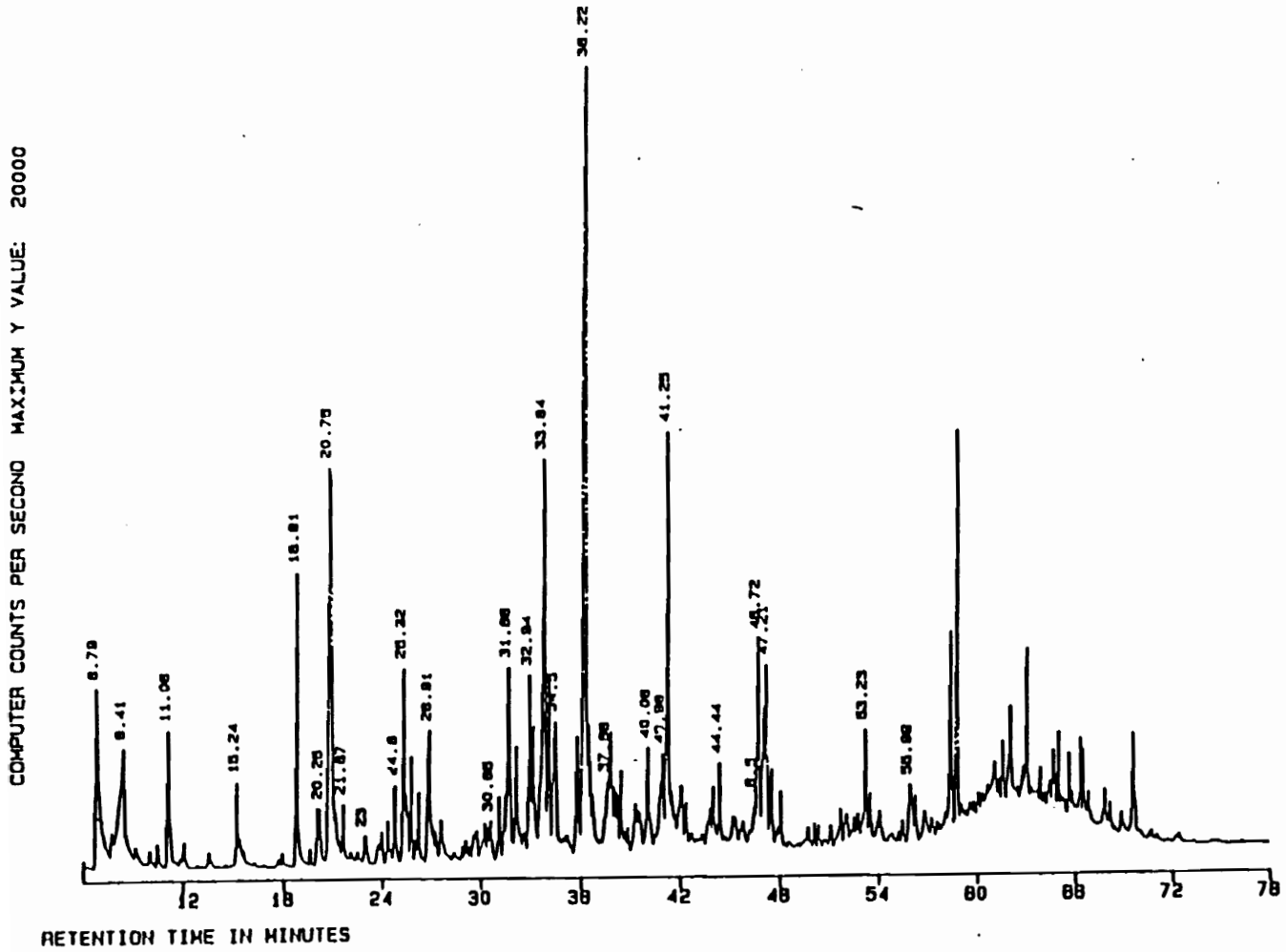


Figure 10. GC analysis from catfish oil rated acceptable: pentane (6.79), 2-butanone (11.08), pentanal (15.24), hexanal (20.75), and nonanal (41.25). (Dupuy and Flick, 1987)

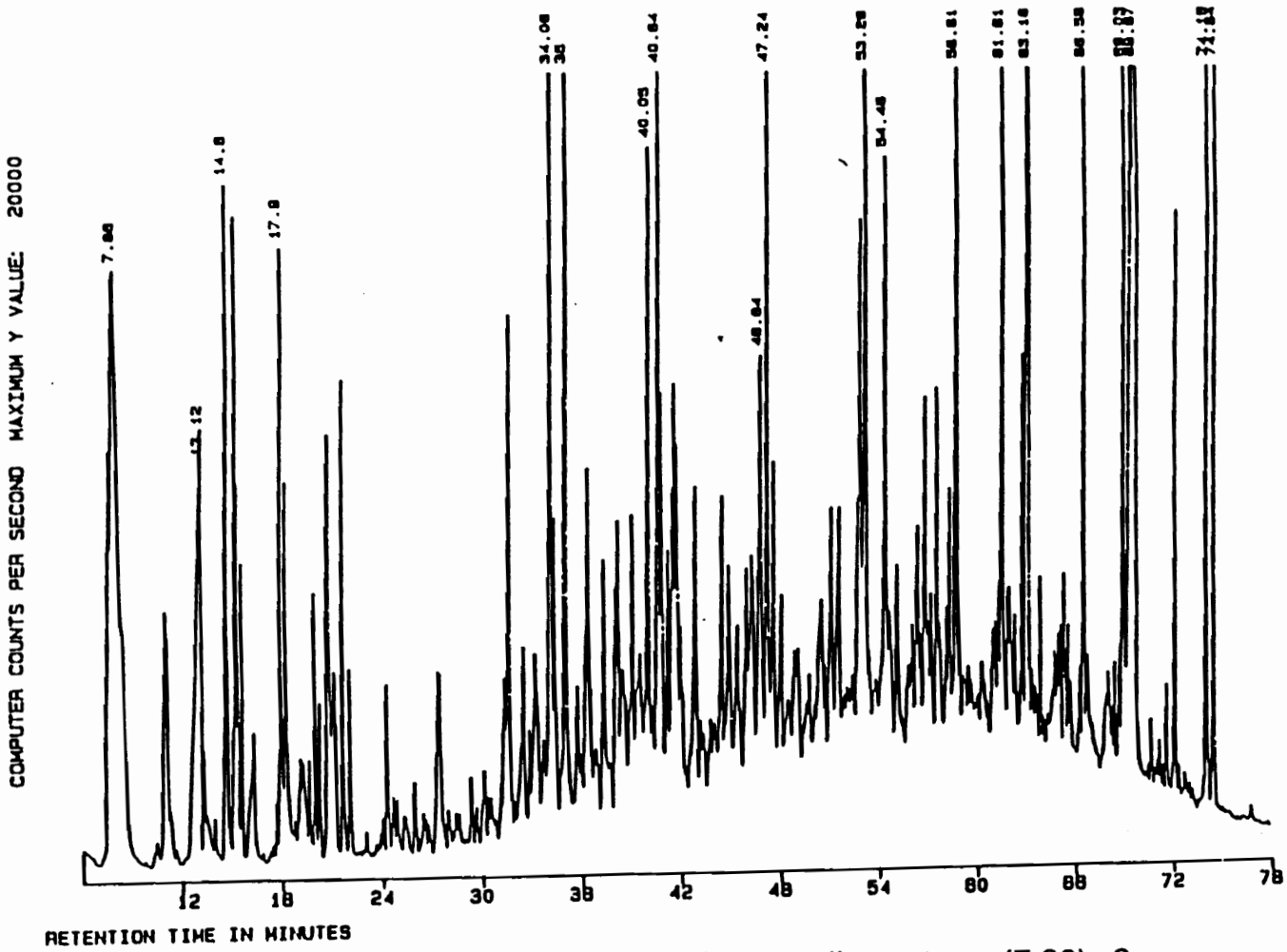


Figure 11. GC analysis of crude menhaden oil: pentane (7.86), 2-butanone (11.02), pentanal (15.13), hexanal (21.16), and nonanal (41.48). (Dupuy and Flick, 1987)

a method combining the concepts of steam distillation with the purge and trap technique for the analysis of volatiles in food and beverage products. The samples were placed in an Erlenmeyer flask and drawn onto a tenax trap under vacuum, (see Figure 12. for illustration). The samples were mildly heated at temperatures ranging from 25 to 60°C. The volatile compounds recovered were comparable or were better than the those found using dynamic or static headspace and purge and trap. Vercellotti, et al. (1988) pointed out that this procedure greatly reduced the degree of volatile compound production that is associated with other methods that involved the use of heat, prolonged light, or air exposure.

Each of the methods previously mentioned have their strengths and weaknesses. For the analysis of vegetable and frying oils, the method of choice for volatile analysis should take advantage of any heating periods common to the oil. Furthermore, the analysis should not influence the rate of autoxidation of the samples to be tested, considering that this is a primary measure of lipid oxidation (Chang, et al., 1977; Fritsch, 1981; Neff and Selke, 1993). Therefore, the purge and trap method, and modifications of the static headspace method are the best methods currently available for the volatile analysis of frying and vegetable oils. For a more comprehensive coverage of gas chromatographic procedures, trouble shooting, and chromatogram interpretation, refer to Basic Gas Chromatography, by H.M. McNair and E.J. Bonelli, 1967, and High Resolution Gas Chromatography, third edition, edited by K.J. Hyver, 1989.

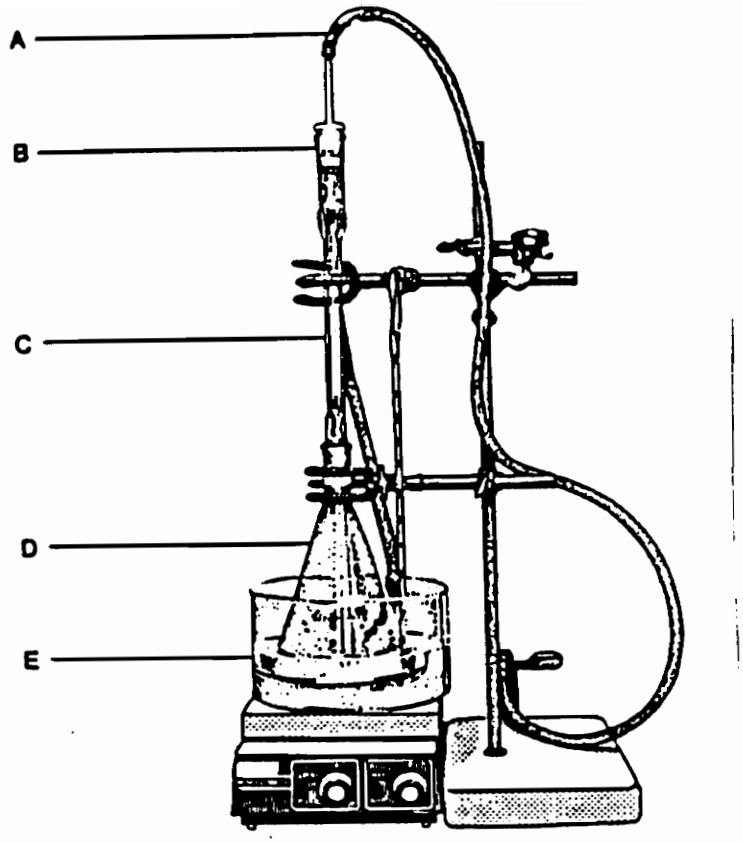


Figure 12. Apparatus for stripping volatiles under vacuum: A= source of vacuum, B= tenax trap, C= tap-water-cooled condenser, D= Erlenmeyer flask, and E= warm water bath. (Vercellotti, et. al, 1988)

B. 6. Polar Component and Polymerization Detection

The class of compounds referred to as polar components in frying oils are monoglycerides, diglycerides, and free fatty acids. The polar components of a frying oil are most often a product of thermal hydrolysis of the triglyceride (Cuesta, et al., 1993) or enzymatic cleavage by lipase Robards, et. al (1988). Polymerization is the result of a conjugated fatty acid (diene) resulting from thermal oxidation, which then interacts with a monounsaturated fatty acid (monoene) producing cyclic dimers, trimers, and triglyceride polymers (Berman and Loeb, 1975). The reaction is characterized by the Diels Alder Reaction, (Solomons, 1992b) which will be covered in greater detail in section C. 2.. The importance of both polar and polymerized products is that they lead to the deterioration of frying oils, (Cuesta, et al., 1993; Dobarganes, et al., 1993; Sanchez-Muniz, et al., 1993). Paulose and Chang (1972) have indicated that the nonvolatile (cyclic dimers) and volatile components may affect human health. Therefore, the detection of polar components and polymerized products are important, not only as a measure of frying oil deterioration, but in addition to consumer safety.

Presently the separation and identification of polymers from heated frying oils is too complex for the typical laboratory. Methods for separation and identification include infrared absorbance (IR), nuclear magnetic resonance (NMR), high performance size exclusion chromatography (HPSEC), and plasma desorption mass spectrometry

(PDMS), (Sanders, et. al, 1992). Berman and Loeb (1975) have illustrated the multitude of cyclic dimers and trimers that can result during the process of polymerization. For this reason the majority of researchers, (Chang, et al., 1977; Frankel and Huang, 1994; Fritsch, 1981; Neff and Selke, 1993) have analyzed the volatile compounds resulting from thermal oxidation. Methods which will be discussed later in this section, (Dobarganes, et al., 1993; Cuesta, et al., 1993; Sanchez-Muniz, et al., 1993) have been used to identify polymers, and polar components separated into classes.

Before covering these methods, traditional and simple tests for the identification of polar components will be addressed. A number of methods have been used to separate the polar and nonpolar classes or to detect the free fatty acid levels. Bati (1989) has listed a variety of thin layer chromatography (TLC) techniques previously used to determine the polar fraction in a frying oil sample. Among these methods are quantitative TLC, (Freeman, 1974) and high performance TLC, (Hauck, et al., 1987). The American Oil Chemists Society lists a procedure for the determination of polar components that involves the use of column chromatography, followed by TLC and AOCS Official Method Cd 20-91 **Determination of Polar Compounds in Frying Fats** (AOCS, 1991). The use of column chromatography and TLC can be time consuming and highly unreliable. Bati (1989) has covered some of the more common problems associated with TLC, and has suggested an outline to

properly select and prepare a TLC plate. Commonly the acid value (AV), a measure of the free fatty acids (FFA) is used due to its simplicity, (Robards, et. al, 1988). The free fatty acids are those fatty acids that are liberated during the frying process from the triglyceride, (Cuesta, et al., 1993) or from enzymatic cleavage, (Robards, 1988). The free fatty acids, expressed as the acid value are often used in conjunction with other measurements to assess the quality of a frying oil (Warner and Mounts, 1993; Warner, et al., 1994). The procedures for conducting an acid value test can be found in AOCS Official Method Cd 3d-63 **Acid Value** (AOCS, 1963).

The method of choice for the detection and determination of polar components and polymers in vegetable oil samples is towards high performance size exclusion chromatography (HPSEC). The use of HPSEC in vegetable analysis yields a profile that includes polymerized products, diglycerides, and free fatty acids. The polar fraction must initially be isolated before HPSEC analysis can be conducted. This is generally accomplished by column chromatography (Dobarganes, et al., 1993). Methods utilizing HPSEC were conducted by Sanchez-Muniz, et al., (1993) to compare the differences in polar components, polymerized triglycerides, dimerized triglycerides, and oxidized triglycerides in sunflower oil during the frying of potato slices. Each of the compounds were well resolved using this system, with notable differences between frying periods and fresh samples. The most noticeable differences were observed in the triglyceride dimers, increasing from 0.1 mg/100 mg to 6.7 mg/100 mg after

fifteen frying periods. Differences were observed in the free fatty acids after the fifteenth frying period, as well as, an increase from 0.4 to 0.6 mg/100 mg of oil sample. Similar results were reported by Dobarganes, et al., (1993) and by Cuesta, et al., (1993). A representation of the HPSEC chromatogram is presented in Figure 13.

C. The Thermal Oxidtive Stability of Vegetable Oils Used For Frying.

C. 1. Autoxidation

Autoxidation is a reaction common to all vegetable oils containing any degree of unsaturated fatty acids. The mechanism of the reaction is complex, still autoxidation occurs readily in vegetable oils. There is a great deal of dispute between the actual mechanism and intermediates involved in the initiation process. However, the majority of researchers who have extensively studied the process agree that it is an autocatalytic free radical chain mechanism (Chan and Coxon., 1987; Adachi, et al., 1995; Frankel and Huang, 1994; Neff and Selke, 1993). The overall reaction is represented below.

Initiation $RH \longrightarrow R\cdot$ (1)

Propagation $R\cdot + O_2 \longrightarrow ROO\cdot$ (2)

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

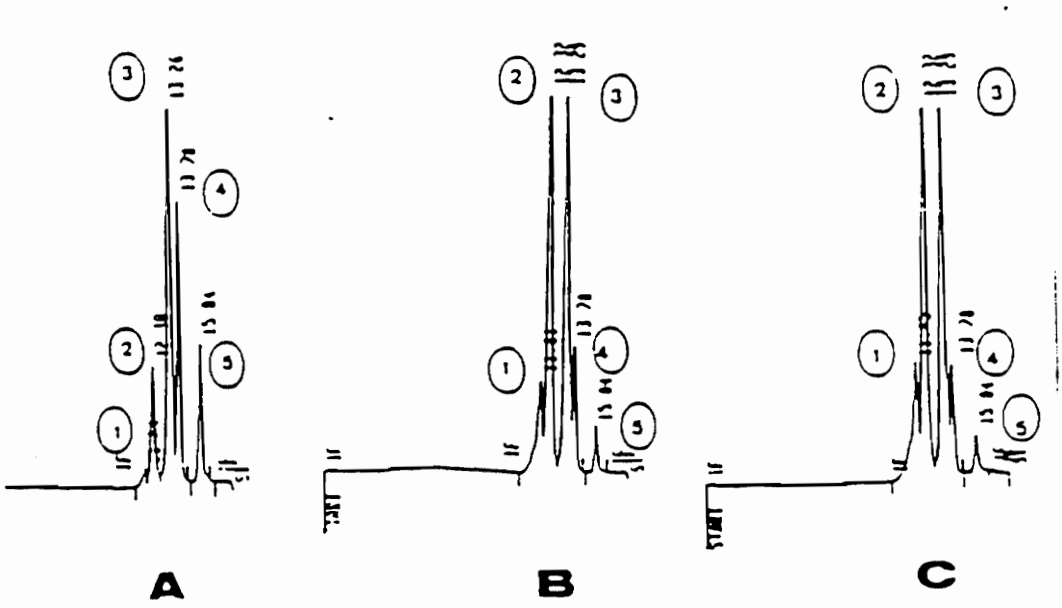


Figure 13. Representative high performance size exclusion (HPSE) chromatograms for sunflower oil. A= fresh sunflower oil, B= sunflower oil heated for 10 hours at 180°C, and C= sunflower oil heated for 20 hours at 180°C. (Sanchez-muniz, et. al, 1993)

Notes:

1= Triglyceride polymers
2= Triglyceride dimers
3= Oxidized triglycerides
4= Diglycerides
5= Free fatty acids

Termination $2R\cdot \longrightarrow R\text{-}R$ (3)

Where,

RH = the alkenoic acid R· = the alkenyl radical

ROO· = the peroxy radical ROOH = the hydroperoxide

Autoxidation is represented by the 3 basic steps above, chain initiation, chain propagation, and chain termination (Simic, et al., 1992; Chan and Coxon, 1987). As previously stated, there is some dispute about the actual initiation of autoxidation. Research (Chan and Coxon., 1987) has shown that molecular oxygen in the triplet state ($^3O_2$) reacting with an olefin (alkenyl) is highly unlikely due to the endothermic energy required, which is reported as approximately 35 kcal/mole.

$RH + {}^3O_2 \longrightarrow R\cdot + HO_2\cdot$ [35 Kcal/mole] (4)

On the other hand, the reaction of molecular oxygen with an already formed radical in the propagation step is highly probable, considering that the spin conservation is maintained (Chan and Coxon, 1987). In this case, the reaction of a triplet state molecule and a doublet state molecule result in a doublet state product. The reaction of molecular oxygen in the initiation of autoxidation is

commonly associated with the iron-containing lipoxygenase enzyme (German, et al., 1992).

Oxygen in the singlet state ($^1O_2$) however, is believed to produce a hydroperoxide which dissociates forming an alkyl hydroxy radical (Nawar, 1985). The spin conservation is maintained in this reaction (S + S = S). However, the activation energy for the cleavage of the hydroperoxide is relatively high (44 kcal/mole), making this explanation appear unlikely (Chan and Coxon, 1987). Research (Neff, et al., 1993) has shown that purified vegetable oils exposed to light had an appreciably higher rate of autoxidation when compared to vegetable oils left in the dark under identical conditions. Therefore, it can be concluded that light exposure interacts with the dissolved oxygen in some manner. The singlet state oxygen mechanism is believed to occur by photoxidation (Neff, et al., 1993) and by photosensitization from pigments in foods (Nawar, 1985). Nawar (1985) has indicated that the most likely spin states of the singlet oxygen are antiparallel occurring in 2 different antibonding pi orbitals. This singlet state is 15 kcal/mole higher in energy than the singlet spin state with both electrons in one pi orbital and 37 kcal/mole higher than $^3O_2$. Nawar (1985) also suggested that certain components such as *beta*-carotene, BHT, BHA, and tocopherols serve as singlet state oxygen quenchers, reducing the $^1O_2$ activity.

Other mechanisms accounting for the initiation step are the presence of transition metals (Chan and Coxon, 1987; Simic, et al.,

1992) and unimolecular and bimolecular reactions (Chan and Coxon, 1987). The unimolecular is the reaction described above, where the hydroperoxide dissociates forming two products, an alkyl and hydroxy radical. The bimolecular mechanism has a much lower activation energy, therefore, it is believed to be a major contributor once the initiation process has begun (Chan and Coxon, 1987).

$$2ROOH \longrightarrow RO\cdot + H_2O + RO_2\cdot \qquad (5)$$

The mechanisms involving the reduction of the metal itself or the reduction of oxygen are also feasible mechanisms. The bond dissociation for the methylene carbon is reported as -15 kcal/mole making the reduction of a transition metal appear as the most likely mechanism (Chan and Coxon, 1987).

$$RH + M^{III} \longrightarrow R\cdot + H^{+} + M^{II} \qquad (6)$$

The oxidation of iron and reduction of oxygen is known to occur in a system containing lipoxygenase, heme iron, and when iron appears alone (Simic, et al., 1992).

$$O_2 + Fe^{II} \longrightarrow O_2\cdot + Fe^{III} \qquad (7)$$

As mentioned previously the initiation process is extremely difficult to define, especially the most likely contributors to

initiation. However, the research previously cited does indicate that chain initiation is a definite reality. In summary, autoxidation can result from a variety of mechanisms (Chan and Coxon, 1987; Neff, et al., 1993; Simic, et al., 1992; Nawar, 1985). The process is largely governed by the bimolecular reaction once initiation has begun (Chan and Coxon, 1987).

Chain propagation and termination are perhaps the two most widely studied steps in autoxidation and are more thoroughly understood. The understanding of these steps are obtained from the analysis of intermediate products (Cuesta, et al., 1993) and termination products (Adachi, et al., 1995; Frankel and Huang, 1994; Neff and Selke, 1993; Wu and Chen, 1992).

Propagation occurs in two distinct steps resulting in the formation of a hydroperoxide (Chan and Coxon, 1987; Simic, et al., 1992). The first step involves the interaction of a free radical and molecular oxygen, resulting in a peroxy radical (Chan and Coxon, 1987). The reaction proceeds rapidly with an activation energy near zero. In addition, the reaction maintains spin conservation (D + T = D) between the reactants and product, where D represents the doublet state and T represents the triplet state.

$$R\cdot + O_2 \longrightarrow RO_2\cdot \qquad (8)$$

In the second step of propagation, the peroxy radical reacts with the

unsaturated fatty acid, resulting in a hydroperoxide and an alkenyl radical. The alkenyl radical then perpetuates the cycle.

$$RO_2\cdot + RH \longrightarrow ROOH + R\cdot \qquad (9)$$

The two reactions are interdependent in that the rapid consumption of the alkenyl radical in the first step maintains an equilibrium between the two steps. Based on this kinetic principal the rate production of the peroxy radical is equal to the rate production of the hydroperoxide.

$$K_o\,[R\cdot]\,[O_2] = K_p\,[RO_2\cdot]\,[RH] \qquad (10)$$

Where,

K_o = the kinetic rate of oxygen

K_p = the kinetic rate of the peroxy radical

Research (Adachi, et al., 1995; Myashita, et al., 1990) has shown that the rates of autoxidation occur more rapidly with the degree of unsaturation. Linolenic acid (C18:3) reacts more rapidly than linoleic acid (C18:2), which reacts more rapidly than oleic acid (C18:1). Similar findings have been found in studies using vegetable oils with high polyunsaturated fatty acid contents (Frankel and Huang, 1994; Neff, et al., 1991; Warner and Mounts, 1993). The

explanation of the higher autoxidation rates for polyunsaturated fatty acids resides in the bond dissociation energies and the process of delocalization (Chan and Coxon, 1987). The bond dissociation energy D(R-H) for alkenyl methylene groups, is on the average of 20 kcal/mole less than the alkyl methylene hydrocarbons. This occurrence alone, makes the abstraction of a hydrogen from a saturated fatty acid highly unlikely. The ability of the polyunsaturated fatty acids to internalize the single electron by delocalization, makes the abstraction of a hydrogen more accessible. Therefore, the greater the degree of unsaturation, the greater the ability for delocalization.

The final step of autoxidation, chain termination, involves the reactions of accumulated reactants and intermediates. For the case of vegetable oils, or more appropriately unsaturated fatty acids, the most important end products result from the cleavage of the hydroperoxide (Chan and Coxon, 1987; German, et al., 1992; Nawar, 1985). The hyroperoxide decomposition occurs in two distinct steps, *beta*-scission and carbon-carbon cleavage.

In *beta*-scission the bond occurring between the two oxygen molecules is cleaved resulting in a hydroxy radical and an alkoxy radical (Nawar, 1985; Neff and Selke, 1993). In the second step, the alkoxy radical is cleaved either on the methyl side resulting in a hydrocarbon and an oxy acid, or on the carboxylic acid side resulting in an aldehyde and an organic acid (Nawar, 1985; Neff and Selke,

1993). It should be noted that these steps and all other steps occurring in autoxidation are generalizations which account for the majority of products resulting from autoxidation.

The process of heating a vegetable oil greatly enhances the rate of autoxidation, determined by the peroxide value, the accumulation of volatile end products, and hydroperoxide intermediates (Chang, et al., 1977; Fritsch, 1981; Neff, et al., 1991; Robards, et al., 1988; Warner, et al., 1994; Wu and Chen, 1992; Myashita, et al., 1990). Research (Yoon, et al., 1988) has also shown that the addition of thermally oxidized compounds, when added to purified vegetable oils increased the rate of volatile production. In general, as the time exposure to frying temperatures increases, the rate of autoxidation increases. Warner and Mounts (1993) observed that the rate of autoxidation increased significantly in modified vegetable oil blends as the hours of heating at 190 degrees Celsius increased. Presently, research is being aimed at the modification of vegetable oils through genetic engineering (Kinney, 1994; Knutzon, et al., 1992), by vegetable oil blending (Frankel and Huang, 1994), and by interesterification (Neff, et al., 1994b). Each of these modifications are designed to reduce the rate of autoxidation by producing vegetable oils with a lower unsaturated fatty acid content.

C. 1. a. Peroxidation of Linoleic, Linolenic, and Oleic Acid.

The points of peroxidation on the fatty acid are largely governed

by the degree of unsaturation. These points of reactivity on the individual unsaturated fatty acids follow a predictable pathway, giving rise to a number of major volatile products (see Appendix K). The 3 major unsaturated fatty acids which will be discussed are oleic, linoleic, and linolenic acid.

Oleic acid contains one double bond at the ninth and tenth carbons. The two bordering methylene carbons, (carbons 8 and 11 of the fatty acid chain) are the points of hydrogen abstraction, either by singlet oxygen (Nawar, 1985) or by a transition metal or dissolved free radical (Chan and Coxon, 1987). The result from the hydrogen abstraction are four possible hydroperoxide isomers. Abstraction at the eighth carbon can lead to a 8-OOH and a 10-OOH hydroperoxide, where abstraction at the eleventh carbon can result in a 9-OOH and a 11-OOH hydroperoxide (Chan and Coxon, 1987). Although linoleic acid has three methylene carbons adjacent to a double bond (carbons 8, 10, and 14), the tenth methylene carbon is the most likely point of attack. Abstraction of a hydrogen at the tenth carbon results in a conjugated structure which is able to stabilize the hydroperoxide through resonance (Chan and Coxon, 1987). The major hydroperoxides formed are (*trans, cis* and *trans, trans*) 13-OOH and (*trans, trans* and *cis, trans*) 9-OOH. Research (Neff and Selke, 1993) has shown that the preservation of the conjugated system is preferred in the peroxidation of linoleic acid. In this case, volatile products resulting from the oxygen insertion at the point of hydrogen abstraction are negligible.

The abstraction of a hydrogen from linolenic acid can occur at four possible methylene carbons (carbons 8, 11, 14, and 17). However, due to the process of delocalization, the two most likely points of abstraction are carbons 11 and 14 on the fatty acid chain. Six major hydroperoxide isomers can result occurring in *trans* or *cis* configurations, 9-OOH, 11-OOH, 13-OOH, 12-OOH, 14-OOH, and 16-OOH (German, et al, 1992, Myashita, et al., 1990). Research (Raghuveer and Hammond, 1966) has shown that the rate of peroxidation increases for the polyunsaturated fatty acids when they are in closer proximity, due to crystal packing. Raghuveer and Hammond (1966) suggested that unsaturated fatty acids in the hexagonal *ortho* array as opposed to the hexagonal *meta* array are more prone to peroxidation. The *ortho* array is characterized as being less random, where an oxidized fatty acid can more easily interact with a neighboring fatty acid. The degree of unsaturation as well as the positioning of the fatty acids had an impact on the rate of autoxidation.

Further research (Myashita, et al., 1990) has clarified the actual positioning of the unsaturated fatty acid and its impact on the rate of autoxidation. Research showed that the polyunsaturated triglycerides experienced the greatest rate of autoxidation when the linolenic acid was located on the *sn* position 2. Triglycerides with a total reduction of double bonds at the *sn* positions 1 and 3 were less prone to autoxidation. However, when the *sn* 2 position was replaced with linoleic acid, the rate of autoxidation decreased even further.

The major volatile products resulting from autoxidation of oleic, linoleic, and linolenic acid are presented in Table 3.

C. 2. Thermal Polymerization

Thermal polymerization is a reaction common to vegetable oils used for frying (Firestone, 1994; Thompson and Aust, 1983; Chang, et al., 1977). Research (Chang, et al., 1977) has shown that the occurrence of thermal polymers, mainly cyclic dimers and trimers, are greater in vegetable oils which are largely polyunsaturated. Thermal polymerization testing conducted on triolein and tristearin revealed that no cyclic dimers or trimers were present after simulated frying periods (Chang, et al., 1977). The concluding results indicated that the rate of thermal polymerization increased with the level of polyunsaturated triglycerides. Therefore, the production of cyclic dimers and trimers must require the presence of polyunsaturated fatty acids.

The Diels Alder reaction is characterized by an interaction between a monoene (monounsaturated compound) and a conjugated diene (polyunsaturated compound) (Berman and Loeb, 1975). In this reaction, which is generally induced by heat and the presence of a clay catalyst, a shifting of double bonds between the two fatty acids produces a cyclic compound. The cyclic compound, which encompasses both fatty acids, can occur in a multitude of isomeric

Table 3. Major volatiles produced during autoxidation of oleic, linoleic, and linolenic acid.

Compound	C18:1	C18:2	C18:3
Hexanal		+	
Heptanal	+	+	
(Z)-2-Heptenal		+	
(Z,Z)-2,4-Heptadienal			+
(Z,Z)-2,4-Decadienal		+	
Nonanal	+		

(Chan and Coxon, 1987)

Notes:

1. C18:1 = Oleic acid
2. C18:2 = Linoleic acid
3. C18:3 = Linolenic acid

forms (Berman and Loeb, 1975). In general, each of the carboxyl ends and methyl ends of both fatty acids can appear in the head first or tail first position when polymerization occurs.

The majority of knowledge concerning the cyclic dimers and trimers comes from industrial research (Berman and Loeb, 1975). The research concluded that dimer production resulted from the heating of polyunsaturated fatty acids and esters. In this case the methylene carbons occurring between the double bonds of the polyunsaturated fatty acid would have to be conjugated to produce a diene before the reaction could occur. A conjugated hydroperoxide (Section C. 1. a.) results from autoxidation of linoleic and linolenic acid. Therefore, it is more likely that polymerization also occurs during the autoxidation of the vegetable oil. In fact, this prerequisite was postulated in the chemical abstracts of Scheiber (1929) and Kappelmeier (1933). Kappelmeier (1933) postulated that the reaction was a Diels Alder type reaction, occurring between a conjugated and nonconjugated fatty acid.

The extent to which thermal polymer products affect the quality of the vegetable oil is often unclear. Research (Thompson and Aust, 1983; Chang, et al., 1977) has suggested that thermal polymers and other oxidative products may induce toxicological effects when consumed in fried foods. However, many researchers (Cuesta, et al., 1993; Dobarganes, et al., 1993; Hansen, et al., 1994; White and Wang, 1986) have focused on the quantification of thermal polymers and

their impact on vegetable oil stability. Research (White and Wang, 1986) has shown that polymerized triglycerides and cyclic dimer levels increased significantly in soybean oil heated for 56 hours at 180 degrees Celsius. In a study conducted by Hansen, et al. (1994), polymerized products from 99% pure triolein were observed. The triolein was heated for 12 hours per day at 190 degrees Celsius, and after 60 hours of heating, the researchers reported 20% or greater polymeric materials. The polymeric components consisted of tetrameres, trimers, dimers, monomers, and low molecular weight products. This research has raised questions about the Diels Alder Reaction being the likely mechanism, considering triolein is composed of monenic fatty acids. However, there was no mention of the presence of conjugated volatile products, or the composition of the other 1%, therefore, the Diels Alder Reaction can not be ruled out. In any case, polymerization occurs in vegetable oils during the frying process and is closely associated with autoxidation. A number of researchers (Hansen, et al., 1994; Cuesta, et al., 1993; Dobarganes, et al., 1993; Firestone, 1994; White and Wang, 1986) have suggested using the presence of polymerized products as an indicator of frying oil quality. Hansen, et al., (1994) suggested that 20% or greater of polymeric materials should be an adequate cut off point. The same research team also indicated that the use of HPSEC for the detection of polymeric materials is far more reliable than the polar component test and free fatty acid/acid value test. As indicated (section B. 6.) high performance size exclusion chromatography (HPSEC) can be utilized to determine the levels of

polar and polymeric compounds simultaneously.

C. 3. Thermal Hydrolysis

Thermal hydrolysis is the breakdown of the triglycerides contained in a vegetable oil during heating, to form monoglycerides, diglycerides, and free fatty acids (Robards, et. al, 1988). This process, in general, requires the presence of moisture and/or the presence of lipase enzymes. The refining process of vegetable oils described by Van Ness (1981) involves the deactivation of lipase enzymes. This deactivation together with the denaturing of the enzymes that would occur at frying temperatures (Nawar, 1985) makes the enzyme-mediated hydrolysis of frying oils highly unlikely. Hydrolysis of a lipid in general is sometimes referred in to literature as hydrolytic rancidity (Nawar, 1985). The term has been used to describe off flavors produced from the cleavage of short chain fatty acids in dairy products. However, in vegetable oils the term rancidity or flavor reversion is associated with the oxidation of linolenic acid and not necessarily the hydrolysis (Chang, et al., 1977).

Thermal hydrolysis is most commonly associated with vegetable oil or shortenings that have been used to fry foods, such as potatoes or chicken (Fritsch, 1981). In the process of frying a food, moisture is released from the food passing through the frying oil as steam. During this process the fatty acids contained on the triglycerides

can be released by the addition of water across the fatty acid-glycerol ester bond. The result is an accumulation of free fatty acids, monoglycerides, diglycerides, and glycerol. These components were defined previously as the traditional polar components in vegetable oils (section B. 6.). However, it should be noted that triglyceride dimers and oxidized triglycerides are sometimes categorized as polar components in literature (Cuesta, et al., 1993; Dobarganes, et al., 1993). Furthermore, the triglyceride dimers and oxidized triglycerides are a result of polymerization and autoxidation, which readily occur at low moisture contents and can occur independently of triglyceride hydrolysis. The polymerized products are generally included with the polar components such as free fatty acids, monoglycerides, and diglycerides since they are all resolved on one chromatogram using HPSEC (section B. 6.).

The accumulation of free fatty acids, monoglycerides, diglycerides, and polymerized components in a frying oil can lead to the rapid deterioration of the frying oil and the quality of fried food products (Fritsch, 1981). These components, once in the frying oil, are absorbed into subsequent fried food products, which will cause undesirable changes in the color and taste of the fried foods (Fritsch, 1981). The exchange between the fried foods and the frying oil is a dynamic relationship which in time will have a negative effect on the vegetable oil and the food. The increased level of polar components in the frying oil have a significant impact on the physical properties of the frying oil. It was established (section A.

3.) that the triglyceride packing of the vegetable oil is disrupted by the presence of monoglycerides, diglycerides, and other impurities (Small, 1986). Research (Raghuveer and Hammond, 1966) has shown that changes in the triglyceride packing structures can lead to changes in the rate of autoxidation. In addition, free fatty acids can form low molecular weight dimers, which have been reported to produce ill health effects when consumed (Thompson and Aust, 1983; Hansen, et al., 1994). The production of off flavors in the frying oil and fried foods have been attributed to high levels of free fatty acids, especially linolenic acid (Warner and Nelson, 1996; Chang, et al., 1977).

Research (Cuesta, et al., 1993; Dobarganes, et al., 1993) has shown that the presence of total polar compounds in a frying oil increases significantly with continued frying. Cuesta, et al., (1993) showed that the total polar content increased from 5.09 mg/100 mg of sunflower oil to 15.99 mg/100 mg of sunflower oil after 20 frying periods using potato slices. However, the diglyceride and free fatty acid levels remained relatively consistent, with only minor increases. The major changes experienced were largely a result of triglyceride polymerization and dimerization. In another study (Dobarganes, et al., 1993), a genetically modified (high oleic acid) sunflower oil was heated to 180°C with and without potato slices. The results indicated that there was a significant increase in the levels of total polar components after 10 hours of heating the vegetable oil alone. The increase was reported as 3.3% of total

composition initially and 29.4% after 10 hours of heating. The vegetable oil used to fry potato slices along with the fat extracted from the fried potato slices contained a lower total polar content after 10 hours of heating. The value was reported as 19.9% of total composition. Again, only minor changes in the free fatty acid and diglyceride levels resulted which were not reported as significantly different. The majority of the changes in total polar content resulted from the production of triglyceride polymers and dimers. The conclusions reached in this study (Dobarganes, et al., 1993) indicated that polymerization and autoxidation are largely responsible for the deterioration of the frying oils. Furthermore, only minor differences were noted between the vegetable oils with and without potato slices during heating (Dobarganes, et al., 1993). Other studies (Cuesta, et al., 1993; Dobarganes, et al., 1993) have indicated that the addition of a food product to a frying oil may not be a driving force for thermal hydrolysis, at least on a small scale. This is especially true, considering that the average moisture content of frying potatoes is 77.3% of total weight (Cuesta, et al., 1993). This amount of moisture exposure to the frying oil on a repeated basis should be more than adequate to induce hydrothermal hydrolysis. However, the amount of free fatty acids, monoglycerides, and diglycerides remaining in the frying oil will depend on the type of fryer used and filtering system (Yates and Caldwell, 1993), the quality of the vegetable oil resulting from the refining process (Dobarganes, et. al, 1993), and the amount of vegetable oil components absorbed into the fried food product

(Thompson and Aust, 1983; Warner and Mounts, 1993).

C. 4. Sensory Characteristics of Heated Frying Oils

The flavor and aromas found in vegetable oils are largely a result of polyunsaturated and monounsaturated fatty acid autoxidation (Warner and Nelson, 1996). Therefore, as the vegetable oil ages or is frequently used, the intensities of the flavors and aromas increase (Warner, et al., 1994). Research (Cowan , et al., 1971; Warner and Mounts, 1993) has shown that the volatiles absorbed into fried food products such as french fries can decrease the acceptability of these food products. Other characteristics such as room odor intensity (Warner and Mounts, 1993) can occur from the thermal oxidation of vegetable oils. Vegetable oils with the highest concentrations of linolenic acid were found to produce the greatest intensities in room odor after heating.

In some cases the flavors imparted to a fried food from the vegetable oil may be desirable. Research (Warner, et al., 1994) has shown that potato chips fried in canola oil heated for 3 to 6 hours at 192°C rated higher in sensory testing than potato chips fried in fresh canola oil. However, potato chips fried in vegetable oils heated for 2 days or more showed a decrease in sensory ratings. Potato chips fried in hydrogenated vegetable oils produced a waxy or characteristic hydrogenated taste in the potato chips, regardless of

the maturity of the oil (Warner, et al., 1994). However, the hydrogenated taste intensity did increase as the frying oil matured. Other researchers (Frankel, et al., 1985) have experienced similar results with hydrogenated vegetable oils. Generally, as the levels of hydrogenation increased in the vegetable oils, an increase in the room odor was a result. Frankel, et al., (1985) demonstrated that vegetable oils partially hydrogenated with a commonly used catalyst such as nickel produced the greatest room odors and flavor intensities.

The autoxidation of the fatty acids contained in a vegetable oil is ultimately responsible for the flavor and aromas. However, it is the specific volatile end products and combinations that produce characteristic and discernable flavors and aromas. Volatile end products from the more common fatty acids linolenic, linoleic, and oleic acid were are listed in Table 3..

Research (Warner and Nelson, 1996) has shown that volatile end products arising from linolenic, linoleic, and oleic acid greatly influence the flavor intensity and acceptability of the vegetable oils they are contained in. In an AOCS collaborative study (Warner and Nelson, 1996) 16 laboratories participated in the sensory analysis of fresh and oxidized vegetable oil samples. The vegetable oils consisted of soybean, sunflower, canola, and corn oil. Each of the oils were distributed to the individual laboratories, identified by a code number. The individual laboratories were then responsible for

the determination of the volatile content and sensory characteristics of each coded vegetable oil. Off flavors such as beany and grassy were present in each of the soybean, sunflower, and canola oxidized samples. The unused samples were described overall as nutty or buttery for each of the vegetable oils. The highly oxidized soybean and canola oils which contained between 5.0 and 7.0% linolenic acid were characterized as having a fishy and painty odor. The objective of the study was to identify 13 major volatiles arising from the breakdown of the major fatty acids. Unfortunately, the only two volatiles that were successfully identified in all of the laboratories were pentane and hexanal.

Hexanal and pentane occur from the autoxidation of linoleic acid (Chan and Coxon, 1987). However, it is unlikely that the flavor intensities resulted largely from 2 volatile components, especially given the degree of linolenic acid present in the soybean and canola vegetable oils. A more even distribution of vegetable oils independently high in oleic acid, linoleic, and linolenic acid would have helped to correlate the sensory scores to the individual fatty acids. In addition, hydrogenated vegetable oils were omitted from the study (Warner and Nelson, 1996), which were previously shown to produce characteristic waxy aromas and flavors (Warner and Mounts, 1993).

Robards, et. al (1988) characterized the aromas of several volatiles produced in standard and hydrogenated vegetable oils. The

hydrogenated aromas were present in (Z)-6-nonenal samples, and the stale and rancid aromas were present in (Z,Z)-2,4-decadienal and (Z,Z)-2,4-hepatadienal samples, respectively. It was reported (section C. 1. a.) that (Z,Z)-2,4-decadienal results from the autoxidation of linoleic acid and that (Z,Z)-2,4-heptadienal results from the autoxidation of linolenic acid. The grassy or green odors were found in volatile samples such as hexenal, heptenal, and (Z)-2-hexenal (Robards, et. al, 1988).

D. Vegetable Oil Modifications

D. 1. Hydrogenation

The most common chemical modification of vegetable oils is hydrogenation. Hydrogenation is generally carried out at the vegetable oil refining plant (Langstraat, 1976). The process of hydrogenation (Van Ness, 1981) involves the saturation of linoleic and linolenic acid to stearic acid. In this first order kinetic reaction hydrogen gas is bubbled through a sieve containing a nickel catalyst and polyunsaturated triglycerides. The result is an addition of hydrogen across the double bonds present in the unsaturated fatty acids.

Partial hydrogenation of unsaturated fatty acids is accompanied by double bond migration and stereo-mutation (Gunstone, 1986d). As

a result of partial hydrogenation both *cis* and *trans* isomers can occur in the vegetable oil. The *trans* isomers increase the vegetables oil's stability and increase the melting point due to a better packing of crystal structures (Gunstone, 1986b). However, vegetable oils containing *trans* fatty acids have been reported to produce waxy and unpleasant hydrogenated odors when used in frying (Warner and Mounts, 1993). The *trans* fatty acids produced from the partial hydrogenation of vegetable oils are also believed to produce ill health effects. Research (Zock and Katan, 1992) has shown that elaidic acid (*trans*-C18:1[n-9]) produced from the partial hydrogenation of linoleic acid decreased high density lipoproteins (HDL) and increased low density lipoproteins (LDL) in healthy male subjects when consumed as 3.8% of a normal diet. Regardless, partially hydrogenated vegetable oils are still one of the most widely used frying oils (Frankel and Huang, 1994).

D. 2. Genetic Engineering of Vegetable Oils

Currently a variety of genetic applications are being used for the modification of seed oils at the biological level. Research (Somerville, 1993) has shown that the core of genetic technology rests upon the expression or nonexpression of proteins and polypeptides. In many cases, it is the deactivation of a particular enzyme that results in phenotypic changes in the plant. The synthesis of selected fatty acids such as oleic acid by the incorporation of an antisense gene would be an example of a

phenotyic change. Kinney (1994) found that the introduction of an antisense mRNA blocking the synthesis of the omega-3-desaturase enzyme into soybean species successfully decreased the linoleic acid content of the seed oil. By using the omega-6-desaturase antisense gene, Kinney (1994) was able to produce a high oleic acid soybean oil. High stearic acid varieties of soybean oil were also produced using a delta-9-desaturase antisense gene.

The general mechanism for the introduction of an antisense gene and enzymatic pathways found in seed oil bearing species have been researched (Kinney, 1994; Knutzon, et al., 1992; Tonnet and Green, 1986; Somerville, 1993; Bier, 1993). The general mechanism for incorporation of an antisense gene into the seed germ involves the use of a bacterial vector. *Agrobacterium tumefaciens* are commonly cloned in a suitable E. *coli* strain. The disarmed T-DNA is introduced into the host cell of the seed germ through viral transposon mutagenesis, via the double recombination mechanism. The T-DNA normally contains phytohormones that produce cancerous cells in the infected plant. Therefore, disarming is a necessary and important step. The result is the introduction of an antisense mRNA that forms a hybrid double-stranded segment with the mRNA coding for the particular target enzyme. Once this hybrid is formed, subsequent delivery of amino acids by the tRNA forming the enzyme is halted. In this case the enzyme is not expressed, resulting in a change of the observed phenotype (Knutzon, et al., 1992).

The general enzymatic pathways in seed oils begin with the linkage of the stearoyl group with an acyl carrier protein (Kinney, 1994). The first desaturation occurs in the plastid where the delta-9-desaturase introduces the first double bond. Subsequent desaturation occurs in the cytosol after the acyl-ACP thioesterase releases the 18:1-CoA. Omega-6-desaturase introduces the second double bond and omega-3-desaturase introduces the third double bond. The resulting fatty acids are then incorporated into the phospholipids, digylcerides, and triglycerides. The degree of desaturation for the individual fatty acids will depend on the activation or deactivation of the given enzymatic pathways. Figure 14. illustrates the generalized enzymatic pathways of developing seed oil. The requirements for polyunsaturated triglycerides in the plant seed is largely regulated by climatic control (Wilcox and Cavins, 1992; McCormik, et al., 1992).

Therefore, genetic modification may not be appropriate for seed crops in cooler climates. However, the overall result of using genetic engineering in seed oils is the production of triglycerides of known fatty acid composition without the occurrence of *trans* fatty acids experienced in vegetable oil hydrogenation (Kinney, 1994; Somerville, 1993; Frankel, et al., 1985). The genetically engineered vegetable oils, particularly high oleic sunflower oils, have been well documented as thermally stable vegetable oils (Dobarganes, et al., 1993; Frankel and Huang, 1994).

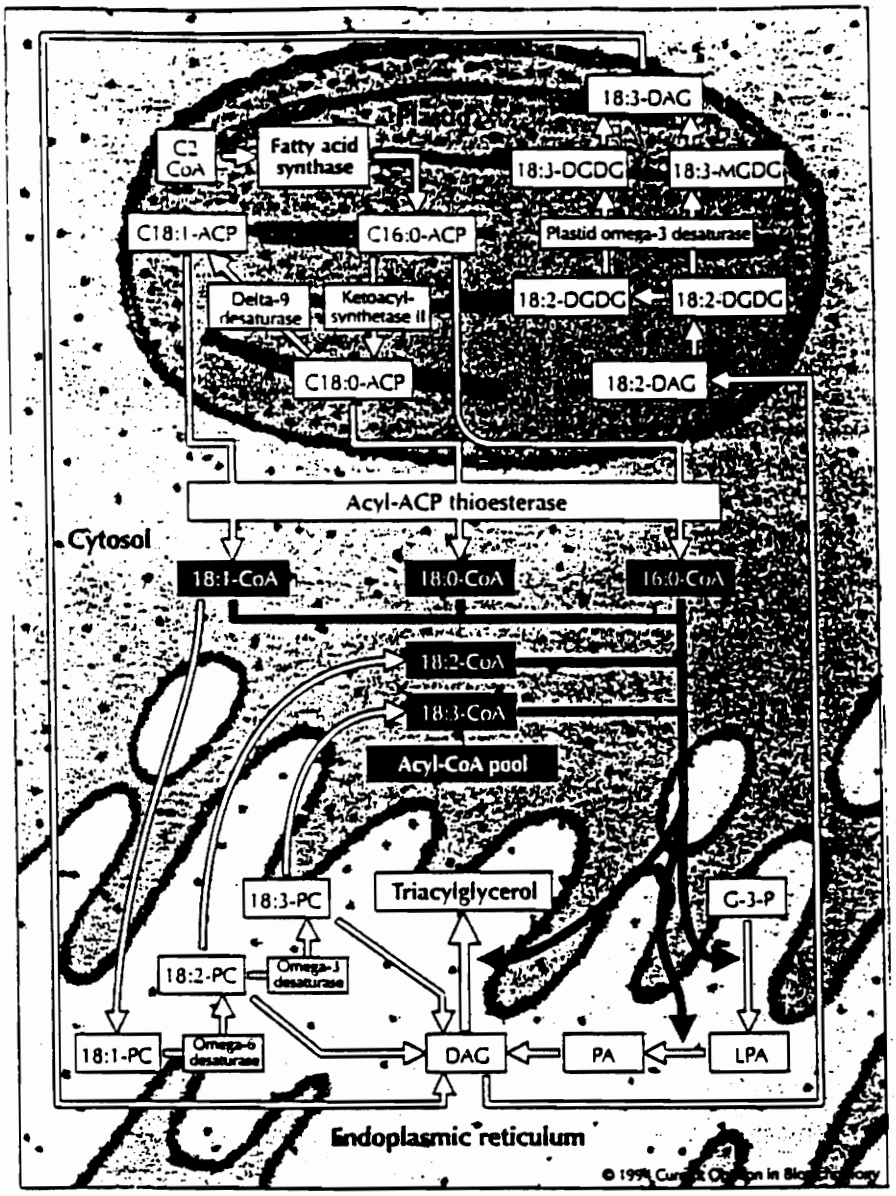


Figure 14. Enzymatic pathways for the synthesis of triglycerides in developing seed oil. (Kinney, 1994)

D. 3. Interesterification and Vegetable Oil Blending

Interesterification of vegetable oils is a process whereby the fatty acids present on the triglycerides are interchanged to produce new triglyceride species (Neff, et al., 1994b). The process is generally carried out using sodium methoxide in the presence of heat, followed by quenching with aqueous citric acid (Neff, et al., 1994b; Raghuveer and Hammond, 1966). The result is a randomized mixture of triglycerides with fatty acids present at different *s n* positions, which normally do not occur during biosynthesis. The goal of interesterification is to produce vegetable oils that are more resistant to autoxidation based on the altering of the *sn* positioning of the fatty acids on the triglyceride (Neff, et al., 1994b; Raghuveer and Hammond 1966; Myashita, et al., 1990).

Vegetable oil blending is the process of blending two or more vegetable oils to obtain a hybrid vegetable oil of specific triglyceride composition (Frankel and Huang, 1994; Neff et al., 1994b). The blending process simply involves mixing the vegetable oils of interest at given concentrations to obtain the desired blend composition. Vegetable oil blending is the simplest of the methods that have been previously reviewed. Vegetable oil blends can be obtained from a variety of vegetable oils including, interesterified blends (Neff, et al., 1994b), hydrogenated blends (Warner and Mounts, 1993), high oleic acid blends (Frankel and Huang, 1994), and low erucic acid rapeseed oil blends (Warner, et al., 1994). Many other

possibilities exist, using virtually any type of fat or vegetable oil imaginable. However, the goal of vegetable oil blending is to produce blends that have specific flavor and aroma characteristics, that have reduced levels of *trans* fatty acids, show an increase in thermal oxidative stability, and are easy to obtain.

E. Current Research Using Modified Vegetable Oils

E. 1. Hydrogenated Vegetable Oil Blends

Current research (Warner and Mounts, 1993; Neff and Selke, 1993) has shown that vegetable oil blends using hydrogenated soybean or canola oil produce off flavors in the vegetable oils and fried foods. These off flavors were characterized as having a painty, fishy, or waxy odor. When high oleic varieties of soybean or canola oils were compared against the hydrogenated blends, there were no significant differences noted in the rates of autoxidation. However, both blend varieties were found to have a greater thermal stability when compared to polyunsaturated vegetable oils. The off flavors produced in the hydrogenated blends were not present in the high oleic blends. These findings indicated that high oleic vegetable oil blends may be more suitable than the hydrogenated vegetable oils, due to the absence of undesirable waxy aromas and flavors.

E. 2. Interesterified Vegetable Oils and Vegetable Oil Blends.

Research (Neff, et al., 1994b) has shown that interesterified blends are not necessarily more stable to thermal oxidation when compared to standard vegetable oil blends. The researchers compared interesterified soybean and palm olein blends with standard palm olein and soybean oil blends. The soybean oil contained a high percentage of linoleic acid (52%) and linolenic acid (7%) while the palm oil contained a high percentage of palmitic acid (40%) and oleic acid (42%). A variety of interesterified and standard blend ratios were established and the thermal oxidative stability was determined. A marked decrease in hexanal production was experienced in both blend types when compared to the soybean oil. However, the standard blends experienced a greater thermal stability when compared to the interesterified blends. These findings suggested that randomization of the fatty acids were not conducive to producing thermally stable vegetable oil blends. The researchers also concluded that the interesterification led to a decrease in trilinolein, which would tend to improve oxidative stability. However, a decrease in palmityldiolein and dipalmitylolein were also experienced, which would tend to decrease the oxidative stability.

Frankel and Huang (1994) tested the thermal oxidative stability of high oleic acid and polyunsaturated vegetable oil blends. High

oleic acid sunflower oil was mixed independently with soybean oil and with canola oil to produce vegetable oil blends containing between 4.5% and 1.0% linolenic acid. A standard blend of hydrogenated soybean oil and high oleic sunflower oil was also established. The soybean/sunflower blends were found to have the greatest thermal stability when compared to the hydrogenated soybean/sunflower blends. Similar results were reported for the canola/sunflower oil blends when compared to the hydrogenated canola/sunflower oil blends. Each of the vegetable oil blends, including the hydrogenated blends, were superior to the polyunsaturated vegetable oils, which experienced thermal oxidation at a lower level. The vegetable oil blends consisting of 1 to 2% linolenic acid experienced the greatest thermal oxidative stability. The results of the study clearly illustrated that vegetable oil blends consisting of high oleic sunflower oil performed greater in thermal oxidation test when compared to polyunsaturated vegetable oils and hydrogenated vegetable oils. However, a comparison of the high oleic sunflower oil to the other blends would have clearly indicated to what degree the thermal oxidative stability was improved.

E. 3. Thermal Stability of Genetically Modified Vegetable Oils.

In a study conducted by Dobarganes, et al. (1993) genetically modified sunflower oil was compared to conventional sunflower and

olive oil. Three varieties of genetically modified sunflower oils were used with triolein contents ranging from 40%, 55%, and 61%. The conventional sunflower oil contained 4.6% triolein and 27% trilinolein, while the olive oil contained 44% triolein and less than 1% of trilinolein. Each of the vegetable oils were heated for 5 and 10 hours at 180°C. The conventional sunflower oil experienced the greatest total polar component increase when compared to the other vegetable oil varieties. The general trend was a marked decrease in polar component production as the percentage of triolein increased. The olive oil performed similarly to the genetically modified sunflower oil containing 55% triolein. The reduced levels of trilinolein found in the olive oil apparently had an impact on increasing the oxidative stability. The overall results of this study indicated that an increase in the levels of oleic acid, led to an increase in the thermal stability of the vegetable oils.

The results from the cited studies indicate that vegetable oils with increased levels of oleic acid tend to perform better in thermal oxidative studies. The increased stability of high oleic acid vegetable oils also appear to be superior to hydrogenated vegetable oils without the waxy or painty aromas. Therefore, research has indicated that a polyunsaturated vegetable oil can be improved by standard blending with high oleic vegetable oils in a manner consisted with that of Frankel and Huang (1994).

CHAPTER III.

Methods and Procedures

F. Experimental Design

The thermal oxidative stability was evaluated in quadruplicate for seven different vegetable oil blends consisting of high oleic sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB). Vegetable oil blends were heated at simulated frying temperatures ranging from 178 to 182°C, without the presence of food particulates. The heating cycle consisted of 5 hours per day for a total of 10 days. Each of the vegetable oil blends were tested in a predetermined randomized order. The change in fatty acid composition, triglyceride composition, peroxide value, and volatile production were used to indicate the relative thermal oxidative stability and to chart trends during the process of heating and cooling. Each of the vegetable oil blends were then rated using the thermal breakdown ranking (TBR) and relative stability of the fatty acids and the triglycerides: triolein and trilinolein.

F. 1. Vegetable Oil Blending and Blend Profile

Seven vegetable oil blends (Table 4.) were established using high oleic sunflower oil *Helianthus annuus L.* (Trisun^{R} 80) obtained from SVO Specialty Products, Inc. (Eastlake OH, 44095, USA [216] 942-0680) and polyunsaturated soybean oil *Glycina max* (Crisco

Vegetable Oil, Proctor and Gamble, Cincinnati, OH) obtained from Kroger Co. (Blacksburg VA, 24060, USA [540] 951-3045). A modification of the vegetable oil blending used by Frankel and Huang (1994) was employed. The modification simply involved the preparation of a greater number of vegetable oil blends at a total volume of 500 ml, representing a wider range of polyunsaturation. In addition, 100% high oleic sunflower and 100% polyunsaturated soybean oil were included in the study.

The vegetable oil blends were prepared immediately after receiving the vegetable oil stocks. The appropriate volume of each vegetable oil was volumetrically delivered to a graduated flask. The blend was carefully swirled and poured into a prelabled storage container. There were four different containers for each of the seven vegetable oil blends. The storage container was then flushed with nitrogen gas, sealed, and placed in a nontranslucent box in the freezer at a mean temperature of minus 40°F. The stored blends were not disrupted nor exposed to light or increased temperatures until the day of their initial testing. The testing order was determined by placing the blend number with an assigned letter from a to d (representing the repetition) on a 1 inch square piece of paper in a closed sack. The slips of paper were drawn out one at a time and recorded in a laboratory journal. Table 4. represents the concentration of each blend and the blend number assignment.

Two hundred and fifty milliliters were simultaneously

Table 4. Blend number assignment and concentration levels of high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends.

Blend & Number	%HOSO	%PUSB	Ratio
1 a,b,c,d	100	0	100:0
2 a,b,c,d	90	10	9:1
3 a,b,c,d	75	25	3:1
4 a,b,c,d	50	50	1:1
5 a,b,c,d	25	75	1:3
6 a,b,c,d	10	90	1:9
7 a,b,c,d	0	100	0:100

Notes:

1. The letters a,b,c, and d represent the number of trial per blend.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

established during the blending process for the development of the blend profile. The characteristic fatty acid composition, triglyceride composition, and characteristic peroxide values were determined by performing each test in triplicate. The peroxide values were performed in triplicate for each sample tested, representing a total of nine peroxide values for each blend.

F. 2. Simulated Frying: Thermal Oxidation of the Vegetable Oil Blends.

F. 2. a. Description of the Thermal Oxidation System.

The simulated frying system was adapted from the methods used by Wu and Chen (1992) and Chang, et al. (1977). Five hundred milliliters of the vegetable oil or vegetable oil blend were poured into a one liter triple neck Pyrex flask. The flask was supported by a stationary clamp, seated directly into a heating mantle (Electromantle Model) purchased from Fisher Scientific (Fair Lawn, NJ). The first port was fitted with a 250°C capacity thermometer. The second port was fitted with a Pyrex gas washing bottle containing a 100 mg tenax-GR (2,6-diphenyl-p-phenylene oxide polymer) chamber (Alltech, Deerfield, IL) supported by clean glass wool on the effluent end. The third port was fitted with a reflux line leading directly into the influx of the gas washing bottle, which circulated back through the vegetable oil. In this way, the system

was closed during the heating process, with the exception of the effluent end of the gas washing bottle, which contained an internal diameter of less than 1 mm. The thermal oxidation system was designed in this manner to prevent outside contamination room air or moisture, to retain the volatiles within the vegetable oil and headspace, and to prevent pressure build up within the flask. The thermal oxidation system is represented in Figure 15., in which there were two identical stations available.

F. 2. b. Simulated Frying: Closed System

The vegetable oil sample was brought up to a temperature of 180°C ± 2°C from 28°C for a 5 hour period at an average rate of 1.60°C/minute. The temperature increment was conducted at a moderate rate to avoid super heating the vegetable oil, and to avoid rupturing the flask. This process was repeated for 3 consecutive days, for a total of 15 hours of heating in a closed system. The vegetable oil sample was allowed to remain without heating for one full day in the closed system before the secondary heating phase. This step was taken to monitor changes resulting from a cooling period of 48 hours, in effect, to evaluate if the vegetable oil continued to breakdown without continued heating.

Thirty five milliliters of vegetable oil sample were removed from

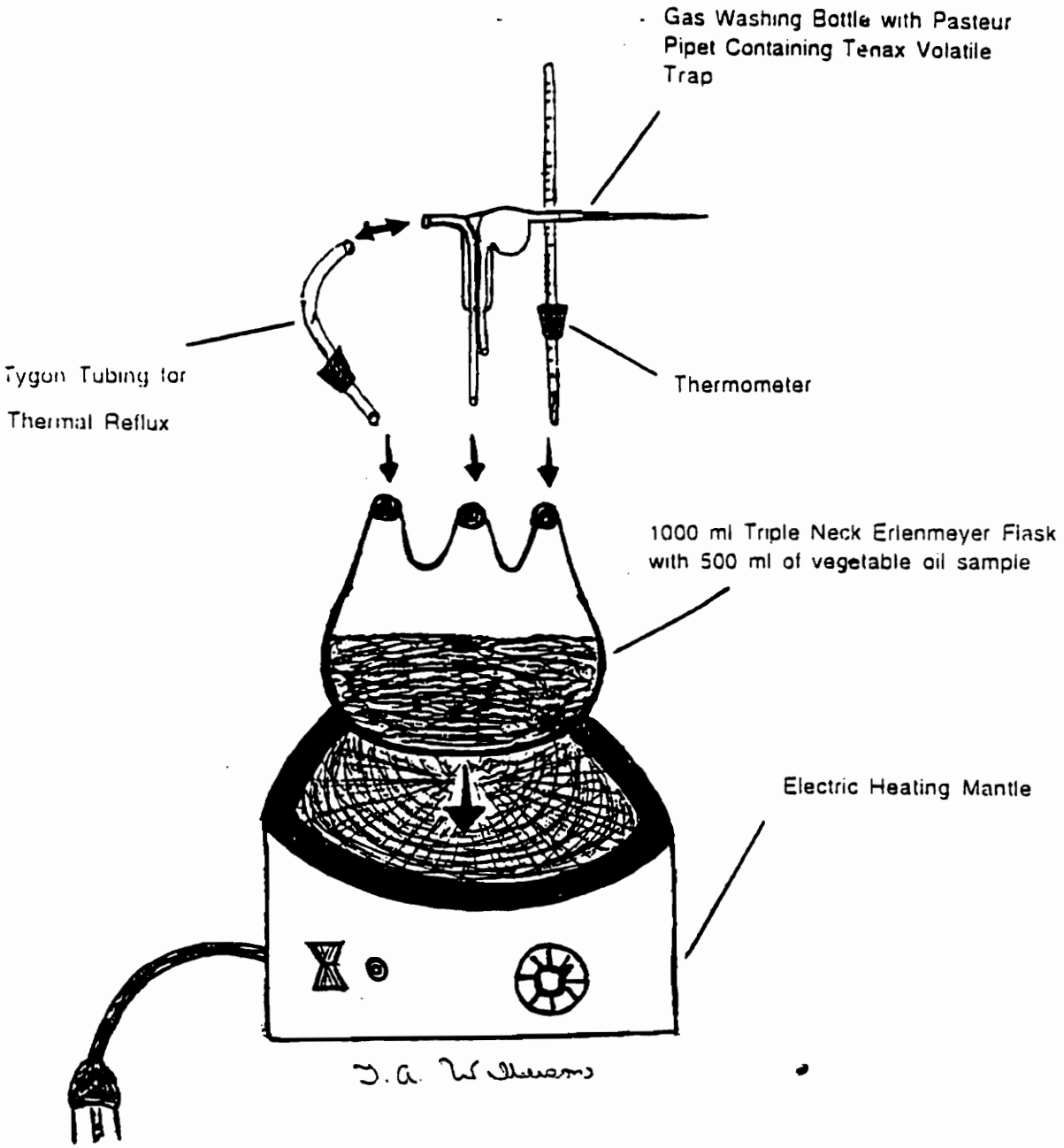


Figure 15. Closed thermal oxidation system for the testing of high oleic acid sunflower and soybean oil blends.

the third port and transferred to a 50 ml graduated cylinder. The sample was then delivered to a prelabled 50 ml vial, flushed with nitrogen gas, sealed and placed in the freezer, prior to testing. The 35 ml of sample were distributed as follows: 25 ml were reserved for volatile analysis, 9 ml for peroxide test, 0.5 ml for triglyceride analysis, and 0.5 ml for fatty acid analysis. Samples were taken from the vegetable oil as follows: before intitiation of heating, designated as time zero, and at the end of each heating period, designated as time 300 minutes. The heating periods were distinguished by the total hours of use and the actual time of heating at 180°C, shown in Table 5.

The peroxide value and triglyceride analyses were conducted on all samples. The volatile analysis was conducted on the fresh samples and on samples after the 300 minute heating period. Samples were allowed to reach a temperature of 28°C for a total of four volatile analyses per vegetable oil blend. The fatty acid analysis was conducted only on the fresh samples prior to heating in the closed system. The headspace volatiles collected in the effluent tenax-GC trap were analyzed on the fourth day.

F. 2. c. Simulated Frying: Open System

The secondary heating involved placing the 255 ml of remaining

Table 5. Identification of high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blend minutes of heating and hours of total usage.

Day & Minutes of Heating (180°C)	Total hours of use & minutes of Heating (180°C)
D1 0	0:0
D1 300	5:300
D2 0	24:300
D2 300	29:600
D3 0	48:600
D3 300	53:900
D4 0	72:900
D11 2100	264:3000

vegetable oil sample in a 500 ml Pyrex beaker and heating at 180°C ± 5°C for 5 hours per day for seven consecutive days. The secondary heating phase was employed to increase the surface air exposure to the vegetable oil during the heating process. The emphasis during the open heating period was to monitor the change in fatty acid and triglyceride composition, and peroxide values. Thirty five milliliters of the vegetable oil sample were removed from the beaker after the seventh day, 25 ml were reserved for volatile analysis, 9 ml for peroxide test, 0.5 ml for triglyceride analysis, and 0.5 ml for fatty acid analysis.

F. 3. Analytical and Chemical Testing.

F. 3. a. Peroxide Value (PV)

The AOCS Official Method Cd 8-53 **Peroxide Value: Acid-Chloroform Method** (AOCS, 1993) was used to determine the peroxide values for all samples tested. Twenty five milliliters of HPLC-grade acetic acid:chloroform [3:2] were added to approximately 3.0 grams of vegetable oil sample in a 250 ml Erlenmeyer flask. Five hundred microliters of freshly prepared saturated potassium iodide (Fisher Scientific, Fair Lawn NJ) were added to the solution and carefully swirled. The sample was placed in the dark for approximately 2 minutes, followed by the addition of 50 ml of

deionized water. A clean magnetic stir bar was placed in the solution, and allowed to moderately stir the mixture. Several drops of starch solution were added and a micro-scale digital burette (Polyscience Model CB, Fisher Scientific, Fair Lawn NJ) was used to titrate the mixture with fresh 0.1 *M* sodium thiosulfate.

The samples were titrated dropwise, about one drop per three seconds, until the disappearance of the purple coloration. The end titration volume was recorded after the solution remained clear for one minute. A reference blank containing no sample was established for each series of vegetable oil samples tested. In addition, rancid peanut oil was used as an indication that the procedure was detecting the presence of peroxides in the samples. The rancid peanut oil was known to have an average peroxide value of approximately two hundred. The rancid peanut oil was analyzed prior to the testing of the vegetable oil samples. Each sample was tested in triplicate and the peroxide values were recorded as the mean peroxide value. The determination of the peroxide value is represented by the following equation:

$$PV\ (u\text{eqv. } O_2\text{/g of vegetable oil}) = \frac{(S-B)(N)(1000)}{\text{Wt. (g)}}$$

Where,

S = Ml of Oil Sample B = Ml of Blank

Wt.= Weight in grams of Oil Sample N = Normality of NaS_2O_3

F. 3. b. Fatty Acid Analysis: Fatty Acid Methyl Esters (FAMES) by Gas Chromatography (GC)

The fatty acid composition for each vegetable oil and blend was determined in triplicate by using the fatty acid methyl ester (FAME) procedure described by Liu (1994). Approximately 50.0 mg of vegetable oil sample were weighed out in a 10 ml Pyrex test tube. One milliliter of an internal standard solution containing 5 mg of heptadecanoic acid (C17) (Sigma Chemical Co., MO) in HPLC-grade chloroform (Fisher Scientific, Fair Lawn, NJ) was added. Two milliliters of HPLC-grade toluene (Fisher Scientific, Fair Lawn, NJ) and 2 ml of methanolinic base (0.5*N* Supelco 3-3080, Bellefonte, PA) were then added. The samples were sealed, vortexed and then heated at 75°C for 15 minutes followed by a 15 minute cooling period. The mixture was poured into a 60 ml separatory funnel with the addition of 6 ml of deionized water followed by the addition of 6 ml of HPLC-grade petroleum ether (Fisher Scientific, Fair Lawn, NJ). The bottom layer was removed after separation and poured through a sodium sulfate drying tube into a 10 ml vial, to remove residual moisture. The remaining solution was prepared for gas chromatography (GC) by concentrating to 4 ml under a steady stream of nitrogen gas.

Approximately 100 *u*l of sample were delivered to a GC vial and

placed in the autoinjector holding tray, with an injection volume of 1.0 *ul*. A Shimadzu GC14A model (Shimadzu, Columbia, MD) equipped with an AOC 14 autoinjector and a chromatopac C-R4AX processor with flame ionization detection (FID) was used for GC analysis. The injector temperature was set at 220°C and the detector temperature at 240°C. The helium carrier gas was approximately 1.0 ml/min, with a make up gas of hydrogen/air at a ratio of 3:30 and a flow rate of 50.0 ml/min. The split ratio was set at 1:20 (20 ml/min) and the attenuation (sensitivity) was set at 3. A SP2330 (Supelco, Bellefonte, PA) capillary column , 30 meters by 0.32 millimeters internal diameter with a 0.20 *um* film thickness was used for FAME separation. The temperature programming consisted of a 5°C/minute temperature ramp from 180°C to 220°C with a 14 minute hold at the final column temperature. The column was conditioned for 30 minutes prior to each initial run by heating at 230°C.

Fatty acid concentrations were determined on a weight % bases, using the internal standard heptadecanoic acid (C17) as a measure to check the percent recovery. The individual fatty acids (FAMES) were identified using a Sigma reference oil standard AOCS 0-7756 (Sigma Chemical Co., St. Louis, MO) see Appendix F (Figure F. 1.). The reference standard was placed in the first and every fifth position of the autoinjector tray, immediately following a solvent blank. In this manner, a shift in retention times, if experienced, could be accounted for by comparing the differences in the reference chromatograms. The solvent blanks were used to insure that

resolved peaks were not a result of column overload or contamination.

F. 3. c. Triglyceride Analysis: Reverse-phase High Performance Liquid Chromatography (HPLC).

The individual triglyceride content of the vegetable oil blends were determined using the AOCS Official Method Ce 5c-93: **Individual Triglycerides in Oils and Fats by HPLC**,(AOCS, 1993) and the methods described by Palmer and Palmer (1989). The reverse-phase isocratic HPLC system was an Automated Baseline 815, with Baseline Software and a NEC Waters Computer System with a Votex ELSD II A mass detector (Burtonsville, MD). A 250 mm by 4.6 mm and 5*u* ODS ultracarb HPLC column with a 30 mm by 4.6 mm guard column was used for the separation of triglycerides (Phenomenex Inc., Los Angeles CA).

Approximately 85.0 mg of vegetable oil sample were dissolved in 10.0 ml of HPLC-grade acetone (Fisher Scientific, Fair Lawn, NJ), capped and vortexed. Approximately 100.0 *u*l were delivered to a HPLC vial and placed in the autoinjector tray, with a 20 *u*l injection volume. The HPLC running conditions consisted of a 1.2 ml/min flow rate in an acetone:acetonitrile [3:1] mobile phase for a total running time of 30 minutes. The column temperature was maintained at 22°C with a back pressure of 1300 psi $\pm$ 200 psi, with

a 2000 psi automatic shut off. The following conditions were used for the mass detector: tube temperature was set at 110°C and the exhaust temperature at 65°C. Nitrogen at 20 psi or 49 mm was used as the nebulizing gas. A wave length of 250 nm was used for detection of the individual triglycerides.

A reference standard consisting of a mixture of individual triglycerides and monoglycerides was used to identify the major triglycerides in the vegetable oil blends and to monitor retention times see Appendix G (Figure G. 1.). The individual triglycerides consisted of triolein (OOO), trilinolein (LLL), trilinolenin (LnLnLn), tristearin (SSS), and tripalmitin (PPP), and the monoglycerides were present as a mixture of racemic 1-mono-oleolyglycerol (Sigma Chemical Co., St. Louis, MO) see Appendix J (Figure J. 1.). The majority of the reference standards consisted of OOO, LLL, LnLnLn, and *r*-1-mono-oleolyglycerol. The exclusion of SSS and PPP was based on previous pilot studies, where these two triglycerides were not present in any of the vegetable oils or blends tested.

The triglycerides in which there were no individual standards were tentatively identified using reference chromatograms of high oleic sunflower and soybean oil (Caboni, et al., 1992) and soybean oil (Neff, et al., 1992), see Appendix B (Figure B. 4. A and B). In addition, the equivalent carbon number (ECN) from AOCS Official Method Ce 5b-89: **Triglycerides in Vegetable Oils by HPLC** (AOCS, 1989) and the FAME comparison method from AOCS Official

Method Ce 5c-93: **Individual Triglycerides in Oils and Fats by HPLC** (AOCS, 1993) were used for additional support in identifying the individual triglycerides. A detailed explanation of the ECN and FAME comparison method is presented in (Chapter II. section A. 3.).

Triolein and trilinolein were the two major triglycerides quantified using a linear regression model based on the individual reference standards at concentrations ranging from 5.0 mg to 100 mg (see Appendix C). These triglycerides were selected due to the availability of individual reference standards and their relatively high percentages in each of the major vegetable oils. Triolein represents roughly 83.0 % of high oleic sunflower oil, and 1.00 % of polyunsaturated soybean oil. Trilinolein represents roughly 31.00 % of polyunsaturated soybean oil and 1.00 % of high oleic sunflower oil. The triolein and trilinolein were positively identified by spiking the vegetable oil samples and then by matching the resulting chromatograms with established reference chromatograms.

The remaining triglycerides peaks were evaluated collectively in groups, based on the number of double bonds present. Each of the triglyceride peaks were characterized by their double bond range. The double bond ranges were 9 to 6, 5 to 3, 3 to 1, and a group for monoglycerides and diglycerides collectively see Appendix B (Figure B. 1.). The double bond ranges were determined using the reference chromatograms (Caboni, et al., 1992; Neff, et al., 1992) and the ECN (AOCS Official Method Ce5b-89) and available reference standards.

All of the available individual reference standards were plotted against the reference chromatograms, and the ranges of double bonds occurring between any two individual reference standards were determined.

All of the possible triglycerides were determined using the fatty acid composition obtained from the GC/FAME analysis (see Appendix F). Triglycerides present in the reference chromatograms which had fatty acid compositions different from the fatty acid profile were eliminated. The remaining triglyceride possibilities were then assigned to their respective double bond range. The triglycerides with the best percentage match up with the fatty acid composition (FAME) and reference chromatograms were recorded as the tentative triglycerides.

F. 3. d. Volatile Preparation: Purge and Trap followed by Solvent Extraction.

The volatiles produced in the vegetable oils and blends during the process of thermal oxidation were isolated from three different sources. The primary source was the vegetable oil in the closed system of the simulated frying. The two secondary sources were obtained from the open system and the headspace of the closed system. Each of the volatile samples were obtained from a purge and trap technique followed by solvent extraction as described by

Olafsdottir, et al., (1985). However, the volatiles isolated from the headspace of the closed system were not purged with nitrogen gas, only by the vapor pressure which drove the volatiles onto the tenax-GR trap as a result of the heating process. This procedure was used to simply estimate the relative loss of the volatiles from the vegetable oil during the heating process in the closed system.

Twenty five milliliters of sample were removed from the vegetable oil or blend after reaching a temperature of 27°C and delivered to a 50 ml Pyrex gas washing bottle. For fresh vegetable oils and blends, the 25 ml sample was taken directly. Nitrogen gas at a flow rate of 100 ml/min was purged through the sample in the dark which passed through a tenax-GR (2,6-diphenyl-p-phenylene oxide polymer) chamber (Alltech, Deerfield, IL) for a 12 hour period. The tenax-GR was supported by clean, silane-treated glass wool (Alltech, Deerfield, IL), which was established in a 6 inch Pasteur pipet (Fisher Scientific, Fair Lawn, NJ) secured to the gas washing bottle by a 1 inch piece of unused tygon tubing. Tygon tubing was also used to deliver the nitrogen gas to the gas washing bottle.

Each of the samples contained a 12.5 ppm *trans*-2-decenal internal standard (Bedoukian, Danbury, CT) in HPLC-grade petroleum ether (Fisher Scientific, Fair Lawn, NJ) which was delivered prior to the purging process. The internal standard was used to determine the purge efficiency which was compared to a standard curve using the internal standard at three different concentration. The 3

concentrations for the standard curve were 12.5, 25.0, and 50.0 ppm, each being tested in triplicate (see Appendix C).

F. 3. e. The Concentration Percent Recovery for trans-2-Decenal in High Oleic Acid Sunflower and Soybean Oil Blends.

The *trans*-2-decenal content was estimated in parts per million (ppm) for each of the volatile purging periods for every vegetable oil blend evaluated in this study using linear regression statistics. Concentrations of 12.5, 25.0, and 50.0 ppm were used to construct the standard curve, with each sample being tested in triplicate. After establishing the standard curve the *trans*-2-decenal concentration was estimated for each of the vegetable oil blends tested. The ppm value was multiplied by the extract volume to obtain the estimated quantity, in nanograms. The nanogram value was then divided by 312,500 ng and multiplied by 100 to determine the % recovery of the internal standard. The value 312,500 ng represents the quantity of trans-2-decenal spiked into the 25 ml volume of vegetable oil, which had a concentration of 12.5 ppm relative to the 25 ml vegetable oil sample. The linear regression equations are represented as follows:

1. $X\text{ppm} = \frac{Y\text{ ng}}{Z\ u\text{l}}$

Where,

X = The ppm value obtained from the standard curve.

Z = The known extract volume of the volatile samples.

X = The estimated quantity in nanograms.

2. % Recovery = $\frac{\text{Y ng}}{\text{312,500 ng}}$ (100)

Where,

312,500 ng is equivalent to 312.50 *u*g spiked into 25.0 ml of vegetable oil, which = 12.5 ppm.

The above equations represent the estimate concentration of the internal standard relative to the extract volume. This value does not represent the estimated or relative concentration of the internal standard to the 25 ml of actual vegetable oil sample. To obtain the estimated concentration of the internal standard relative to the 25.0 ml of vegetable oil purged, the following equation can be employed:

3. Xppb = $\frac{\text{Y ng}}{\text{25.0 ml of vegetable oil}}$

Where,

ppb = Parts per billion

After the 12 hour purging period the tenax-GR chamber was removed and extracted twice with a 1 ml aliquot of HPLC-grade petroleum ether into a 2 ml nontranslucent vial. The solvent extract was evaporated down to approximately 100 *u*l under a steady stream of nitrogen gas consisting of a flow rate of 50 ml/min. The 100 *u*l of solvent extract were then carefully delivered to a pre-weighed HPLC insert using a mechanical pipet and further concentrated to a final volume of 3 to 6 *u*l. The HPLC insert was then weighed and immediately sealed with a teflon liner.

F. 3. f. Volatile Analysis: Gas Chromatography (GC)

GC analysis of the volatiles was conducted in accordance with AOCS Recommended Practice Cg 4-94: **Volatile Organic Compounds (VOC) in Fats and Oils by Gas Chromatography** (AOCS, 1994). Eight tenths of a microliter from the 3 to 6 *u*l extract solvent was manually injected into a Shimadzu GC 9A with flame ionization detection (FID) (Columbia, MD). A 60 m by 0.25 mm internal diameter with a 0.25 *u*m film thickness Supelcowax SP10 capillary column (Supelco, Bellefonte, PA) was used for volatile separation. The following temperature program was used: 50°C

initial temperature held for 1 minute, 2°C/minute up to 150°C and then 4°C/minute to 220°C held for 10 minutes. The injector and FID were set at 240°C and the carrier gas, helium, contained a head pressure of 1.8 Kg/cm^2 and a linear flow rate of 20 cm/sec.

The column was conditioned before each use by heating to 240°C and holding for 30 minutes. In addition, the glass insert found below the injector port was checked and cleaned on a regular basis and the septum was checked and replaced as needed. Petroleum ether solvent blanks were run on a regular basis between samples to check for solvent purity and possible column contamination. Reference standards consisting of *trans*-2-decenal, heptanal, hexenal, (Z)-2-heptenal, nonanal, (E,E)-2,4-heptadienal, and (E,Z)-2,4-decadienal (Bedoukian, Danbury, CT) were used to tentatively identify the major volatiles found in the vegetable oil and blend samples.

A standard curve consisting of 6.25, 12.5, and 25.0 ppm was constructed for each reference standard dissolved in petroleum ether, also consisting of a manual 0.8 *u*l injection volume. The standard curves proved to be ineffective due to the low purity of the individual standards. No similarities between the concentration of the individual standards and relative peak areas could be established. However, the base peaks produced in the reference chromatograms were sufficient for tentative identification with the peak areas found in the volatile samples, which were later confirmed by gas chromatography/mass spectrometry.

F. 3. g. Volatile Identification: Gas Chromatography with Mass Spectrometry (GC-MS).

Volatile extracts were prepared in the same manner as previously stated and were identified using a GC/MS (Hewlett Packard 5890, Series II Plus, Little Falls Site 4300, DE) system with a National Bureau of Standards mass spectra library (NBS) and a Mass Selective Detector, HP5972A MSD Hardware purchased from Hewlett Packard Co., Little Falls Site 4300, DE. Two columns were used in the analysis: (a) the Supelcowax SP10 capillary column was used for initial volatile analysis;and (b) a nonpolar HP5 column (Hewlett Packard Co., Little Falls Site 4300, DE) with a 30.0 m length and a 0.530 mm internal diameter and a 0.25 *um* film thickness. The HP5 column was used for secondary confirmation of the volatile reference standards and volatiles extracted from the vegetable oil samples. This measure was taken to insure that the GC/MS system was responding properly by giving the same NBS library match for a compound run on both columns.

The temperature programming was identical to the temperature programming used for the primary volatile analysis. The carrier gas, helium, was set at a flow rate of 1.0 ml/min at a linear velocity of 25.6 cm/sec. The injector temperature was set at 250°C and the injector purge valve at 0.75 seconds, following injection. The mass spectrometer's ion acceleration voltage was set at 1635.3 V, with

scan parameters of 26 for low mass, 350 for high mass, and a threshold of 500.

Confirmation of the individual reference standards was first obtained on both columns prior to the analysis of the volatile extracts. The impurities found in the individual standards were a result of volatile breakdown, isomerization, and cyclic dimerization in some cases, producing a variety of peaks for each standard based on the NBS library identifications. Volatile extracts obtained from high oleic sunflower oil, polyunsaturated soybean oil, and a 1:1 mixture heated for 3 days in the closed system were each analyzed in triplicate. A printout of the 3 best matches for each volatile peak and mass spectra was obtained. Only volatile identifications with an 80.0% or greater quality match with the NBS library were considered.

The major volatiles produced from the three fatty acids oleic, linoleic, and linolenic acid where compared by the peak area percentages, with the exclusion of the internal standard *t*-2-decenal after the percent recoveries were determined. The internal standard was factored out considering that it was not produced as a result of thermal oxidation. The major volatiles produced during thermal oxidation of the vegetable oils were hexanal, heptanal, (Z)-2-heptenal, nonanal, (E,E)-2,4-heptadienal, and (E,E)-2,4-decadienal, which were reported as the percent major volatile production (%MVP). Table 3. (Section C. 1. a.) listed the volatile breakdown

products common to each fatty acid.

F. 4. Statistical Analysis

F. 4. a. t-Test: Paired Two Sample for Means.

A t-Test: Paired two Sample for Means, using the Excell microsoft 5.0 computer software (Grey Matter International Inc., Cambridge MA) was performed on the blend profile and fresh vegetable blends prior to heating. Triolein (OOO), trilinolein (LLL), and the characteristic peroxide values (PV) were evaluated. The levels of significance were set at a p value of 0.01 for the analysis between the blend profile and the fresh vegetable oil blends prior to use. The comparison between the blend profile and fresh vegetable oil blends was used to note any differences that may have resulted from freezer storage. In this respect, changes resulting from freezer storage or the onset of autoxidation before the actual testing period could be taken into account.

F. 4. b. Simultaneous Confidence Intervals: Mean Separation

The differences occurring in the vegetable oils from the fresh samples and final samples were compared using the simulataneous

confidence intervals analysis (Lentner, 1986; Lentner, 1993). The level of significance was set at a p value of 0.01. The individual fatty acids, triolein, and trilinolein were evaluated. The SAS software (SAS Institute Inc., Cary, NC) was used for the mean separation and the blend:time interaction. The simultaneous confidence intervals were used to assess the relative thermal stability of each of the fatty acids, triolein, and trilinolein by indicating whether or not changes in the concentration levels occurred as a result of heating. The simultaneous confidence intervals were also used to indicate whether the mean averages for each component were similar or different before and after heating.

The simultaneous confidence intervals (SCI) are defined by the (k_i) value. When the mean average (Y_i) is greater than the K_i for two or more blends, these blends have mean averages which are larger than the mean averages of the other blends. A 2 Way ANOVA with a level of significance set at ($p = 0.01$) was used to indicate the level of significance occurring between and within the blends prior to calculating the SCI values. The SCI for the separation of the largest means can be calculated by hand as follows:

1. S_d = The square root of 2(MSE)/N
2. $K_i = M_i - S_d(D)$
3. ($Y_i > K_i$) = The mean average is greater than the mean averages for the other blends.

Where,

S_d = Standard deviation

MSE = Mean sum of squares

N = Number of trials

D = Degrees of freedom from the Dunett's Table (Lentner, 1993)

K_i = Simultaneous confidence intervals for largest means

M_i = The largest mean average for all samples

$_i$ = Sample

2 = Simultaneous confidence interval constant

Example: Oleic acid before heating for blend one (100% HOSO), where m_i = 77.56, Y_i = 83.47, D= 2.385, MSE= 1.5970, and N= 3.

1. S_d = The square root of 2(1.597) = 1.0645
2. 1.0645(D) = 2.5387
3. $K_i = M_i$ - 2.5387 = 75.00
4. (83.47 > 75.00), the largest mean

A trend analysis was constructed for all of the individual components, comparing the differences occurring between the fresh samples and samples heated at 180°C for 3000 minutes. A Paired t-Test: Assuming Equal Variance was used in addition to determine the level of signficance occurring within individual components between

the seven different vegetable oil blends. These values were then plotted using the mean averages and ranked according to their relative thermal satbility. The relative thermal stability ranking was determined by summing the total of positive and negative values. A positive value resulted from an increase in the component percentage after heating, where a negative value resulted from a decrease in the component percentage as a result of heating. The possible values ranged from a positive seven to a negative seven, where the seven represented the number of blends. The negative seven value was assigned a number value of one and the positive seven a number value of fourteen to create a ranking scale, ranging from one to fourteen. Zero was not assigned a number value, since it was not a possibility with an odd number of blends.

F. 4. c. Trend Analysis: During and After Heating.

A trend analysis with a 2 Way ANOVA was performed on the peroxide values and major volatile products using the Excell microsoft 5.0 computer software (Grey Matter International, Inc., Cambridge, MA). A comparison for each blend was conducted between the fresh samples and samples heated at 180°C for 3000 minutes, with a level of significance set at a p value of 0.01. A Paired t-Test: Assuming Equal Variance with a level of significance set at a p value of 0.01 was used to determine if trends having similar values were significantly different.

Trends occurring during the heating and cooling cycle for the peroxide values were plotted using the mean averages . Trends occurring in the production of the monoglyceirdes and diglycerides were also plotted using the mean averages obtained from the final heated samples.

F. 4. d. Standard Linear Regression Models.

A linear regression using the SAS software (SAS Institute Inc., Carry, NC) was used to construct a standard curve for triolein, trilinolein, and *trans*-2-decenal. The triolein and trilinolein standard curves (Appendix C) were used to determine the linear response of the HPLC mass detector and to determine the approximate levels of these components occurring in the vegetable oil blends. The *trans*-2-decenal standard curve (Appendix C) was used to compare the percent recoveries and the purge efficiency of the volatile extractions.

CHAPTER IV.

Results and Discussion

G. 1. Comparison of the Blend Profile and the Fresh Vegetable Oil Blends Prior to Heating.

The characteristic peroxide values (PV), trilinolein (LLL), and triolein (OOO) contents were compared between the blend profile and each vegetable oil blend prior to heating . No significant differences were observed ($p > 0.01$) for any of the three components (see Table 6.). Based on these results, there was no evidence indicating the onset of autoxidation prior to the heating of each of the vegetable oil blends.

The fatty acid composition of the vegetable oil blends consisted primarily of oleic, linoleic, palmitic, stearic, and linolenic acid. Traces (less than 1.0%) of eicosanoic acid (C20:0) and docosanoic acid (C22:0) were present in a few of the high oleic acid sunflower oil samples, primarily the 90.0% and 100.0% blend levels.. However, the majority of the fatty acid methyl ester (FAME) gas chromatograms consisted of the five primary fatty acid listed above. The average fatty acid content for each vegetable oil is presented in Table 7. and representative chromatograms for each blend are located in (Appendix F).

Table 6. Blend profile and fresh starting vegetable oil blend comparison of the peroxide values (PV), % triolein (OOO), and % trilinolein (LLL).

	(PV)		(OOO)		(LLL)	
Blend	I.	II.	I.	II.	I.	II.
1	0.91	0.84	83.19	83.68	1.24	1.21
2	0.97	1.09	80.14	80.40	2.40	2.56
3	1.11	1.26	70.85	70.85	6.05	6.13
4	1.31	1.57	53.52	50.11	12.22	14.29
5	0.70	0.93	16.94	18.50	24.70	25.95
6	0.72	1.36	10.75	8.84	27.59	28.94
7	0.70	0.61	1.10	1.47	30.85	29.69
p value:	(0.4672)		(0.2769)		(0.2236)	

Notes:
1. I. = Blend profile vegetable oil blend samples.
2. II. = Fresh starting vegetable oil blend samples.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Table 7. Average fatty acid content for high oleic acid sunflower and soybean oil blends.

Blend	C16:0 %	C18:0 %	C18:1 %	C18:2 %	C18:3 %
1	2.88	4.00	82.84	9.78	0
2	3.46	4.02	76.70	14.27	0.73
3	4.28	4.06	68.08	20.87	1.69
4	5.92	4.12	56.18	30.43	3.07
5	8.48	4.30	33.52	47.56	5.77
6	9.06	4.30	28.90	51.52	6.29
7	9.49	4.30	23.51	55.97	6.98

Notes:

1. C16:0 = Palmitic acid
2. C18:0 = Stearic acid
3. C18:1 = Oleic acid
4. C18:2 = Linoleic acid
5. C18:3 = Linolenic acid

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

G. 1. a. Comparison of the Blend Profile Triolein and Trilinolein Content with the Standard Curves.

The standard curves for triolein and trilinolein were based on reference standards ranging from 5.0 to 200.0 mg/10 ml acetone, with the majority falling between 5.0 and 100.0 mg/10 ml acetone (Appendix C, Tables C. 6. and C. 7.). The peak areas of each triglyceride were plotted against the concentration using a linear regression model. Triolein experienced a multiple R value of (0.9995) and trilinolein (0.9996).

After the linear regression curve was established the blend profile triglycerides, triolein (OOO) and trilinolein (LLL) were plotted and the average percentages in each vegetable oil blend were determined for both triglycerides. The average percent correlation for OOO to the standard curve was 96.0 % or greater, and was 100.0% for LLL. In each case, the peak areas obtained from the blend profile chromatograms were plotted on the OOO and LLL standard curves to obtain the percentage of each component (Appendix C, Tables C. 7. and C. 8.). The value obtained was then divided by the respective vegetable oil weight (in milligrams) and compared to the peak area percent of the HPLC triglyceride chromatogram. The final percent values with a $\pm$ 4.0% deviation indicated that the HPLC mass detector responded similarly to the triolein and trilinolein

triglycerides in both the individual standards and in the vegetable oil blends. On the average the OOO percentages were slightly less and the LLL percentages were slightly greater for the comparison of the blend profile HPLC chromatograms to the standard curves. The detector response was linear for the maximum concentrations experienced in each vegetable oil blend. Triolein occurred at a maximum level of 84.0 mg per 100.0 mg of vegetable oil sample. Individual standards were tested up to 200.0 mg, in the event that a relative increase of triolein exceeded 100.0 mg as a result of heating. However, this was not the case. The highest values experienced for OOO were approximately 85.0% with an average vegetable sample weight of 85.0 mg. Trilinolein standards were tested at a maximum concentration of 100 mg/10 ml of acetone considering that the highest percentages found in the vegetable oil blends did not exceed 31.0% of the total composition. The average percent of OOO and LLL are presented in Table 8.

G. 1. b. Tentative Triglyceride Composition of High Oleic Acid Sunflower and Soybean Oil.

The remaining triglycerides were tentatively identified using the equivalent carbon number (ECN) , a FAME balance sheet, and triglyceride reference chromatograms. The ECN, represented in Appendix B (Table B. 1.) is a value which provides an approximate resolution order for the triglycerides based on the number of double bonds present. However, the ECN value was ineffective when used

Table 8. Average triolein (OOO) and trilinolein (LLL) content for high oleic acid sunflower and soybean oil blends.

Blend	OOO%	LLL %
1	83.20	1.24
2	80.14	2.40
3	70.85	6.05
4	53.52	12.22
5	16.94	24.90
6	10.75	27.59
7	1.10	30.85

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

alone, due to more than one tentative triglyceride having the same ECN value.

The available reference chromatograms ([Appendix B]; Caboni, et. al (1992) and AOCS Official Method Ce 5b-89 [AOCS, 1989]) posed several problems in the tentative identification of the individual triglycerides. Each of the reference chromatograms represented different triglycerides for the same vegetable oil. In addition, each source listed a profile of triglycerides that were inconsistent with the fatty acid profile obtained in this study and in literature (Mead, et. al, 1986). However, the fatty acid profiles for soybean oil and high oleic acid sunflower oil blends produced in this study were consistent with the soybean and high oleic acid sunflower oil fatty acid profiles provided by Mead, et. al (1986). The only explanation, assuming that each triglyceride reference chromatogram was correct, is that each of these vegetable oils can exist with a variety of different triglycerides composing the vegetable oil.

The reference chromatogram provided by Caboni, et. al (1992) contained a number of oversights. The reference table listed over 15 commonly occurring vegetable oils with the potential triglycerides presented in the first column. The triglyceride LLnLn was not accounted for in this table but was labeled on the chromatogram for soybean oil. The triglyceride LLnL was misrepresented as containing 8 double bonds instead of 7 and LLL was misrepresented as containing 7 double bonds instead of 6. Stearic acid was not

accounted for in any of the vegetable oils, particularly high oleic acid sunflower and soybean oil. The peaks identified as LLnO in the reference chromatograms were determined to be LLL by the use of individual triglyceride reference standards in this study. Even with these minor oversights, the reference chromatograms provided some useful data, and the isocratic reverse-phased HPLC method described produced exceptional results in this study.

The reference triglyceride chromatograms for soybean oil provided by Neff, et. al (1992) [Appendix B, Figure B. 4. B.] had the closest similarity to the tentative triglyceride chromatograms produced in this study. However, the peak resolution had less clarity when compared to the chromatograms of this study (Appendix G, Figures G. 1. to G. 7.). This difference may have been attributed to the selection of the mobile phase, which consisted of acetonitrile:methylene chloride (70:30 v/v and 40:60). The use of a gradient solvent system and a flame ionization detector (FID) opposed to a light scattering detector may have impaired the peak resolution as well. Based on these subtle differences these triglyceride reference chromatograms were not used in the tentative identification of the triglycerides.

The overall best method for the tentative identification of the individual triglycerides was the use of the FAME balance sheet (Appendix B, Tables B. 2. And B. 3.). A list of all potential triglycerides for soybean and high oleic acid sunflower oil were

constructed and assigned an ECN value. The list of individual triglycerides were systematically compared to the fatty acid profile of each vegetable oil. The overall best comparisons for high oleic acid sunflower and soybean oil were reported as the tentative triglyceride composition, which are presented in Appendix B (Figures B. 2. And B. 3.).

G. 1. c. Triglyceride Categorization by Double Bond Number Grouping.

Another approach for determining the approximate content of high oleic acid sunflower and soybean oil was based on double bond number grouping. Individual reference standards were used to establish peak boundaries for all of the potential triglycerides, monoglycerides and diglycerides common to both vegetable oils. After establishing these peak boundaries, all of the potential triglycerides that could occur between any two reference peaks were assigned to a double bond group. The double bond groups were represented as 9 to 6, 5 to 3, and 3 to 1 double bonds. In addition, a category for monoglycerides and diglycerides was established.

Categorization of the triglycerides by double bond number was in agreement with the ECN value and the reference chromatograms provided by Caboni, et. al (1992) and Neff, et. al (1992). Furthermore, this system of classification may prove to be useful when the absolute triglyceride composition is not of importance but

rather the response of the triglycerides to oxidation based on the level of unsaturation. A representation of the categorization by double bond number is presented in Appendix B (Figure B. 1.).

G. 2. Estimation of the Concentration and Percent Recovery for *trans*-2-Decenal in High Oleic Acid Sunflower and Soybean Oil Blends.

The *trans*-2-decenal content was estimated in parts per million (ppm) for each of the volatile purging periods for every vegetable oil blend evaluated in this study. The linear regression statistics (Appendix C, Table C. 1.) indicated a multiple R value of (0.9899) for the individual reference standards evaluated. Concentrations of 12.5, 25.0, and 50.0 ppm were used to construct the standard curve, with each sample being tested in triplicate.

The average concentration range for the *trans*-2-decenal purged from the vegetable oil samples was between 34 and 67 ppm relative to the extract volume. The average concentration for *trans*-2-decenal relative to the 25 ml of vegetable oil sample was 9.36 ppb. The % recoveries ranged from 0.038 to 0.110 % with an average of 0.069 % recovery. Table 9. illustrates the estimated concentrations and estimated percent recoveries for the vegetable oil blends evaluated in this study.

Table 9. Estimated percent recovery of *trans*-2-decenal in high oleic acid sunflower and soybean oil blends evaluated for thermal oxidative stability.

Blend	trans-2-decenal Peak Area	Extract volume (*u*L)	ppm	(ng)	Estimated % Recovery
1	232817	6.57	52.65	345.9	0.070
2	185333	6.70	41.91	280.8	0.090
3	235880	6.65	53.34	354.7	0.110
4	183004	3.55	41.39	146.9	0.047
5	148160	4.80	33.51	160.8	0.051
6	163633	3.22	37.00	119.1	0.038
7	148368	6.85	33.55	229.8	0.073

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

The overall percent recoveries were less than those reported by Olafsdottir, et. al (1985). The percent recoveries reported in the cited literature had a range between 0.4 and 5.7%. The reproducibility for the volatile recovery was reported in the range of 5 to 22%. The low percent reproducibility would account for the wide range of the *trans*-2-decenal ppm values. Inherently, there are many potential areas for deviation in volatile purging. Among these areas are differences in the liquid's viscosity, polarity, and affinity for individual volatiles. Other potential problems may arise with the purging apparatus itself, where nondetectable leaks can lead to an increased loss of the volatiles from the purging system.

Olafsdottir, et. al (1985) primarily used low molecular weight volatiles which were moderately polar, such as butanol, to estimate the percent recoveries. In addition, the volatiles were purged from an aqueous medium, which has a higher polarity when compared to mediums such as vegetable oils. Based on these differences alone, it is difficult to compare the percent recoveries on a one to one basis. The differences in percent recoveries may be attributed to volatile breakthrough, volatile retention in the vegetable oil, or differences in molecular weight.

Under most circumstances a % recovery of less than 1.0% would be considered poor by most researchers. However, the resolution pattern of the major volatiles, hexanal, heptanal, (Z)-2-heptenal, nonanal, and 2,4-decadienal were consistent within each blend and

experienced similar changes during the process of heating. The major difference experienced within and between blends was the purge efficiency, indicated by the differences in the percent recoveries of the internal standard (Table 9.).

G. 2. a. Comparative Purging Methods for Volatile Extraction.

In addition to the regular purging method which used a nitrogen flow rate of 100 ml/minute for a 12 hour purging period, volatiles were collected by 2 additional methods. The first method (as described in the Methods and Procedure section), the volatiles were extracted with petroleum ether from a tenax-GR trap after being purged by the thermal effluent produced during the heating process. The second method consisted of purging the volatiles immediately after heating at 180°C for 300 minutes. The same nitrogen flow rate was used as for the 12 hour purge of the cooled vegetable oil, however, the 25 ml of heated vegetable oil sample was purged for only 1 hour. Before covering these methods, the choice of elution solvent will be addressed.

Typically, ethyl ether has been used as an elution solvent for volatiles (Olafsdottir, et. al, 1985). Petroleum ether was used in this study for 2 primary reasons. In pilot studies resolution of the volatiles and reproducibility of the volatile chromatograms was complicated by the use of ethyl ether. Similar vegetable oil samples

of similar treatment often produced different chromatogram profiles. The second problem associated with the ethyl ether was the characteristic split solvent peak. This second peak had a tendency to mask volatiles resolving close to the solvent peak, which were readily discernable when using petroleum ether. Representations of the ethyl ether and petroleum ether solvent peak are located in Appendix L (Figures L.1. and L. 2.).

The chromatograms for the thermally purged volatiles from the closed heating system (Appendix L, Figure L. 3.) contained volatile contents similar to those found in the regular 12 hour purge. However, the overall peaks areas were on the average of 10 to 100 times less. To indicate whether this was a result of poor volatile purging or volatile breakthrough, an internal standard was spiked into an additional series of vegetable oils and was heated for 300 minutes per day at 180°C for three consecutive days. A (9:1) high oleic acid sunflower to soybean oil blend series consisting of 500 ml with a 312.5 *u*g internal spike of trans-2-decenal was used. A % recovery of 0.37% was observed after the third day of heating. This value was approximately 5 times greater than the average % recovery for the 12 hour-purged samples (see Table 10.) . However, the major volatile contents were still on an average of 10 to 100 times less (see Appendices H and L, Figures H. 1 to H. 16. and Figures L 3. and 4). These results suggested that nitrogen purging for prolonged periods of time may have been conducive to the volatile breakthrough for compounds such as *trans*-2-decenal. It is

unclear whether the low peak areas of the major volatiles were a result of retention in the vegetable oil resulting from the closed heating apparatus, or whether these volatiles were lost during the course of heating. However, it is apparent, that the tenax-GR trap was successful in retaining some of the volatiles.

The results of the samples purged for one hour immediately following the first 300 minute heating period, indicated that volatile retention in the vegetable oil sample had occurred. The samples purged immediately following heating had the same major volatile profile as those experienced in the other purging methods. However, volatiles generally resolving around baseline where on the average of 50 times greater than those experienced in the 12 hour purged-samples. The average peak areas for nonanal, 2,4-decadienal, and *trans*-2-decenal were compared with the 12 hour purged-samples. The peak area for nonanal was on the average of 23.0 times greater, 2,4-decadienal was on the average of 68.2 times greater, and the % recovery for trans-2-decenal was on the average of 8.0 times greater (shown in Table 10.). It should be noted that the same extract concentration and injection volumes were used for both the 12 hour purged-samples and the heated 1 hour purged-samples. These results clearly show that the volatile contents of the vegetable oil are far greater than the average purging technique can detect. In addition, it is apparent the volatile binding capacity of the tenax-GR polymer was not a limiting factor in the extraction of volatiles. More appropriately it was the inefficient volatile

Table 10. Volatile peak area comparison for volatiles extracted from high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends using different purge and trap techniques.

Component/ Value	Thermal Effluent [1]	Thermal 1h purge [2]	12h purge [3]
T-2-Decenal peak area	774733	1494770	185314
Nonanal peak area	24101	1057958	45998
2,4-Decadienal peak area	16256	13196524	193458
T-2-Decenal % recovery	0.37%	0.64%	0.08%

Notes:

1. [1] = Volatiles extracted from a tenax-GR trap placed on the effluent end of a closed heating system containing 500 ml of (9:1) HOSO to PUSB with a 312.50 *u*g spike of *trans*-2-decenal. The blend was heated for a total of 900 minutes at 180°C and allowed to cool to 26°C before extracting the volatile chamber.

2. [2] = Volatiles extracted from a tenax-GR trap placed on the effluent end of a gas washing bottle containing 25 ml of (1:1) HOSO to PUSB with a 312.50 *u*g spike of *trans*-2-decenal. Volatiles were purged for 1 hour at a nitrogen flow rate of 100 ml/minute immediately following a 300 minute heating period at 180°C in a closed system.

3. [3] = Volatiles extracted from a tenax-GR trap placed on the effluent end of a gas washing bottle containing 25 ml of (1:1) HOSO to PUSB with a 312.50 *u*g spike of *trans*-2-decenal. Volatiles were purged for 12 hours at a nitrogen flow rate of 100 ml/minute. The blend was heated in a closed system for a total of 900 minutes at 180°C and allowed to cool to 26°C before purging the sample.

purging from the vegetable oil and volatile breakthrough resulting from extended nitrogen purging that resulted in lower recoveries of the volatile components. The results of the heated 1 hour purged-samples also indicated that volatile retention had occurred in the closed system, however, to what degree is uncertain.

G. 3. Trends Occurring in the Triolein (OOO), Trilinolein (LLL), and FAME Contents after 3000 Minutes of Heating at 180°C.

The level of significance for each vegetable oil component between each blend was determined to have a p value (≤ 0.01) with the exception of stearic acid. This level of significance was not surprising considering that the vegetable oil blends were inherently different in their composition of fatty acids and triglycerides. However, with the onset of thermal oxidation many potential changes could result in the composition of the individual vegetable oil blends. The simultaneous confidence intervals located in Appendix D (Tables D. 1. to D. 14.) indicated that only moderate changes were experienced for the fatty acids and triglycerides after the 3000 minute heating period at 180°C. However, certain trends were discernable for the saturated fatty acids, monoenic fatty acids and triglycerides, and the polyenic fatty acids and triglycerides.

G. 3. a. Saturated Fatty Acid Trends after 3000 minutes of Heating at 180°C.

Palmitic and stearic acid experienced the greatest level of stability (see Table 11.) of all of the fatty acids and triglycerides tested. The levels of palmitic acid were significantly different between blends ($p < 0.01$). The levels of palmitic acid progressively increased with the addition of soybean oil to the blends, with 100% soybean oil having the highest content, however, the percent increase was not significant ($p > 0.01$). The relative percentage of palmitic acid increased for each blend after the heating period, with an average percent increase of 8.0% (See Figure 16.).

Stearic acid levels were not significantly different between the blends ($p > 0.01$). The levels of stearic acid increased for each blend after the 3000 minute heating period at 180°C (see Figure 17.), however, the increase in relative percent levels was not significant ($p > 0.01$). The increase in the relative percentage of stearic acid was the greatest in blends with 75%, 90%, and 100% soybean oil, blends 5, 6, and 7 respectively. The mean percent value for these blends was 4.30% and was distinguished from the other blends as having the largest means after heating by using the simultaneous confidence intervals (Appendix D, Table D. 4.). The increase of stearic acid levels in the blends with 75.0% or greater of soybean oil was most likely a result of the increased breakdown of the polyenic fatty acids, found in higher concentrations in these blends (Table 7.).

Table 11. The average saturated fatty acid percentages for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends before and after heating at 180°C for 3000 minutes.

	(% Palmitic acid)		(% Stearic acid)	
Blend	I.	II.	I.	II.
1	2.90	2.90	3.91	3.99
2	3.50	3.56	3.89	4.01
3	4.30	4.70	3.92	4.06
4	5.90	6.68	4.06	4.12
5	8.50	6.68	4.11	4.27
6	9.00	9.66	4.02	4.26
7	9.50	10.60	4.04	4.32
p value [1]:	(< 0.0001)		(0.0442)	
p value [2]:	(0.0142)		(0.0141)	

Notes:
1. I. = Before heating
2. II. = After 3000 minutes of heating at 180°C
3. [1] = Significance levels between blends
4. [2] = Significance levels before and after heating for each blend

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

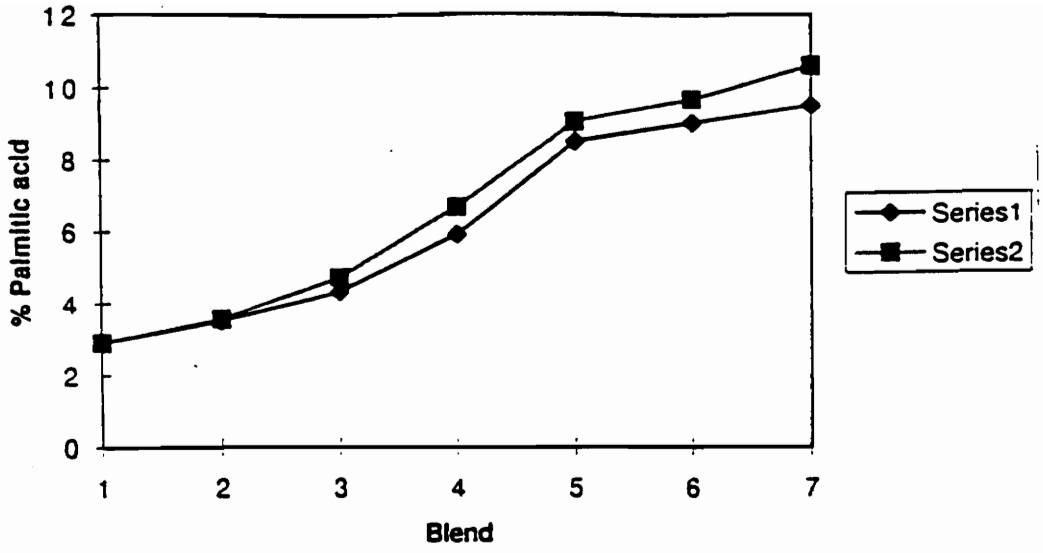


Figure 16. Trends in Palmitic acid contents occurring in high oleic acid sunflower and soybean oil blends before and after heating for 3000 minutes at 180oC.

Notes:

1. Series 1 = Before heating
2. Series 2 = After heating for 3000 minutes at 180°C

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

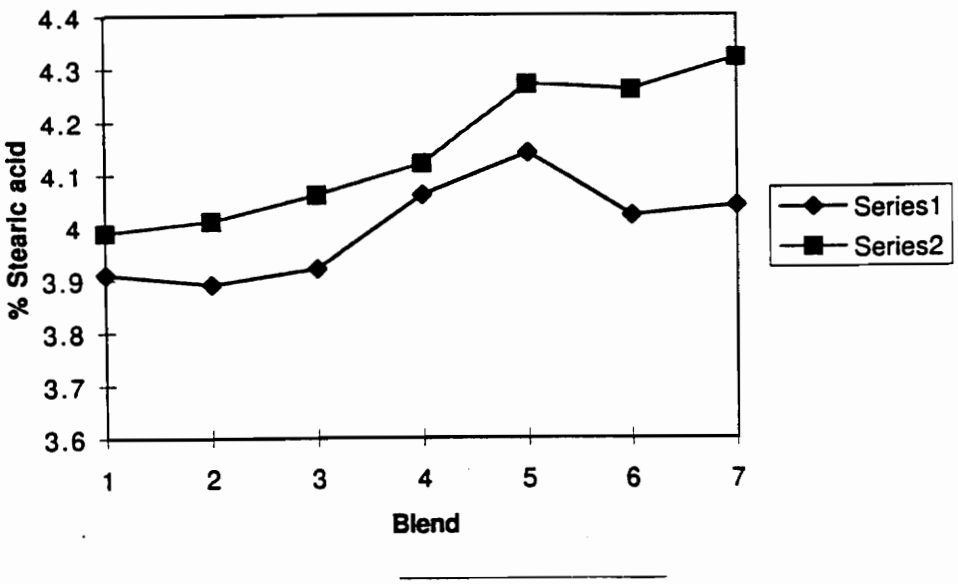


Figure 17. Trends in stearic acid contents occurring in high oleic acid sunflower and soybean oil blends before and after heating for 3000 minutes at 180°C.

Notes:

1. Series 1 = Vegetable oil blend before heating.
2. Series 2 = Vegetable oil blend after 3000 minutes of heating at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

G. 3. b. Monoenic Fatty Acid and Triglyceride Trends After 3000 minutes of Heating at 180°C.

The monoenic fatty acids and triglycerides, oleic acid and triolein both experienced similar results after heating, which was a relative percent increase for most blends (see Table 12.). The monoenic components represented the group with the second overall highest relative stability, following the saturated fatty acids. The triolein and oleic acid levels were significantly different between each blend ($p < 0.01$). Oleic acid experienced an increase in relative concentration for each blend with the exception of blend 4, [(1:1) high oleic acid sunflower to soybean oil]. Blend 4 experienced a 6.76 % decrease in oleic acid content. These results indicated that a one to one blend of high oleic acid sunflower to soybean oil was not conducive to the preservation of oleic acid during heating.

Potentially, the decrease in oleic acid content experienced in the one to one blend (Figure 18.) may have been associated with the fatty acid positioning on the triglyceride. Research (Neff, et. al, 1992; Raghuveer and Hammond ,1966) has shown that the substitution of a polyenic fatty acid on the *sn*-2 position of a triglyceride may lead to an increase in the rate of oxidation. This explanation offers some insight for the results experienced in this study, considering that the triolein content increased for blend 4 (50% high oleic acid sunflower oil). Based on this information it can be deduced that the decrease in oleic acid was not associated with

Table 12. The average monoenic fatty acid and triglyceride percentages for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends before and after heating at 180°C for 3000 minutes.

	(% Oleic acid)		(% Triolein [OOO])	
Blend	I.	II.	I.	II.
1	82.84	83.47	83.68	84.92
2	76.70	77.56	80.40	81.72
3	68.08	69.52	70.88	74.52
4	56.18	52.36	50.11	50.87
5	33.52	35.13	18.50	19.15
6	28.90	30.02	8.84	11.05
7	23.51	24.90	1.47	1.21
p value [1]:	(< 0.0001)		(< 0.0001)	
p value [2]:	(0.5481)		(0.0279)	

Notes:
1. I. = Before heating
2. II. = After 3000 minutes of heating at 180°C
3. [1] = Significance levels between blends
4. [2] = Significance levels before and after heating for each blend

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

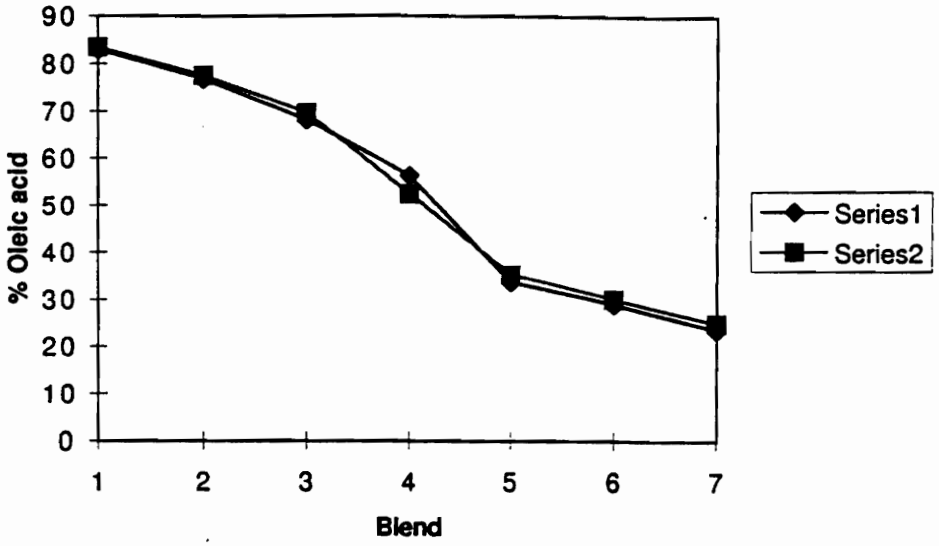


Figure 18. Trends in oleic acid contents occurring in high oleic acid sunflower and soybean oil blends before and after heating for 3000 minutes at 180°C.

Notes:

1. Series 1 = Vegetable oil blend before heating.
2. Series 2 = Vegetable oil blend after 3000 minutes of heating at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

the triglyceride triolein. Furthermore, the increased levels of palmitic acid and stearic acid indicated that these fatty acids were not associated with the triglyceride containing oleic acid. Considering that linoleic acid has the second highest concentration for blend 4 (30.43%) it is most likely the fatty acid that was present with oleic acid on the triglyceride that experienced a relative decrease in concentration. Triglycerides consisting largely of oleic acid and linoleic acid fall into the 3-5 double bond range. The two potential triglycerides, determined by the FAME balance sheet are OLO, and LLO (Appendix B, Figure B.1.). Unfortunately, the individual reference standards were not available for these two triglycerides nor other analytical test such as high performance liquid chromatography mass spectrometry (HPLC-MS). Therefore, the exact triglyceride could not be isolated.

Triolein experienced a relative % increase in concentration for each blend with the exception of blend 7 (100% soybean oil), where a 20.0% decrease was experienced (see Figure 19.). The triolein content of blend 7 was naturally occurring, but at low levels, between 1.40% and 1.50% of total composition (see Table 12.). The impact of autoxidation on the predominant polyenic fatty acids may have had a domino effect on the triolein contained in the soybean oil. It would appear that more than the fatty acid positioning on the triglyceride has an impact on the rate of autoxidation. In this case the oleic acid levels increased but the levels of triolein decreased for blend seven (100% soybean oil). Potentially, the triglyceride

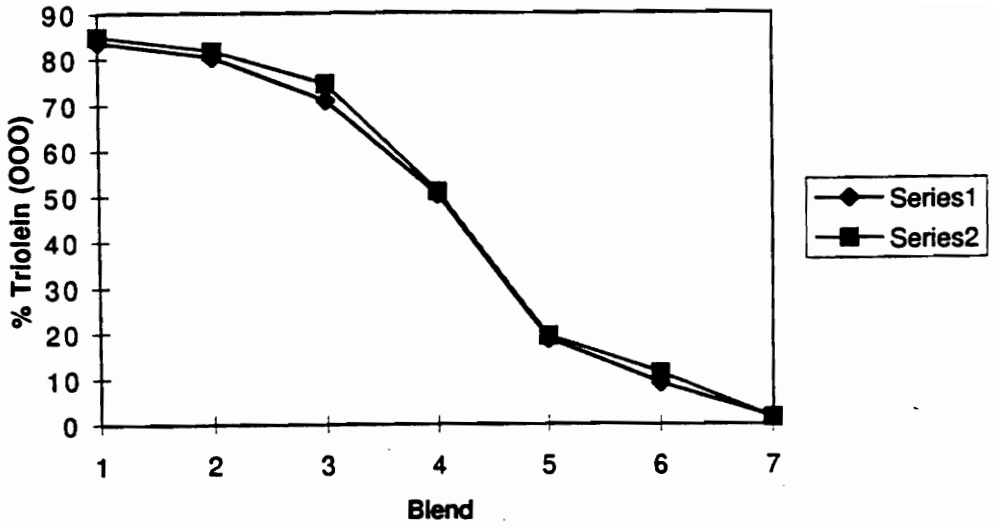


Figure 19. Trends in triolein contents occurring in high oleic acid sunflower and soybean oil blends before and after heating for 3000 minutes at 180°C.

Notes:

1. Series 1 = Vegetable oil blend before heating.
2. Series 2 = Vegetable oil blend after 3000 minutes of heating at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

packing after heating and cooling may have had an impact on triolein degradation. Clearly, the fatty acid positioning is not a viable explanation in this case. More research is needed in the area of vegetable oil composition and the relationship between polymorphism and autoxidation.

G. 3. c. Polyenic Fatty Acids and Triglycerides Trends After 3000 Minutes of Heating at180°C.

The polyenic fatty acids and triglycerides experienced the overall greatest decrease in the concentration levels after the 3000 minute heating period at 180°C (See Table 13.). The levels of linolenic acid and linoleic acid decreased in every blend with the exception of blend 4, [(1:1) high oleic acid sunflower to soybean oil] (see Figures 20. and 21.). Linolenic acid was not present in the 100% high oleic acid sunflower oil (blend 1), therefore, no changes were observed. The levels of linolenic and linoleic acid were significantly different between each blend ($p < 0.01$). Linolenic acid experienced an average decrease of 0.14 mg/1.0 mg of linolenic acid present in the vegetable oil, however, the decrease was not significant ($p > 0.01$). Blend 4 [(1:1) high oleic acid sunflower oil to soybean oil] experienced a 9.5% and 4.7% relative increase for linoleic and linolenic acid respectively. The increase in polyenic fatty acid levels were associated with an increase in triolein and a decrease in

Table 13. The average polyenic fatty acid and triglyceride percentages for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends before and after heating at 180oC for 3000 minutes.

	(% Linoleic acid)		(% Linolenic acid)		(% Trilinolein [LLL])	
Blend	I.	II.	I.	II.	I.	II.
1	9.78	8.73	0	0	1.21	0.85
2	14.27	13.22	0.73	0.46	2.56	1.99
3	20.87	19.79	1.69	1.44	6.13	4.73
4	30.43	32.92	3.07	3.22	14.29	13.16
5	47.56	46.19	5.77	5.09	25.95	24.04
6	51.52	50.08	6.29	5.61	28.94	26.76
7	55.97	54.11	6.98	6.10	29.69	28.71
p value [1]:	(< 0.0001)		(< 0.0001)		(< 0.0001)	
p value [2]:	(0.2159)		(0.0423)		(0.0029)	

Notes:
1. I. = Before heating
2. II. = After 3000 minutes of heating at 180°C
3. [1] = Significance levels between blends
4. [2] = Significance levels before and after heating for each blend

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

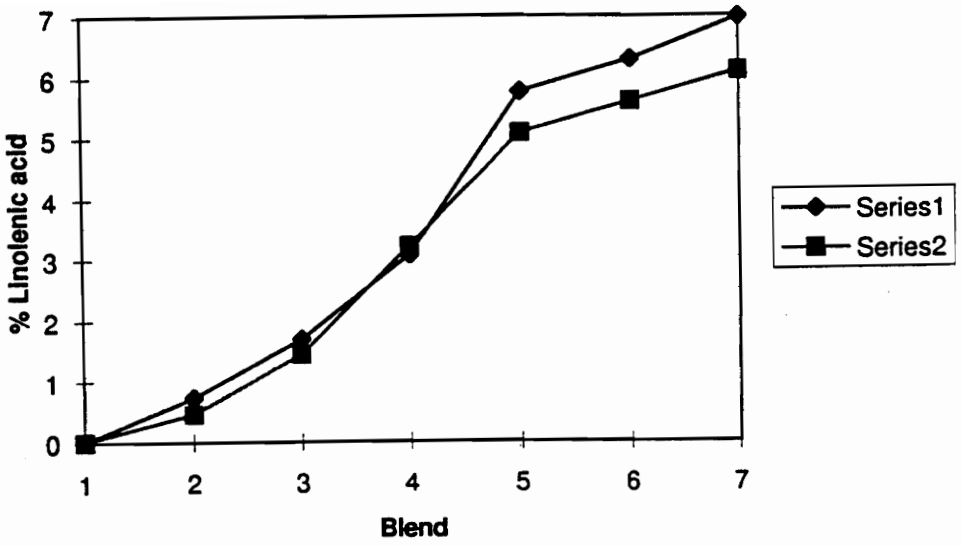


Figure 20. Trends in linolenic acid contents occurring in high oleic acid sunflower and soybean oil blends before and after heating for 3000 minutes at 180°C.

Notes:

1. Series 1 = Vegetable oil blend before heating.
2. Series 2 = Vegetable oil blend after 3000 minutes of heating at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

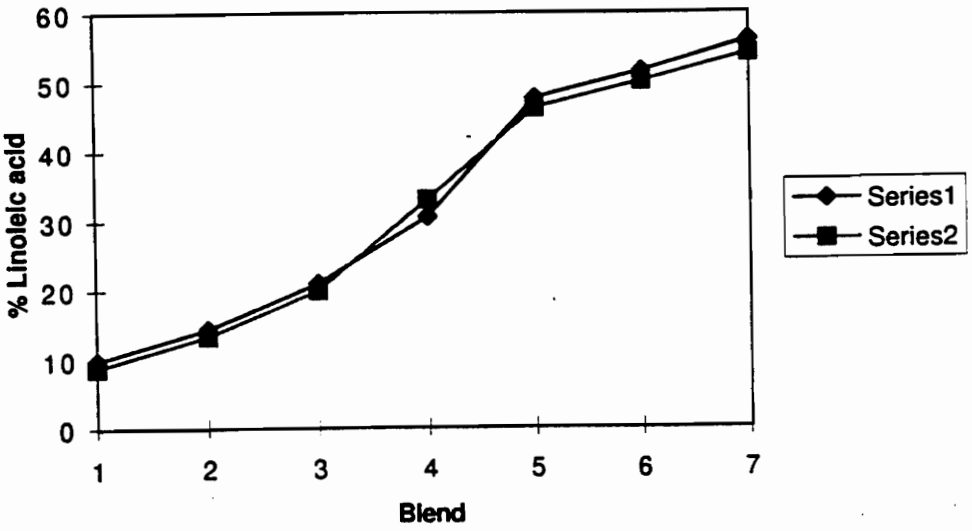


Figure 21. Trends in linoleic acid contents occurring in high oleic acid sunflower and soybean oil blends before and after heating for 3000 minutes at 180°C.

Notes:

1. Series 1 = Vegetable oil blend before heating.
2. Series 2 = Vegetable oil blend after 3000 minutes of heating at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

trilinolein and oleic acid level. The presence of polyenic fatty acids at the *sn*-2 of the triglyceride appears to be the most likely explanation for this occurrence. Raghuveer and Hammond (1966) and Neff, et. al (1992) reported similar results, which were previously covered in section (G. 4. b.).

Apparently the vegetable oil blend with a (1:1) ratio of high oleic acid sunflower to soybean oil are more selective to the degradation of oleic acid compared to the polyenic fatty acids. The answer may reside in the crystal packing structures of the triglycerides after heating and cooling periods. Small (1986) indicated that impurities such as monoglycerides and diglycerides can interrupt the packing structures of the triglycerides. Similar occurrences may have resulted in blend 4 [(1:1) high oleic acid sunflower to soybean oil], where the triglyceride packing structures were disrupted by a heterogeneous mixture of triglycerides more pronounced in this blend than in the other blends. Unfortunately, there is minimal research in this area, especially in the area of crystallography, therefore only speculations can be made.

The levels of trilinolein (LLL) experienced a decrease in every blend with an average percent decrease of 26.32% for blends with an average LLL content of 1.27%, 13.12% for blends with an average LLL content of 6.87% , and 5.92% for blends with an average LLL content of 28.16% (see Table 13.). The percentages of LLL were significantly different between each blend before and after heating

($p < 0.01$), with the exception of blends 6 and 7 which were not significantly different from one another before heating (see Appendix D, Table D. 13.). The levels of LLL represented the only vegetable oil component that experienced a decrease after 3000 minutes of heating at 180°C that was significantly different ($p < 0.01$) from the initial value. Figure 22. illustrates the decrease experienced for LLL in each of the blends. Based on the results of this study, LLL was clearly the least stable of all of the vegetable oil components analyzed, including linolenic acid. However, if linolenic acid had occurred at higher concentration levels and was present on a triglyceride with 6 or more double bonds, the thermal stability ranking may have been different.

G. 3. d. Stability Ranking of the Fatty Acids and Triglycerides After 3000 Minutes of Heating at 180°C.

A stability ranking system for the fatty acids and triglycerides contained in the vegetable oil blends was established to collectively summarize the overall trends occurring after 3000 minutes of heating at 180°C (see Figure 23.). Each of the fatty acids and triglycerides were assigned a (+) value if no change or an increase was experienced for each blend. A (-) value was assigned if a decrease was experienced for each blend. The plus and minus values were summed for each blend and assigned a number from one to

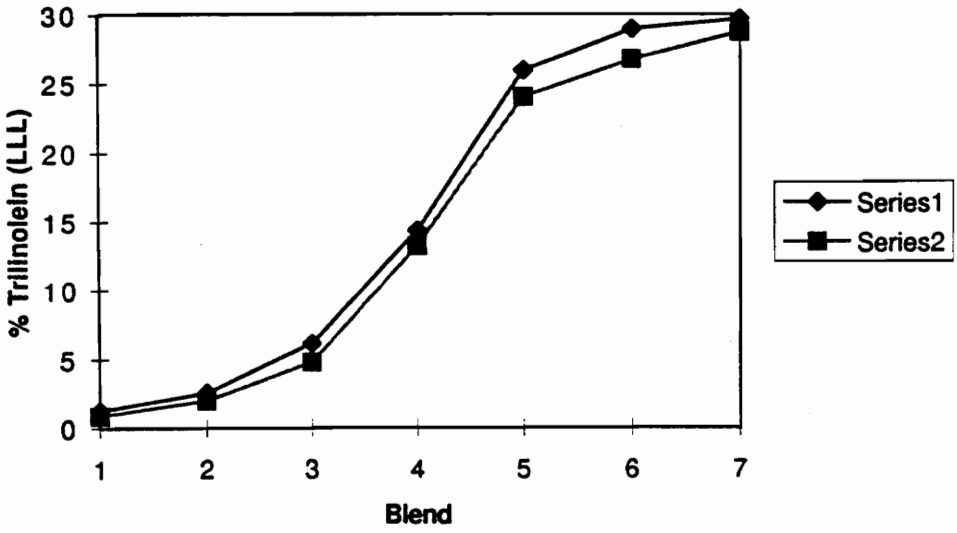


Figure 22. Trends in trilinolein contents occurring in high oleic acid sunflower and soybean oil blends before and after 3000 minutes of heating at 180°C.

Notes:

1. Series 1 = Vegetable oil blend before heating.
2. Series 2 = Vegetable oil blend after 3000 minutes of heating at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

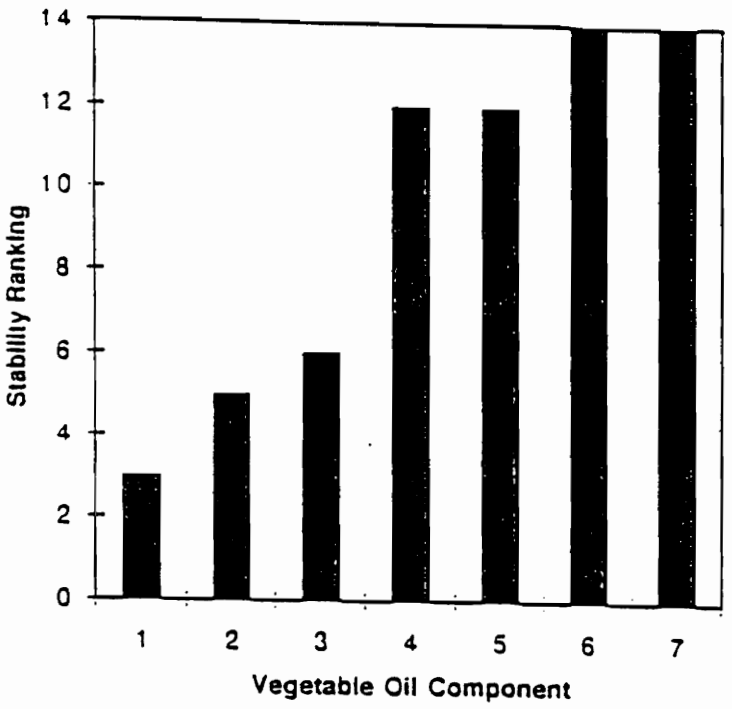


Figure 23. Relative stability ranking of high oleic acid sunflower and soybean oil blend triglycerides and fatty acids after 3000 minutes of heating at 180°C.

Vegetable Oil Components:

1 = LLL
2 = L
3 = Ln
4 = OOO
5 = O
6 = P
7 = S

fourteen to establish the ranking scale. Zero was omitted from the scale considering that this value was not possible with an odd number of blends. A representation of the ranking scale and balance sheet are presented in Appendix D (Figure D. 1.).

The general trend observed after all the values were plotted was that the relative stability increased as the degree of saturation increased. The saturated fatty acids experienced the highest ranking values, (14, on a scale of 1 to 14), followed by the monoenic group (ranking value of 12), and the polyenic group (ranking value of 4). Other researchers (Adachi, et. al 1995; Frankel and Huang 1994; Raghuveer and Hammond 1966) have found similar results. In each case, the free fatty acid or intact triglyceride showed a decrease in oxidative stability as the level of saturation decreased. Representative HPLC triglyceride chromatograms before and after heating are presented in Appendix G (Figures G. 1. To G. 7.) for each of the seven vegetable oil blends.

G. 4. Peroxide Value Trends Following 3000 Minutes of Heating at 180°C.

The differences in peroxide values (PV) for the fresh vegetable oil blends and vegetable oil blends heated for a total time of 3000 minutes at 180°C were significantly different ($p < 0.01$). The peroxide values for the fresh blends ranged from 0.61 to 1.57, with an average peroxide value of 0.88 (Table 14.). The low initial

peroxide values were an indication that the vegetable oil blends had not experienced autoxidation at advanced levels, which was supported by the preservation of OOO and LLL levels during freezer storage, where significant differences were not observed between the blend profile and fresh starting oils (see Table 6.).

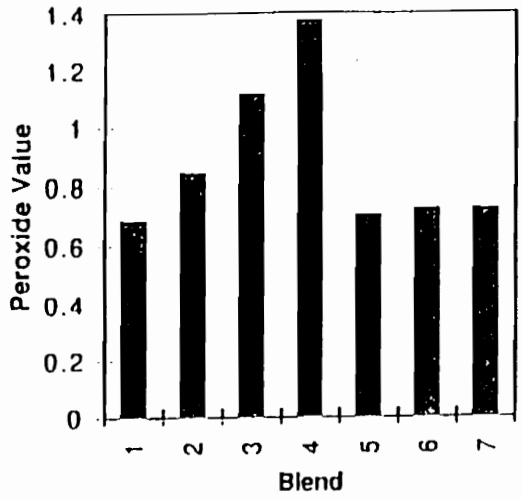
The vegetable oil blends with 75.0% or greater of soybean oil possessed the overall lowest peroxide values between the blends, with an average peroxide value of 0.70. The vegetable oil blends with 50.0% and 75.0% high oleic acid sunflower oil experienced the highest initial peroxide values, 1.57 and 1.26 respectively (see Figure 24. B.). This slight increase in peroxide values for blends 3 and 4 (75% and 50% high oleic acid sunflower oil) may have been associated with the decrease in oleic acid content and increase in the polyenic fatty acid contents occurring in blend 4 after the final heating period consisting of a total of 3000 minutes. The (1:1) ratio of high oleic acid sunflower to soybean oil may have been conducive to an increased rate of autoxidation, based on the results obtained in this study. The association of blend 4 (50% high oleic acid sunflower oil) with an increase in autoxidation indicators such as the peroxide values (refer to Table 14.), major volatile products (refer to Table 17.), and decreases in monoenic fatty acids (refer to Table 12) apparently are related to the mixture of triglycerides, considering that no other differences exists between the vegetable oil blends. Furthermore, each blend was prepared independently, yet the same general trend occurred for all blend 4 samples.

Table 14. Peroxide value (PV) trends before and after 3000 minutes of heating at 180°C for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends.

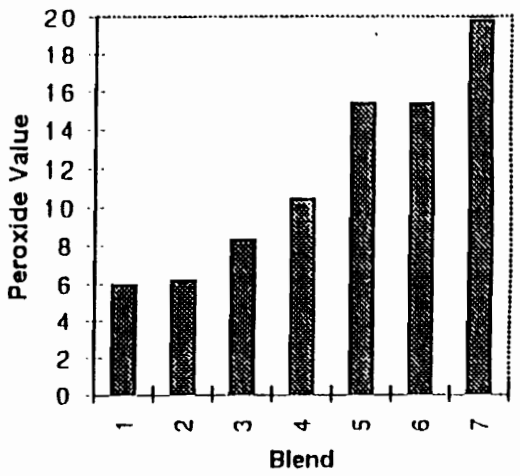
Blend	PV (Fresh)	PV (after 3000 min.)
1	0.84	5.92
2	1.09	6.16
3	1.26	8.28
4	1.57	10.40
5	0.93	15.35
6	1.36	15.34
7	0.61	19.76

* p value: (0.0009)

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100



(B)



(A)

Figure 24. Peroxide values before (B) and after (A) heating for 3000 minute at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

After a total heating time of 3000 minutes at 180°C the peroxide values increased in a linear fashion, where an increase was associated with an increase in the level of soybean oil (see Figure 24. A.). High oleic acid sunflower oil experienced the overall lowest peroxide value of 5.92 and soybean experienced the overall highest value of 19.76. Blend 4 [(1:1) high oleic acid sunflower to soybean oil) experienced the fourth highest value of 10.40, which would indicate that the addition of high oleic acid sunflower oil to the polyunsaturated soybean oil had some impact on reducing the rate of peroxidation, regardless of the triglyceride interactions.

Based on these results, initial peroxide values were not good indicators of how a given vegetable oil will perform in a stability test. If predictions were made based on the initial peroxide values (Figure 24. B.), the blends highest in polyenic fatty acid content would have appeared to be the most stable. However, this was not the case and for this reason peroxide values alone are not good indicators of vegetable oil stability. Frankel and Huang (1994) correlated the peroxide values with the hexanal peak areas for high oleic acid sunflower and soybean oil blends. The hexanal peak areas experienced an increase in the vegetable oil blends that positively correlated with an increase in peroxide values. This correlation was an acceptable approach to complementing the peroxide value. However, hexanal was not the best possible volatile of choice. Hexanal, a major breakdown product of linoleic acid, is not a good indicator for linolenic acid or oleic acid breakdown. Research

(Adachi, et. al ,1995) has shown that linolenic acid experiences autoxidation at a higher rate when compared to linoleic acid. In addition, the blends were predominately high in oleic acid and no indicators were proposed for their breakdown. A more appropriate approach would have been to correlate the peroxide values with the major volatiles produced from each of the major fatty acids (see Table 3.). This approach would have represented the intermediate products (peroxides) and the final breakdown products (volatiles).

G. 4. a. Peroxide Value Trends Occurring During Heating and Cooling Cycles.

The peroxide values fluctuated between the blends during the first 4 days of heating, with values occurring between 0.60 to 5.00 (see Table 15.). The peroxide values were relatively consistent between the blends for the first two heating periods of 300 minutes at 180°C each. The peroxide values were on an average of 2.0 for blends with 75.0% or greater of high oleic acid sunflower oil and on the average of 2.37 for blends with 50.0% or greater of soybean oil. However, by the third heating period the 75.0% or greater high oleic acid sunflower oil blends began to stabilize and the blends highest in soybean oil began to experience a steady increase in peroxide values. After the third heating period the first 3 blends stabilized with an average peroxide value of 3.11 or less where blends 4 through 7 (50.0% or greater of soybean oil) each experienced a peroxide value of 3.6 or greater (see Figure 25.).

Table 15. Peroxide value (PV) trends during heating and cooling cycles.

Blend	0:0	5:300	24: 300	29: 600	48: 600	53: 900	72: 900	264: 3000
1	0.84	1.55	1.78	1.90	1.81	1.56	1.95	5.92
2	1.09	2.51	2.00	2.72	2.87	2.53	2.90	6.16
3	1.26	2.07	2.61	3.33	3.31	3.11	2.37	8.28
4	1.57	2.47	2.51	2.93	2.75	3.70	4.48	10.40
5	0.93	2.12	1.81	1.98	2.18	2.88	3.24	15.35
6	1.36	2.89	2.94	2.70	2.78	3.67	4.98	15.34
7	0.61	1.86	2.22	2.65	3.00	3.85	3.63	19.76

Notes:

1. The first number followed by the colon represents the time in hours for the use of each vegetable oil blend.
2. The second number after the colon represents the time in minutes for the vegetable oil blends heated at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

The basic trends for the peroxide values during the heating and cooling cycle were similar for each blend. The peroxide values increased immediately following heating and continued to increase after 24 hours of cooling (see Figures 25. and 26.). This occurrence indicates that peroxide formation (peroxidation) continued as the vegetable oil returned to a less random state. These findings are in agreement with those reported by Raghuveer and Hammond (1966). The lower peroxide values experienced in the vegetable oil blends highest in triolein content (62.50% or greater) were also in agreement with the results reported by Dobarganes, et. al (1993) and Frankel and Huang (1994). In each of these studies vegetable oils with the greatest level of high oleic acid sunflower oil experienced the lowest peroxide values. The continued change in peroxide values indicates that a peroxide value taken in a single time frame may not be an accurate representation of the level of peroxidation experienced by a vegetable oil. More appropriately, peroxide values should be taken over the course of 12 to 24 hours to insure that the vegetable oil is not in a state of transition.

G. 5. Monoglyceride and Diglyceride Trends Occurring for Vegetable Oils Heated for 3000 Minutes at 180°C.

The relative percentage of monoglycerides and diglycerides were collectively determined by the same HPLC method used for triglyceride analysis. The relative retention time and resolution

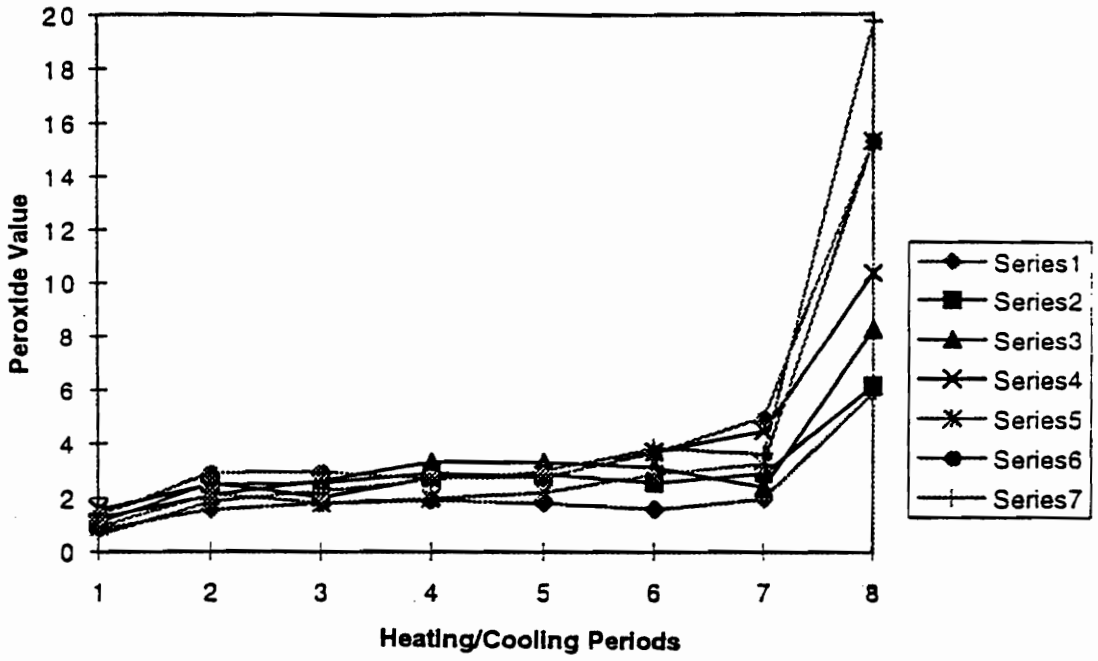


Figure 25. Peroxide Trends for high oleic acid sunflower and soybean oil blends occurring during heating and cooling cycles.

* Series correspond to blend number

Heating and Cooling Periods: (at 180°C)

1 = Day 1 Time 0

2 = Day 1 300 minutes

3 = Day 2 Time 0

4 = Day 2 300 minutes

5 = Day 3 Time 0

6 = Day 3 300 minutes

7 = Day 4 Time 0

8 = Day 11, 3000 minutes total heating

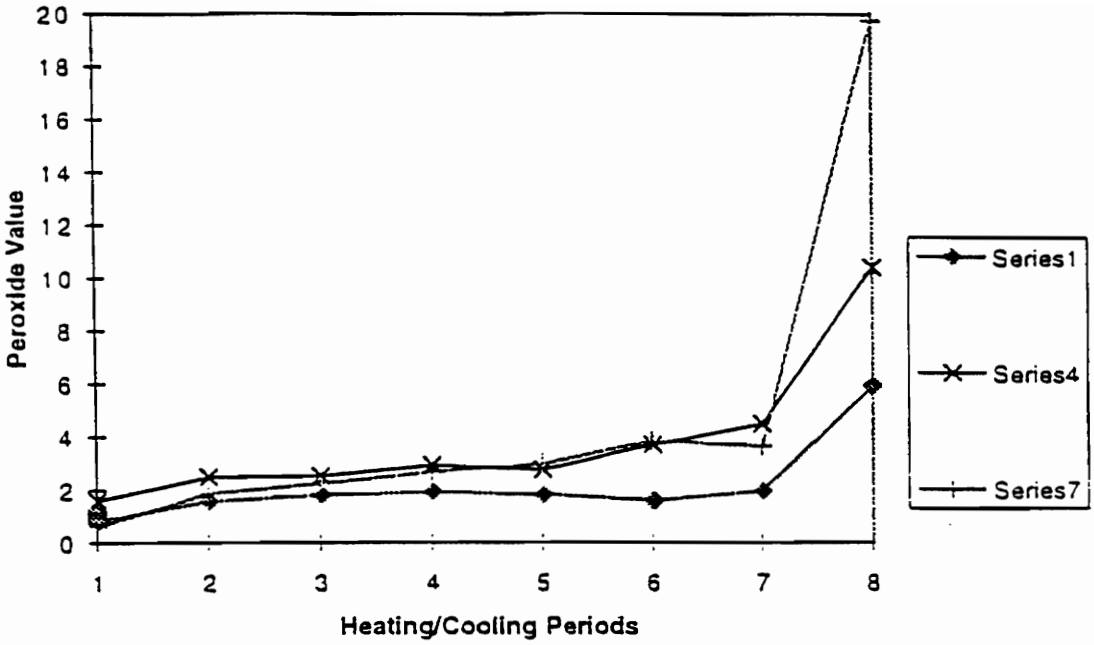


Figure 26 Peroxide trends occuring for high oleic acid sunflower and soybean oil blends 1, 4, and 7 during heating and cooling cycles.

* Series correspond to blend number

Heating and Cooling Periods: (at 180°C)

1 = Day 1 Time 0

2 = Day 1 300 minutes

3 = Day 2 Time 0

4 = Day 2 300 minutes

5 = Day 3 Time 0

6 = Day 3 300 minutes

7 = Day 4 Time 0

8 = Day 11, 3000 minutes total heating

order were determined using reference chromatograms (AOCS, 1989) and an individual reference standard consisting of a racemic mixture of mono-olelyglycerol. Individual vegetable oils were spiked with the racemic mixture to insure that the HPLC system would detect the presence of monoglycerides (see Appendix J). Standard curves were not prepared considering that reference standards were not available for all the potential monoglycerides and diglycerides that could result from the degradation of the vegetable oil blends. Therefore, the results are collectively listed as relative percentages of monoglyceride/diglycerides (MG/DG).

Monoglycerides and diglycerides were not present in the fresh vegetable oil blends, with the exception of an occasional occurrence in blends 2 through 7, with levels no greater than 0.05% of total composition. The vegetable oil blends remained relatively free of MG/DG content through the first 900 minutes of heating. At the conclusion of 3000 minutes the levels of MG/DG reached their peaks for blends 2 through 7 (10.0% or greater of soybean oil). No levels of monoglycerides or diglycerides were detected in the 100% high oleic acid sunflower oil (blend 1). Therefore, the increased levels of MG/DG were associated with the addition of soybean oil to the blends.

The average MG/DG % levels ranged from 0.13% to 0.41% for blends 2 through 7 (see Table 16.). Soybean oil (blend 7) experienced the overall highest value of 0.41%. Blends 2 and 4 experienced an

Table 16. The average monoglyceride and diglyceride percentages (MG/DG%) after 3000 minutes of heating at 180°C for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends.

Blend	MG/DG%
1	0
2	0.13
3	0.28
4	0.13
5	0.31
6	0.30
7	0.41

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

average MG/DG % of 0.13%, while blends 3, 5, and 6 experienced an average MG/DG % of 0.30% (see Figure 27). The peak areas collectively representing the MG/DG group were indigenous to the vegetable oil blends. These peaks were not present in the acetone solvent blanks or the triglyceride reference standards. Furthermore, each of these peaks resolved before the trilinolenein (LnLnLn) reference standard. The LnLnLn represents the first major triglyceride with a total of 9 double bonds to resolve on the HPLC chromatogram using an isocratic reverse-phased system. Potentially, triglycerides with fragmented fatty acid side chains resulting from autoxidation would resolve in this region according to the ECN value. However, the FAME analysis showed no evidence of short chain or fragmented fatty acids. If the fragmented fatty acids were present as part of the triglyceride, the methanolinic base used in the FAME preparation would have reacted with these fragments in a similar fashion as the intact fatty acids. The reaction would occur at the carbonyl group of the alkanoic/alkenoic carbon chain, producing methylated ketones, with a total carbon composition of 18 carbons or less (Liu, 1994). Regardless, of the exact composition of the MG/DG peaks, it is evident that the increase was associated with the degradation of the vegetable oil blends, indicated by an increase in peroxide values (Table 15.) and a decrease in the polyenic fatty acid levels (see Table 13.).

The low levels of monoglycerides and diglycerides produced in the vegetable oil blends may have suggested that hydrothermal

Figure 27. Monoglyceride and diglyceride (MG/DG) trends occurring after 3000 minutes of heating at 180°C for high oleic acid sunflower and soybean oil blends.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

hydrolysis was not a primary reaction when food particulates or moisture were absent from the vegetable oil blends. Research (Cuesta, et. al, 1993; Fritsch, 1981) has shown that the moisture exposure to the vegetable oil increases significantly with the addition of food particulates such as potato slices. Cuesta, et. al (1993) found that the addition of 500 g of potato slices to 3 L of sunflower oil did not significantly increase the levels of polar components such as free fatty acids, and monoglycerides and diglycerides after a total of 75 frying periods of 8 minutes each at 180°C. The primary breakdown products experienced in the sunflower oil were triglyceride dimers, polymers, and oxidized triglycerides. The Diels Alder reaction (see Appendix M) was implicated as the primary reaction leading to the increase in total polar component values. Similar findings were reported by Dobarganes, et. al (1993) and Sanchez-Muniz, et. al (1993). The exact level of total polar components, particularly triglyceride dimers and polymers is uncertain for the vegetable oil blends evaluated in this study.

G. 6. Major Volatile Products (MVP) from the Thermal Oxidation of High Oleic Acid Sunflower and Soybean Oil Blends.

The major volatiles used for comparison between the vegetable oil blends were extracted from 25 ml samples which were subjected to the 12 hour purging period specified in the Methods and

Procedures section. The volatile profiles were similar for each blend (see Table 17.), with the major differences occurring in the volatile peak areas and the point at which the volatile concentrations began to increase (Appendix H, Table H. 1.). The major volatile peak areas continued to increase for the GC chromatograms with continued heating of the vegetable oil blends in the closed heating system. Each vegetable oil blend experienced a volatile profile that was unique with respect to that blend, representing the same major volatiles but at varied concentrations for different heating times. Eight major volatiles were positively identified by individual reference standards and gas chromatography mass spectrometry (GC-MS). However, before covering the major volatiles used for comparison within and between blends, the total amount of volatiles tentatively identified by GC-MS and their respective spectra will be addressed.

G. 6. a. Volatile Identification by Gas Chromatography Mass Spectrometry (GC-MS).

Over 90 volatile peaks were tentatively identified using both an HP5 column (nonpolar) and a Supelcowax SP10 capillary column. The compound identification was similar between the two columns, however, the volatile resolution order and retention times were vastly different. The Supelcowax SP10 capillary column produced chromatograms with sharper peaks which resolved to baseline. This occurrence was not experienced for all the volatile components

Table 17. Percent major volatile production (%MVP) in high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends before and after 900 minutes of heating at 180°C in a closed system.

Blend & Day	% Toluene	% Hexanal	% 2-Heptenal	% Nonanal	% 2,4-Heptadienal	% 2,4-Decadienal
1 D1	32.06	0.83	0	0.85	0	0
1 D4	4.87	5.10	23.36	19.84	2.38	2.06
2 D1	32.50	0.60	0	0	0	0
2 D4	3.15	4.58	24.39	14.04	7.54	2.53
3 D1	24.16	0	0	0	0	3.76
3 D4	2.94	4.21	4.87	27.78	12.09	4.05
4 D1	36.27	0	0	0	0	0
4 D4	3.13	2.95	2.23	9.18	21.39	4.45
5 D1	28.70	0	0	0	0	0
5 D4	4.25	6.00	29.53	8.04	13.40	3.53
6 D1	38.36	0	0	0	0	0
6 D4	3.00	4.54	29.15	3.69	20.59	5.11
7 D1	17.24	0	0	0	0	0
7 D4	1.33	1.60	28.85	3.49	18.65	6.53

Notes:
1. D1 = Fresh samples prior to heating
2. D4 = Samples heated for 900 minutes at 180°C and allowed to stand for 24 hours.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

using the HP5 column. The HP5 column was used as a secondary measure to insure that the mass spectrometer was appropriately identifying the individual reference standards. A positive identification was obtained for each of the volatile standards using both columns. Appendix I (Figure I. 1.) illustrates a representation of a GC-MS chromatogram for blend 4 using the Supelcowax SP10 capillary column, which was used for the comparison of the volatile contents of all the vegetable oil blends. Appendix I, Table I. 1. lists the top 30 peak areas and retention times for those volatiles resolving in Figure I. 1..

A total of 19 volatile peaks (Appendix I, Table I. 2.) were identified using the National Bureau of Standards Mass Spectra Library (NBS) with a percent quality match of 92.0% or greater, with the exception of nonanal, in which there was an 80.0% quality match. These volatiles ranged in relative concentrations from 0.26% to 7.3%. The detection of volatiles included low molecular weight volatiles (1-pentenol), high molecular weight volatiles (hexadecene), heterocyclic volatiles (pentyl-furan), cyclic compounds (toluene), polar compounds (benzyl alcohol), and hydrocarbons and aldehydes (tridecane and nonanal).

The presence of carboxylic acids were absent in each of the volatile chromatograms obtained. Carboxylic acids represent one of the many breakdown products produced from free fatty acid autoxidation (Nawar, 1985). The carboxylic acids result from the

cleavage of the free fatty acid chain, where lower molecular weight organic acids are produced in addition to aldehydes and hydrocarbons from the methyl-terminal end of the fatty acid. The lack of carboxylic acids in the volatile chromatograms may have been attributed to several factors. Olafsdottir, et. al (1985) indicated that the tenax polymers used for volatile trapping have a low affinity for polar and aqueous compounds. The low affinity of the tenax-GR for polar compounds and the use of a nonpolar solvent such as petroleum ether (with a polarity index of 0.0) may have greatly diminished the recovery of the carboxylic acids if they were present. The study conducted by Olafsdottir, et. al (1985) indicated that lower molecular weight volatiles such as acetic acid were extracted from the tenax trap in low quantities. However, in this case ethyl ether was used as an extracting solvent, which has a polarity index of 2.8 (Solomons, 1992c). In addition, the low molecular weight carboxylic acids were extracted from aqueous mediums.

The larger molecular weight volatiles such as 1-hexadecene does indicate that the liberation of free fatty acids may have occurred. The relative peak area percents for these components were each less than 1.0% of the total composition. These results together with the low percentages experienced in the monoglyceride and diglyceride values indicate that the liberation of free fatty acids during the process of thermal oxidation was minimal. Research (Cuesta, et. al ,1993; Dobarganes, et. al ,1993) has shown that the production of free fatty acids and monoglycerides and diglycerides after prolonged

heating at 180°C with and without food particulates are on the average of 2.0% or less. Therefore, it appears that the majority of volatiles produced during the thermal oxidation of the vegetable oil blends resulted from the autoxidation of the fatty acids intact on the triglycerides or polymerized triglycerides based on the results obtained in this study and previous research (Cuesta, et. al, 1993 and Dobarganes, et. al, 1993). The production of triglyceride polymers and dimers would account for the absence of fragmented fatty acids in the FAME analysis and the decrease in triglyceride levels experienced without the occurrence of an increased level of monoglycerides and diglycerides peak area percentages.

G. 6. b. Trends in the Major Volatile Products (MVP) after 900 Minutes of Heating at 180°C in a Closed System.

The trends were observed for a total of 8 volatile compounds in the vegetable oil blends. The internal standard (*trans*-2-decenal) was previously covered in section G. 2. where the percent recoveries were detected for each vegetable oil blend. The remaining 7 volatile compounds were: Toluene, hexanal, (Z)-2-heptenal, (E,E)-2,4-heptadienal, nonanal and 2,4-decadienal. The mass spectra for each of these compounds are presented in Appendix I (Figures I. 2 through I. 9.). The lowest quality match with the NBS mass spectra library was for nonanal (80.0%). The fragmentation pattern is similar

between the observed spectrum and the library mass spectrum. The major difference experienced between the two, is that a mass to charge ratio (M/Z) of 29 occurred in the volatile spectrum obtained from the vegetable oil sample and not the library mass spectrum. The mass value of 29 most closely matches the mass of the carbonyl aldehyde (-COH) which has a molecular weight of 29. If the molecular weight of one methylene carbon ($-CH_2$) is added to 29, the next highest mass fragment of 43 is obtained ($-CH_2-COH$). Therefore, the low quality match is most likely due to the absence of the aldehyde fragment M/Z of 29 from the NBS mass spectra library. The remaining volatiles experienced a quality match of 90.0% or better, both for individual reference standards and within the vegetable oil blends.

Four of the 7 volatiles were used to obtain the major volatile percent (MVP%), which represented the summation of the peak area percents for: (Z)-2-heptenal, (E,E)-2,4-heptadienal, nonanal, and 2,4-decadienal. These volatiles were selected considering that they experienced the greatest increase in peak areas and peak area percentages for each vegetable oil blends as the heating process continued. Research (Chan and Coxon,1987) has shown that these volatiles are major breakdown products of the major fatty acids oleic acid, linoleic acid, and linolenic acid. The peak area percentages and not the peak areas were used due to potential differences occurring in the peak areas as a result of slight differences in the extract volumes. In this respect, the relative

percentages of the major volatiles should remain constant. However, differences in the extract volumes would lead to differences in the peak areas, considering that the concentration of the solute (volatiles) directly determines the magnitude of the peak areas.

By taking the total peak area percent for these 4 volatiles, each of the monoenic and polyenic fatty acids are accounted for (see Table 3.). The relative percents for 2,4-decadienal (from linolenic acid breakdown) and (E,E)-2,4-heptenal (from linoleic acid breakdown) experienced the highest levels for blends with 50.0% or greater soybean oil content after heating (see Table 17.). The highest percent value for (Z)-2-heptenal (from linoleic acid breakdown) was experienced in 75.0% soybean oil, with a peak area percent of 29.53%. The peak area percents for nonanal (from oleic acid breakdown) increased dramatically as the level of high oleic acid sunflower oil increased in the blends. The percentages of nonanal ranged from 3.49% to 27.78% between the blends, with blend 3 (75.0% high oleic acid sunflower oil) having the highest percent value.

The MVP% experienced an overall increase as the level of soybean oil increased in the vegetable oil blends (see Table 18.). High oleic acid sunflower and (9:1) high oleic acid sunflower to soybean oil experienced the overall lowest values of 52.0% for the seven different blends. The major volatile contributors for blends 3

Table 18. Percent major volatile production (%MVP) for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends heated for a total of 900 minutes at 180°C in a closed system.

Blend	%MVP
1	52.10
2	52.25
3	62.07
4	65.86
5	56.73
6	60.41
7	65.73

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

and 4 were (Z)-2-heptenal, nonanal, and (E,E)-2,4-heptadienal (see Table 17.). These results indicated that the breakdown of linoleic acid was more evident than the breakdown of oleic acid and linolenic acid in blends 3 and 4 (see Figure 28.). The levels of 2,4-decadienal progressively increased as the levels of polyunsaturated soybean oil increased in the blends, suggesting that the increase in linolenic acid contents (refer to Table 13.) was responsible for the increase in 2,4-decadienal (refer to Table 17.).

Individual volatiles such as hexanal or heptanal were not selected as indicators of vegetable oil stability considering that they were not a representation for the breakdown products of each of the major fatty acids (see Table 3.). Research (Chan and Coxon, 1987) has shown that hexanal is a breakdown product of linoleic acid primarily and heptanal a breakdown product of oleic acid. Neither of these two volatiles would account for the breakdown occurring in the linolenic acid for the blends with 10.0% of soybean oil or greater. The general trend for hexanal experienced in all of the vegetable oil blends was that the hexanal peak area appeared after 300 minutes of heating at 180°C and began to decrease with a total heating time of 900 minutes. The hexanal peak areas reached the greatest heights for the 100.0% soybean oil after 300 minutes of heating (Appendix H, Table H. 1.). The results experienced in this study indicate that hexanal alone would not be a good indicator for vegetable oil stability, considering that a low hexanal peak area percent can also be associated with an increase in the peak areas for

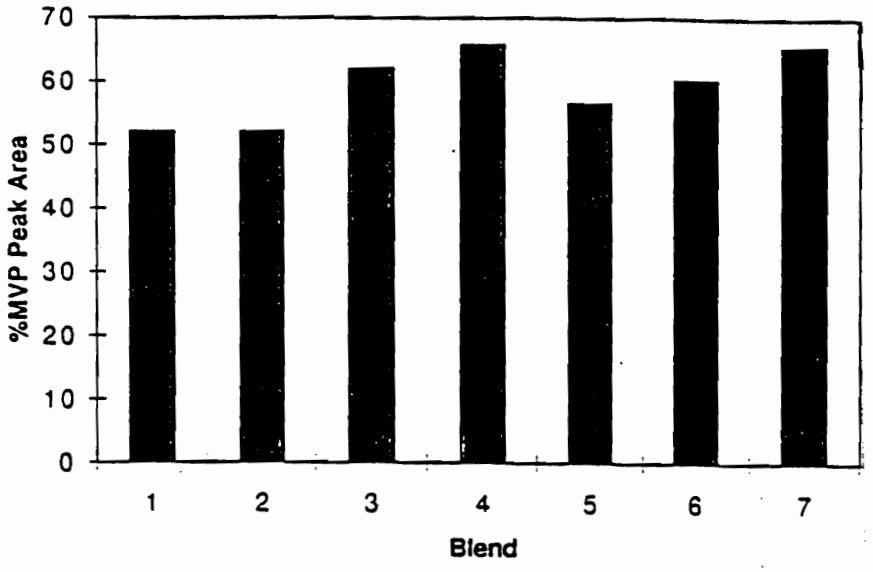


Figure 28. Major volatile products (MVP) for high oleic acid sunflower and soybean oil blends after 900 minutes of heating at 180°C in a closed heating system.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

the major volatiles. However, if the hexanal peak areas are correlated with the major volatile peak areas the following generalizations can be made: (1) a small hexanal peak area with a low MVP% would indicate that the vegetable oil has not experienced extensive levels of autoxidation, and (2) a large hexanal peak area with a low MVP% would indicate a transitional state. Hexanal peak areas that correlate to 25.0 ppm or greater with the *trans*-2-decenal standard curve would be considered large for the observations made in this study.

The toluene peak areas experienced an interesting but predictable trend in each of the vegetable oil blends. The toluene peak areas were the greatest for each of the fresh vegetable oil blends (see Table 17.). The peak areas for toluene decreased after the first 300 minute heating period at 180°C for every blend. Subsequent heating periods lead to a further decrease in the toluene levels for each of the blends. The NBS mass spectra library indicated that toluene was associated with the fatty acid contents of the vegetable oils and provided no further information. However, available research (Chan and Coxon, 1987) shows no indication that toluene is a breakdown product of fatty acid polymerization, dimerization, or autoxidation. The aromatic ring structure and methyl group ($-CH_3$) most closely resembles the first aromatic ring structure in the hydroxylated dihydrochroman ring of the tocopherol isomers (see Appendix M). Furthermore, toluene most closely resembles the alpha tocopherol isomer, which has three methyl

groups associated with the aromatic ring (Pokorny, 1987). The alpha tocopherol isomers are reported to be in the highest concentration (ppb) for sunflower oil (Ackman, 1983). The levels of toluene began to increase again after 2100 minutes of heating at 180°C in the open heating system, representing the largest volatile peak areas and peak area percentages (see Table 19. A. and 19. B.)

Potentially, the initial levels of toluene were associated with the breakdown or dehydration of the alpha tocopherol isomers. Research (Pokorny, 1987) has shown that the alpha tocopherols are the least stable of the tocopherol isomers. This explanation appears reasonable considering that the rate of major volatile production increased as toluene levels decreased. Other research (Nawar, 1985) has suggested that toluene is a breakdown product of *beta*-carotene. Carotenoids such as *beta*-carotene are present in the crude vegetable oils before refinement (Langsraat, 1976), however, the levels after refinement were uncertain in this study. Regardless of the origin of toluene, increased levels were associated with low levels of major volatiles, and decreased levels were associated with increased levels of major volatile products.

G. 6. c. Major Volatile Product Trends for Open Versus Closed Heating Systems.

The volatiles collected from the closed heating system and the open heating system were compared on a peak area percent bases

Table 19. A. Toluene peak areas and peak area percents for volatiles collected from an open heating sysyem containing high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends.

Blend	Toluene Peak Area	Toluene Peak Area %
1	123556	15.68
2	165970	15.02
3	213047	17.93
4	151078	22.75
5	219383	21.72
6	235296	28.26
7	331432	20.42

Table 19. B. Toluene peak areas and peak area percents for volatiles collected from a closed heating system containing high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends.

Blend	Toluene Peak Area	Toluene Peak Area %
1	22568	4.87
2	74075	3.15
3	25440	2.94
4	65906	3.13
5	66486	4.25
6	29380	3.00
7	11029	1.33

Notes:
1. See Table 4. for vegetable oil blend levels.

(see Table 20). Volatiles extracted from the closed heating system were obtained from the blends after heating for 900 minutes at 180°C. Volatiles extracted from the open heating samples were collected after a total heating time of 3000 minutes, with a total 900 minutes of closed heating followed by 2100 minutes of open heating.

The MVP% were approximately half the value experienced for each blend in the open heating system when compared to the closed heating system (see Table 21). The MVP% ranged from 23.77% to 41.0% for the open heating system versus 52.10% to 65.73% for the closed heating system, which were significantly different ($p < 0.01$). Based on these results it would be easy to conclude that open heating was conducive to a reduction in the rate of autoxidation. However, the final peroxide values taken at 3000 minutes (see Table 22) were greater and significantly different ($p < 0.01$)) from the peroxide values taken at 900 minutes in the closed system. In addition, the relative decrease for trilinolein is greater at 3000 minutes when compared to 900 minutes (Appendix G, Tables G. 2. and G. 3.).

When the MVP% were plotted (Figure 29.) for the open heating system, the highest points were experienced for 100.0% high oleic acid sunflower oil (Blend 1) and 100.0% soybean oil (Blend 7). The MVP% values for blend 1 for the open heating samples largely consist of nonanal, a breakdown product of oleic acid (Appendix K,

Table 20. Comparison of the individual percent major volatile products (%MVP) from high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends purged from closed and open heating systems.

	(% 2-Heptenal)		(% Nonanal)		(% 2,4-Heptadienal)		(% 2,4-Decadienal)	
Blend	C-sys	O-sys	C-sys	O-sys	C-sys	O-sys	C-sys	O-sys
1	23.36	12.26	19.84	17.80	2.38	0	2.06	5.27
2	24.39	8.79	14.04	11.35	7.54	1.89	2.53	4.30
3	4.87	12.15	27.78	9.78	12.09	4.00	4.05	3.55
4	2.23	9.14	9.18	8.05	21.39	2.78	4.45	7.86
5	29.53	11.28	8.04	3.43	13.40	4.20	3.53	10.59
6	29.15	12.41	3.69	3.42	20.59	6.65	5.11	12.24
7	28.85	13.58	3.49	2.45	18.65	5.75	6.53	13.21
p value:	(0.0689)		(0.3051)		(0.00363)		(0.0242)	

Notes:
1. C-sys = Closed heating system
2. O-sys = Open heating system

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Table 21. Percent major volatile production (%MVP) for open versus closed heating.

Blend	%MVP [Open]	%MVP [Closed]
1	41.04	52.10
2	25.76	52.25
3	23.77	62.07
4	27.21	65.86
5	30.29	56.73
6	35.70	60.41
7	37.30	65.73

p value: (0.00022)

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Table 22. Comparison of peroxide values (PV) between high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends heated for 900 minutes and 3000 minutes at 180°C.

Blend	PV: 900 min (180°C)	PV: 3000 min(180oC)
1	1.95	5.92
2	2.90	6.16
3	2.37	8.28
4	4.48	10.40
5	3.24	15.35
6	4.98	15.34
7	3.63	19.76

p value: (0.0018)

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

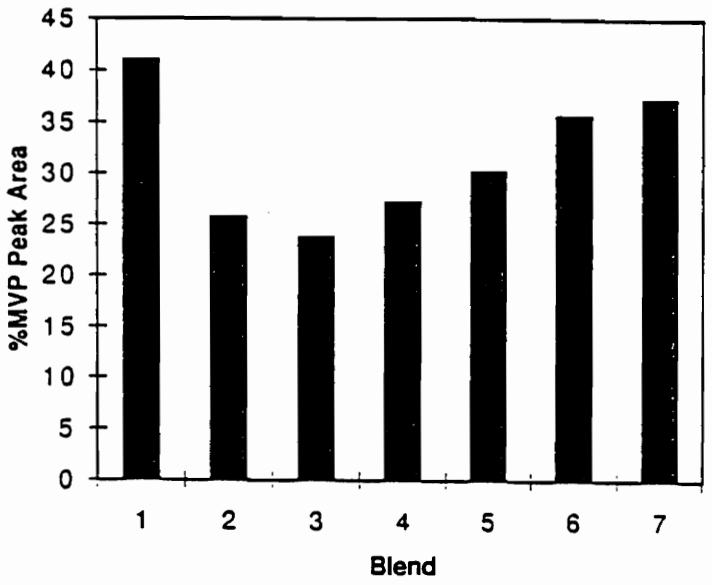


Figure 29 Major volatile products (MVP) for high oleic acid sunflower and soybean oil blends after 2100 minutes of heating at 180°C in an open heating system.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Figure K. 2.). The levels of nonanal decreased as the levels of soybean oil reached 50.0% or greater. The levels of 2,4-decadienal increased as the level of soybean oil increased in the blends. The percent levels of 2,4-decadienal ranged from 13.21% for blend 7 (100.0% soybean oil) to 1.13% for blend 1 (100.0% high oleic acid sunflower oil). The increased levels of 2,4-decadienal and nonanal may have been attributed to two factors: (1) an increased boiling point, and (2) an increased rate of production. Nonanal experienced an average retention time of 27 minutes which correlated to an approximate boiling point of 102°C with the GC temperature program used for volatile elution (see section G. 3. e.). The volatile 2,4-decadienal had an average retention time of 53 minutes which correlated to an approximate boiling point of 158°C. Therefore, it is likely that the equilibrium between the volatiles lost in the open heating system and volatiles produced was shifted towards a greater level of volatile production for nonanal and 2,4-decadienal.

These results indicated that the purging method can greatly influence the volatile profile obtained. The differences between the open and closed heating were only significant ($p < 0.01$) for 2,4-heptadienal percentages between the open and closed heating (see Table 20.). However, the 2,4-heptadienal were on the average of two times greater in the closed heating system with the exception of blends 3 and 4 (75% and 50% high oleic acid sunflower oil), which may have been attributed to the selective autoxidation of oleic acid.

The goal of this study was not to compare the differences between open and closed heating, otherwise each blend would have been heated for exactly the same time frame. However, the information obtained from the comparisons provided some insight into the breakdown patterns of the fatty acids, volatile retention in the vegetable oil, and techniques for maximizing the recovery of vegetable oil volatile products.

G. 7. Overall Ranking of the Thermal Oxidative Stability of High Oleic Acid Sunflower and Soybean Oil Blends.

The peroxide value (PV), percent monoglyceride and diglyceride (%MG/DG), and the percent major volatile production (%MVP) were collectively summarized to produce an overall ranking of the vegetable oil blends used in this study (see Table 23.). Each of the values for each analyses was assigned a number from one to seven, with one representing the lowest value observed in the given blend. The numerical values were then summed together to obtain the overall thermal breakdown ranking (TBR). The lower TBR values correspond to a greater thermal oxidative stability, where the lowest values are the most stable.

The 100% high oleic acid sunflower oil (blend 1) performed the best in each of the individual analyses and possessed the overall lowest TBR ranking of 3 on a scale of 1 to 21. The 100% soybean (blend 7) experienced the largest PV, %MG/DG, and the second largest

Table 23. The thermal breakdown ranking (TBR) for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends using the peroxide value (PV), percent major volatile production (%MVP), and the percent monoglyceride and diglyceride (%MG/DG).

Blend	PV	%MG/DG	%MVP	Overall Ranking
1	5.92	0	52.10	3
2	6.16	0.13	52.25	6
3	8.28	0.28	62.07	11
4	10.40	0.13	65.86	12
5	15.35	0.31	56.73	14
6	15.34	0.30	60.41	13
7	19.76	0.40	65.73	20

* p value: (0.9890)

Notes:
1. The values for each analyses were assigned a number ranking between 1 and 7, with seven representing the highest value experienced in the seven blends.
2. The ranking values were then summed together to obtain the final ranking value for each blend.
3. A larger TBR corresponds to a decrease in thermal oxidative stability.

* A One Way ANOVA was performed on blends 3 through 6 to determine if these blends were significantly different from one another based on the summation of the PV, %MG/DG, and %MVP.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

%MVP, and possessed the overall largest TBR value. The largest TBR value experienced by 100% soybean clearly indicates that the polyunsaturated vegetable oil is less stable than the high oleic acid vegetable oil. Furthermore, the decrease in the polyenic components compared to the monoenic and saturated components supports the TBR value for soybean oil.

The overall thermal oxidative stability of the vegetable oil blends containing between 75.0% and 10.0% high oleic acid sunflower oil are less discernible between these blends (see Figure 30.). The TBR values for these 4 blends ranged between 11 and 14, with blend 6 (10% high oleic acid sunflower oil) having a lower value than blend 5 (25% high oleic acid sunflower oil). However, certain observations can be made. The blends with 90.0% or greater of high oleic acid sunflower oil were clearly more stable than the blends falling into the range of 75% to 10% high oleic acid sunflower oil. The blends in the 75% to 10% high oleic acid range where not significantly different ($p > 0.01$) from one another based on the summation of all of the analyses. When the peroxide values are evaluated independently, the thermal oxidative stability decreased as the level of high oleic acid progressively decreased with each blend. The %MG/DG basically indicated that the addition of soybean oil to high oleic acid sunflower oil lead to an increase in the production of monoglycerides and diglycerides, which were not present in the 100% high oleic acid sunflower oil. The %MVP indicated that the addition of 25.0% or greater of soybean oil to the blends lead to an

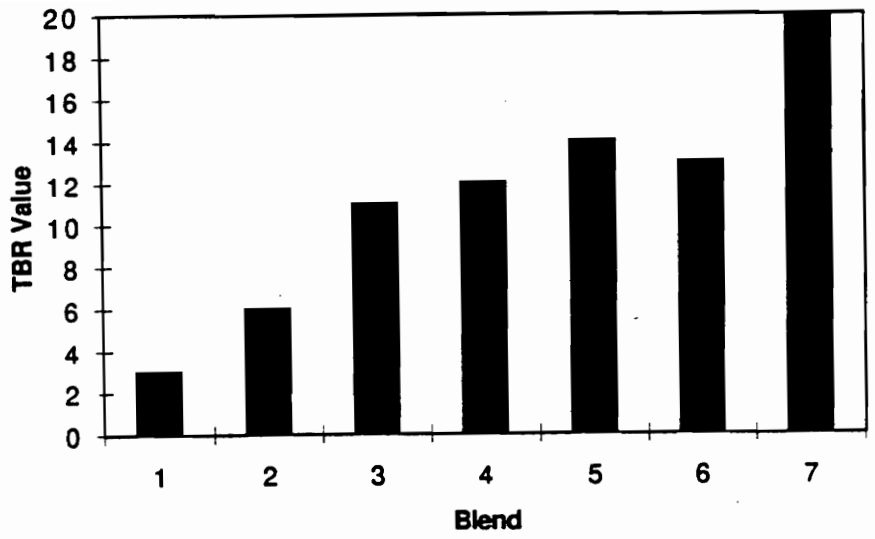


Figure 30. The thermal breakdown ranking (TBR) for high oleic acid sunflower oil (HOSO) and polyunsaturated soybean oil (PUSB) blends: Based on the the peroxide value (PV), % monoglyceride and diglyceride (%MG/DG) after 3000 minutes of heating at 180°C ,and the % major volatile production (%MVP) after 900 minutes of heating in a closed system at 180°C.

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

CHAPTER V.
CONCLUSIONS

The results of this study indicated a number of conclusions regarding the vegetable oil blend's thermal oxidative stability based on the levels of high oleic acid sunflower and soybean oil present in each blend. Conclusions were also reached for the analytical methods used to evaluate the vegetable oil blends in this study. All of the conclusions reached are in general agreement with those presented in the literature review chapter. In a few cases the conclusions were drawn largely from the results of this study, due to the lack of available research literature. In each case the conclusions were supported by the best statistical analysis and research pertaining to vegetable oil stability, composition, and analysis available.

The individual vegetable oil triglycerides and fatty acids showed similar trends in each of the blends. The relative stability ranking system was useful in illustrating these trends between and within the vegetable oil blends. The saturated fatty acids, palmitic and stearic acid, were the most stable fatty acids to the thermal oxidative treatment. The monoenic fatty acids, present as oleic acid and triolein experienced the second highest stability ranking value and the polyenic fatty acids and triglycerides linoleic and linolenic acid, and trilinolein represented the least stable group. One major exception occurred between the monoenic and polyenic group. In

blend 4 ([1:1] high oleic acid sunflower to soybean oil), the polyenic fatty acids experienced a relative percent increase where oleic acid experienced a relative percent decrease. The occurrence was observed in all four trials of blend four. Furthermore, each of the blends were prepared independently of one another. The only difference occurring between the blends was the concentration levels of the fatty acids and triglyceride. The triglyceride composition was the same for each vegetable oil blend, stemming from high oleic acid sunflower and soybean oil. Naturally, blends seven and one (100.0% soybean and 100.0% high oleic acid sunflower oil) possessed triglyceride profiles which were unique to the parent vegetable oils. No unusual triglyceride peaks were noted in any of the HPLC triglyceride chromatograms, only differences in the relative peak areas.

Therefore, it can be concluded that the (1:1) blend experienced a breakdown pattern that was unique to blend four. More specifically, the selectivity for oleic acid breakdown had to be related to the interaction of the triglycerides at the (1:1) blend level with 50.% high oleic acid sunflower oil. Previous research (Raghuveer and Hammond, 1966) has indicated that the differences occurring in triglyceride crystal packing structures can result in an accelerated rate of oxidation. Unfortunately, the literature involving vegetable oils blends or vegetable oils of varied composition related to triglyceride crystal packing and oxidative breakdown is not available at this time. This would be an area of worthy research, an

area that would fill in the missing link of triglyceride stability and response to oxidation. In reality, it is uncertain as to how the triglyceride packing structures effect the rate of oxidation. Other indicators that singled out blend 4 from the other blends was the initial increase in the peroxide value after blending and the overall increase in the percentage of major volatiles. Even though blend 4 showed signs of increased levels of oxidation and a different breakdown pattern, it was still more stable than the 100.0% soybean oil and was compatible with blends containing 10.0% and 75.0% high oleic acid sunflower oil.

The addition of high oleic acid sunflower oil improved the stability of the polyunsaturated soybean oil at each blend level. None of the vegetable oil blends experienced a higher peroxide value or a major volatile production percentage greater than 100.0% soybean oil. In addition, the monoglyceride/diglyceride percentages were lower for each of the blends when compared to the 100.0% soybean oil. The increase in stability for the vegetable oil blends were also evident by the increase in triolein stability and decrease in trilinolein stability. Triolein was the dominant triglyceride of high oleic acid sunflower oil, representing approximately 82.0% of the vegetable oil. Therefore, it can be concluded that the addition of triolein to the vegetable oil blends was largely responsible for the increase in thermal oxidative stability. Similar results were obtained by Frankel and Huang (1994) in the blending of high oleic acid sunflower oil to canola oil. It is apparent based on this study

and on previous research that triolein is clearly more stable than triglycerides containing more than 3 total double bonds. However, there is a need for more research to be conducted comparing the oxidative stability of vegetable oils rich in triolein to hydrogenated vegetable oils and triglycerides containing oleic acid and saturated fatty acids such as palmitic or stearic acid.

The thermal oxidative stability was determined based on the following observations: (1) a decrease in polyenic fatty acid and trilinolein (LLL), (2) an increase in oleic acid and triolein (OOO), (3) an increase in peroxide value (PV), and (4) an increase in the production of major volatiles (%MVP). Each of these tests served as an integral part of the analysis as a whole. However, no individual test individually formed a complete picture of how the vegetable oils were decomposing and what products were being produced. The HPLC triglyceride analysis was effective at showing the stability of OOO and LLL in addition to the emergence of monoglyceride and diglyceride peaks. However, without the GC/FAME analysis the tentative triglyceride identification would have been impossible, and the relative decrease in oleic acid and relative increase in the polyenic fatty acids occurring in blend 4 (50.0% high oleic acid sunflower oil) would have gone undetected. The GC/FAME analysis served as an excellent complement to the HPLC triglyceride analysis. Collectively, the GC/FAME and HPLC triglyceride analyses were effective at showing which substrates were more prone to thermal degradation. The PV was a quick and relatively simple analysis

which showed a general trend for the thermal oxidation of the vegetable oil blends. The peroxide values alone would have also neglected to show the unique breakdown pattern of blend four. However, when the PV was correlated to the %MVP the test collectively had a powerful effect in illustrating the thermal breakdown pattern of the vegetable oil blends. The PV generally corresponded with the continued increase of the %MVP as the accumulated heating time increased.

The %MVP also showed a good correlation to the GC/FAME analysis. The major volatile breakdown products were those produced from the major fatty acids comprising the vegetable oils. The relative percent levels of the fatty acids reflected the percent increases in the volatile end products appearing on the GC/volatile chromatograms. The blends richest in oleic acid content, such as 100.0% high oleic acid sunflower oil, produced the greatest levels of nonanal, where as the blends richest in linoleic acid content, such as 100.0% soybean oil produced the greatest levels of 2,4-decadienal. The exact loss of each volatile from the vegetable oil blends during the purging process is uncertain, even with the use of the internal standard. However, the trends were similar for each of the four trials for each of the individual blends.

The low percentages of monoglyceride and diglyceride peak areas indicated that hydrothermal hydrolysis was not a major reaction in

the oxidation of the fatty acids. The decrease in triglyceride substrates was not associated with an increase in monoglyceride or diglyceride percentages. It can only be theorized that the decrease in triglyceride content was associated with autoxidation of the intact triglyceride fatty acids, triglyceride polymerization, and dimerization, considering that the monoglyceride and diglyceride peak area percents were less than 0.1% of the total composition for each of the blends. Previous researchers (Dobarganes, et. al 1993; Cuesta, et. al, 1993) have found similar results using methods to detect the levels of polar components and polymerized products. The results indicated that the majority of the breakdown products were in the form of triglyceride polymers and dimers.

The use of a closed heating system and 4 trials per blend eliminated a great deal of uncertainty in this study. Four trials for each blend were adequate in demonstrating that each respective blend responded similarly to the testing conditions. Each blend experienced approximately the same PV, %MVP, %MG/DG, and increase or decrease in fatty acids and triglycerides. Therefore, it can be concluded that the results are a fairly reliable accurate representation of the thermal oxidative stability for each of the vegetable oil blends. The closed heating system without the addition of food particulates or any other components not inherent to the vegetable oils helped to correlate the thermal oxidative stability directly to the blend composition. In addition, volatile recovery was greatly enhanced in the closed heating system,

evidenced by the overall decrease occurring in the open heating system with the exception of 2,4-decadienal, which had the highest boiling point of all of the major volatiles. The open heating system served an important role in the study. The purpose of the open heating system was to increase the rate of thermal oxidation by increasing the oxygen exposure to the vegetable oil. This goal was accomplished, whereas the decrease in the polyenic fatty acids, and trilinolein were significantly different from those found in the closed heating system. The trends were the same for both the closed and open heating periods with the exception of the decrease in %MVP experienced in the open heating system. This result was anticipated considering that some of the volatiles would be lost from the vegetable oil sample into the surrounding atmosphere during open heating. Therefore, the %MVP values obtained from the open heating were not used in the ranking of the vegetable oil blends.

The methods for volatile collection greatly influenced the recovery of the volatile components from the vegetable oil samples. The research conducted in this study clearly indicated that the volatiles collected immediately after heating with a short nitrogen purging period produced the greatest recoveries for the internal standard and the major volatiles. The recoveries were no less than 20.0 times greater for the major volatiles. It is evident that the levels of volatiles in the vegetable oil samples were far greater than the average volatile extraction method could detect. The use of thermal purging of volatiles is potentially the best method for

producing the most accurate volatile profile and abundance levels occurring in the vegetable oil. Unfortunately, this method is only applicable to vegetable oils that require a heating period in their evaluation. Therefore, this method could be extended to frying oils and thermal oxidative studies.

The analyses used in this study were collectively effective in determining the levels of thermal oxidation experienced in each vegetable oil blend and making comparisons between blends. However, like other published procedures no one value can be used to estimate the thermal oxidative stability of a vegetable oil under a given set of conditions. Frankel and Huang (1994) correlated the hexanal peak areas with the peroxide values for high oleic acid sunflower and canola oil blends. This was a step in the right direction. Unfortunately, hexanal is only a good indicator for linoleic acid. The hexanal peak areas in this study were the largest at the onset of vegetable oil breakdown, however, the levels decreased with subsequent heating. The major volatile peak areas represented collectively by (Z)-2-heptenal, nonanal, (E,E)-2,4-heptadienal, and 2,4-decadienal experienced an increase in peak area percent with continued heating in this study.

The final stability ranking of the vegetable oil blends collectively using the PV, %MVP, and %MG/DG showed that blends containing between 75.0% and 10.0% high oleic acid sunflower oil had overall ranking values which were between 11 and 14, on a scale

of 1 to 20. Therefore, it can be concluded that the addition of 10.0% to 75.0% of high oleic acid sunflower oil to polyunsaturated soybean oil will improve the thermal oxidative stability equally as well. However, and addition of 90.0% high oleic acid sunflower oil to polyunsaturated soybean oil will greatly improve the thermal oxidative stability, indicated by the ranking value which was half of that experienced by blends with 75.0% and 10.0% high oleic acid sunflower oil.

The next step in research will be to compare these blends under frying conditions with the addition of food particulates such as potato slices or chicken strips. After evaluating the performance of these blends under frying conditions, a comparison to hydrogenated vegetable oils can be made. Potentially, the high oleic acid sunflower oil blends can be used to replace the hydrogenated vegetable oils currently dominating the market, especially if they experience similar changes in the thermal oxidative stability. At the very least, hydrogenated vegetable oil and high oleic acid sunflower oil blends can be considered as a means to reduce the levels of *trans*-fatty acids occurring in the hydrogenated vegetable oils. The consumer awareness of nutrition and food composition is growing, and within time, the general public will become aware of the high levels of hydrogenated vegetable oils used in the fast food and snack food industries.

Recently, high oleic acid vegetable oils have appeared on the

grocery store shelves. The results of this study indicates that vegetable oil blends with a minimum of 10.0% high oleic acid sunflower oil can be expected to last longer under thermal oxidative conditions such as frying and in storage when compared to the polyunsaturated soybean oils. If the composition is any indication of the vegetable oil's stability, then polyunsaturated vegetable oils with similar composition to soybean oil can be expected to be improved by an addition of 10.0% or greater of high oleic acid sunflower oil. Furthermore, based on the results of this study, vegetable oils with approximately the same concentration of triolein (OOO) can be expected to improve the quality of polyunsaturated vegetable oils by a 10.0% addition or greater. However, it should be recognized that other factors such as vegetable oil refinement, storage conditions, and the presence of antioxidants will have a great impact on the quality of any vegetable oil.

The final considerations that need to be taken into account are the consumers' preference for these vegetable oil blends. It is the consumer that ultimately determines the marketability of a product. If the general public does not like a fried food, snack food, or even the smell of a vegetable oil blend, they will not use it for long. Therefore, sensory test should be conducted for both the fried foods, snack food, and vegetable oil blends before trying to market any of the vegetable oil blends.

In summary, it is apparent that there are viable solutions to vegetable oil hydrogenation, at least at the initial stages. The results produced in this study are promising for the future of *trans*-fatty acid free vegetable oils. High oleic acid sunflower oil, attributed to the triolein content, has the potential to improve lower quality polyunsaturated vegetable oils by blending. In the near future high oleic vegetable oils and vegetable oil blends may be the dominant vegetable oils used in the fast food and snack food industries, and also in the home.

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Appendix A

Blend Profile Data for Peroxide Values, Triolein, and Trilinolein.

Table A. 1. Peroxide values (PV) for blend profile high oleic acid sunflower and soybean oil vegetable blends.

(PV = *u*eqv. O_2/g of vegetable oil blend)

Blend	Trial 1	Trial 2	Trial 3
1	1.03	0.99	0.70
2	0.85	0.87	0.89
3	1.12	1.30	0.90
4	1.58	1.12	1.23
5	0.73	0.69	0.68
6	0.56	0.92	0.68
7	0.91	0.67	0.53

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Table A. 2. Blend profile HPLC triglyceride analysis: % weight based on 85.0 mg samples.

Blend	LLL	OOO	DB 6-9	DB 3-5	DB 1-3
1a	1.27	83.23	0	8.22	91.56
1b	1.22	83.28	0	8.02	91.69
1c	1.22	83.07	0	8.09	91.61
	(1.24)	(83.19)	(0)	(8.11)	(91.62)
2a	2.51	79.42	0.35	11.84	87.79
2b	2.31	80.79	0.37	10.95	88.67
2c	2.37	80.21	0.40	11.26	88.33
	(2.40)	(80.14)	(0.37)	(11.35)	(88.26)
3a	5.97	71.38	0.91	21.02	78.10
3b	6.16	70.46	1.04	21.67	77.27
3c	6.03	70.70	1.04	21.46	77.50
	(6.05)	(70.85)	(1.00)	(21.38)	(77.62)
4a	12.28	53.61	2.41	38.76	58.80
4b	12.20	53.74	2.42	38.83	58.96
4c	12.18	53.21	2.50	38.92	58.51
	(12.22)	(53.52)	(2.44)	(38.57)	(58.76)
5a	24.64	17.07	5.96	74.52	19.26
5b	24.98	16.82	5.92	75.49	18.49
5c	25.07	16.92	5.83	75.47	18.66
	(24.70)	(16.94)	(5.90)	(75.16)	(18.80)
6a	27.33	11.03	6.76	80.39	12.63
6b	27.58	10.84	6.78	80.67	12.44
6c	27.87	10.37	6.91	80.87	11.60
	(27.59)	(10.75)	(6.82)	(80.64)	(12.22)
7a	30.86	1.11	8.05	87.54	4.39
7b	30.89	1.11	7.90	87.70	4.38
7c	30.79	1.08	7.92	87.66	4.38
	(30.85)	(1.10)	(7.96)	(87.63)	(4.38)

Appendix B

Tentative Triglyceride Content of High Oleic Acid Sunflower and Polyunsaturated Soybean Oil.

Table B. 1. Equivalent carbon numbers (ECN) for potential triglycerides occurring in high oleic acid sunflower and soybean oil blends.

Triglyceride	Double Bond #	ECN
LnLnLn	9	36
LLnLn	8	38
LLnL	7	40
LLL	6	42
LLnP	5	42
LLO	5	44
OLnL	5	44
LLP	4	44
OLnP	4	44
OLO	4	46
LLS	4	46
OLP	3	46
PLP	3	46
SLnP	3	48
OOO	3	48
OLS	3	48
OOP	2	48

Table B. 1. (Cont.)

Triglyceride	Double Bond Number	ECN
PLS	2	48
POP	1	48

Table B. 2. Triglyceride balance sheet for high oleic acid sunflower oil.

Triglyc-eride	Peak Area % (HPLC)	% C18:2 (FAME)	% C16:0 (FAME)	% C18:1 (FAME)	% C18:0 (FAME)
*LLL	1.27	1.27			
LLP	1.28	0.58	0.422		
LLS	5.47	3.66			1.80
*OOO	83.23			83.23	
OLS	2.83	0.934		0.934	0.934
PLS	5.43	1.79	1.79		1.79
Totals:		8.51	2.21	84.16	4.50

* = Positively identified by individual reference standards.

Table B. 3. Triglyceride balance sheet for soybean oil.

TG	AREA %	C18:2	C18:3	C18:1	C18:0	C16:0
LLnL	7.62	5.10	2.51			
*LLL	30.86	30.86				
OLnL	1.15	0.38	0.38	0.38		
LLO	21.50	7.09		14.40		
POL	15.71	5.18		5.18		5.18
POL,LLS	6.94	3.47		1.14	1.14	1.14
SLnP	10.92		3.60		3.60	3.60
PPL	0.46	0.15				0.31
*OOO	1.11			1.11		
OLS	3.05	1.00		1.00		1.00
Totals:		53.23	6.49	23.21	5.74	0.23

* = Positively identified by individual reference standards.

Table B. 4. Nomenclature for fatty acids contained in vegetable oil Triglycerides.

Fatty Acid	Letter Symbol	Carbon # to Double Bond #
Palmitic	P	C16:0
Stearic	S	C18:0
Oleic	O	C18:1
Linoleic	L	C18:2
Linolenic	Ln	C18:3

Table B. 5. Triglyceride reference table showing the potential resolution of triglycerides occurring in high oleic acid sunflower oil and soybean oil (Caboni, et. al, 1992).

Triglyceride & Resolution Order	% Soybean Oil	% High Oleic Acid Sunflower Oil
LnLnLn	0	0
LLnL	0.4	0.4
LLL	8.3	1.2
LLnO	32.8	5.4
LLnP	2.2	0
LLO	20.5	0
LLP	17.7	0
OLP	10.2	0
PLP	4.6	0
OOO	0.6	80.3
OLS	2.5	0
OOP	0	6.6
POP	0	5.6

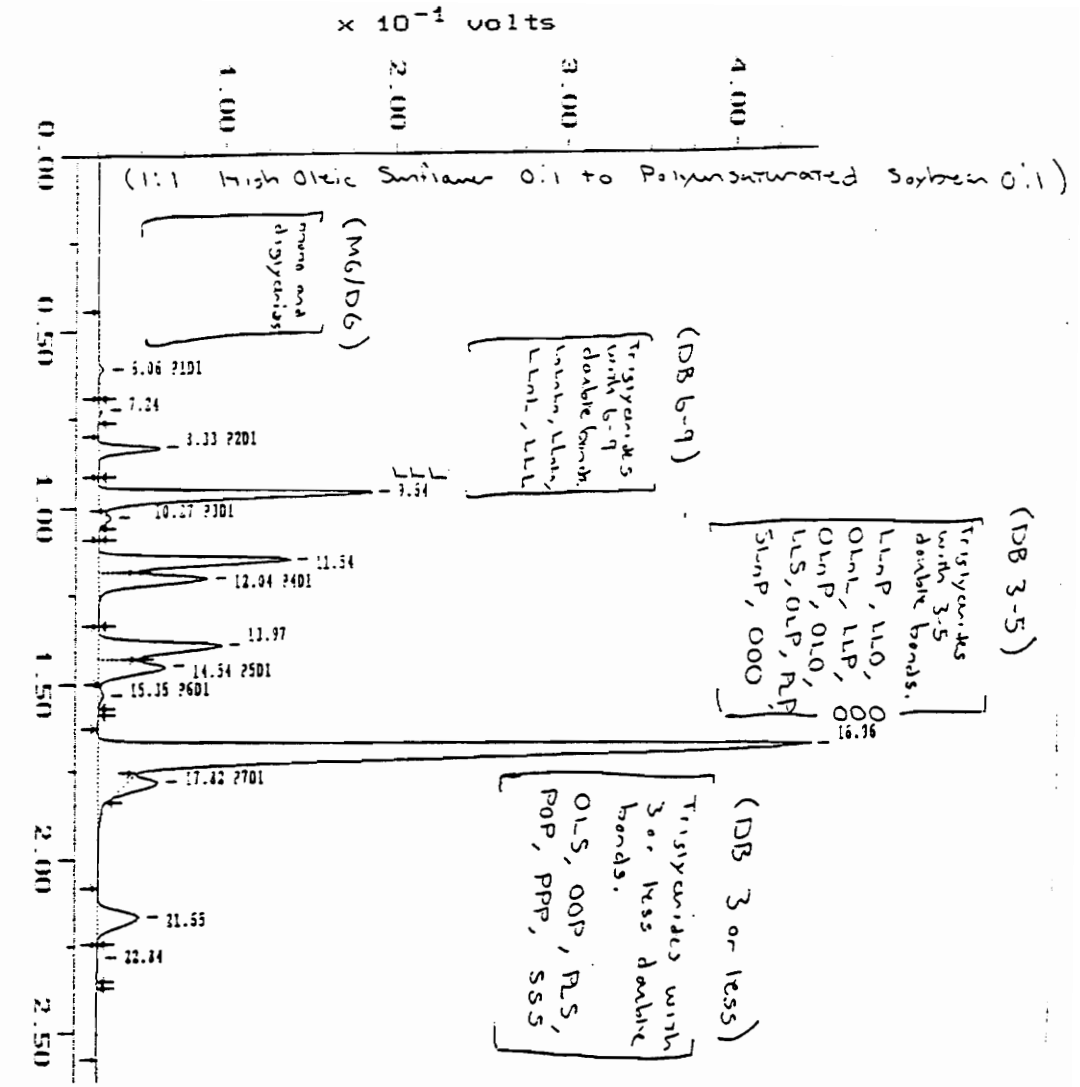


Figure B. 1. Triglyceride categorization by double bond number.

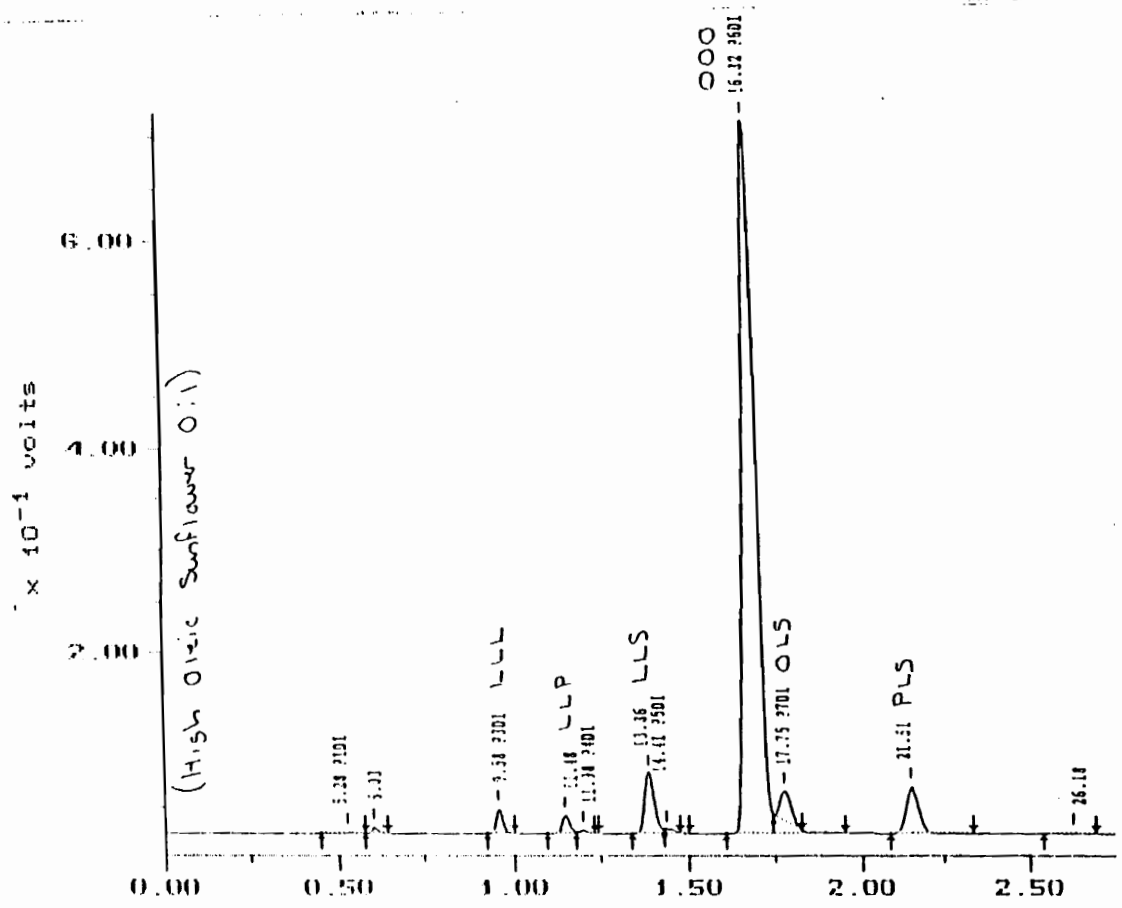


Figure B. 2. Tentative triglyceride composition of high oleic sunflower oil.

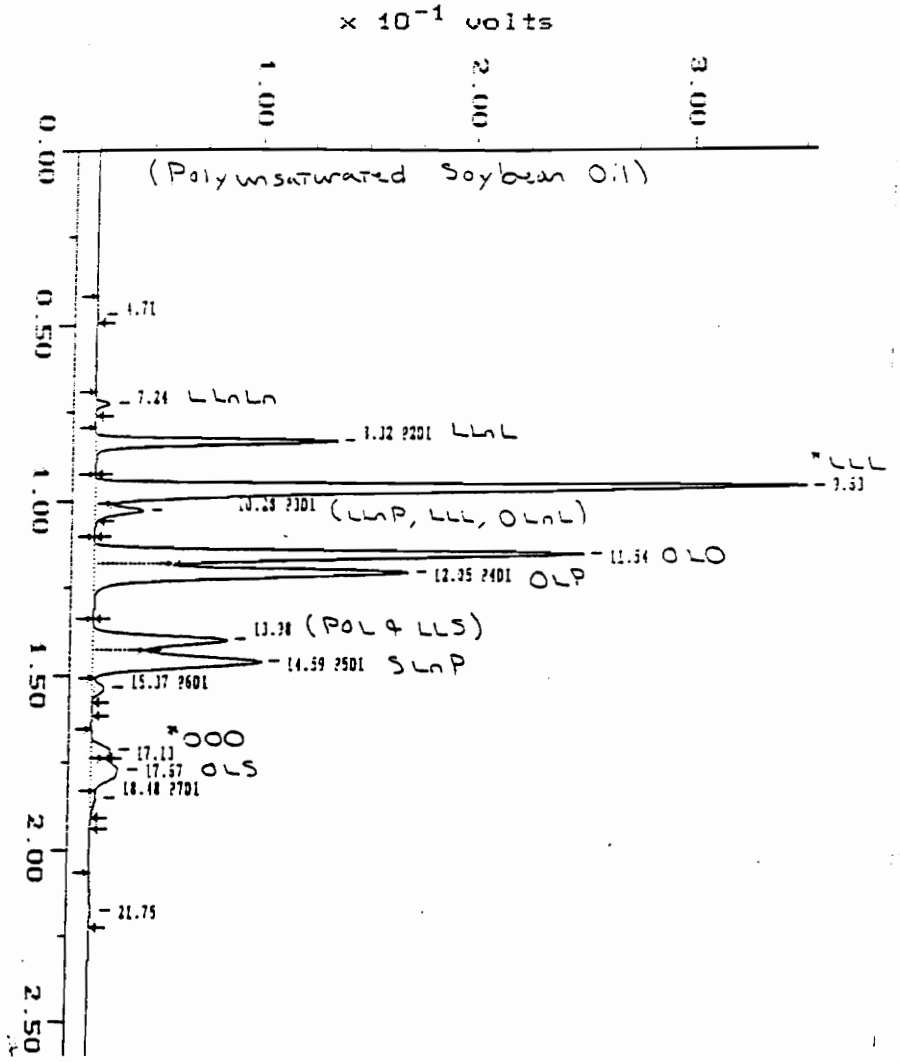
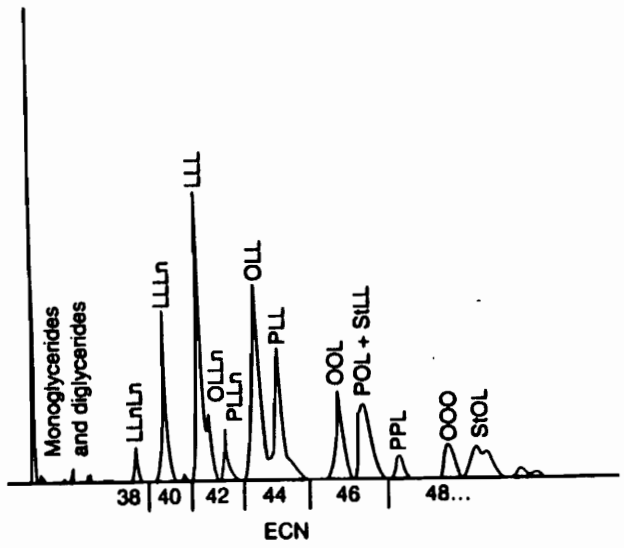
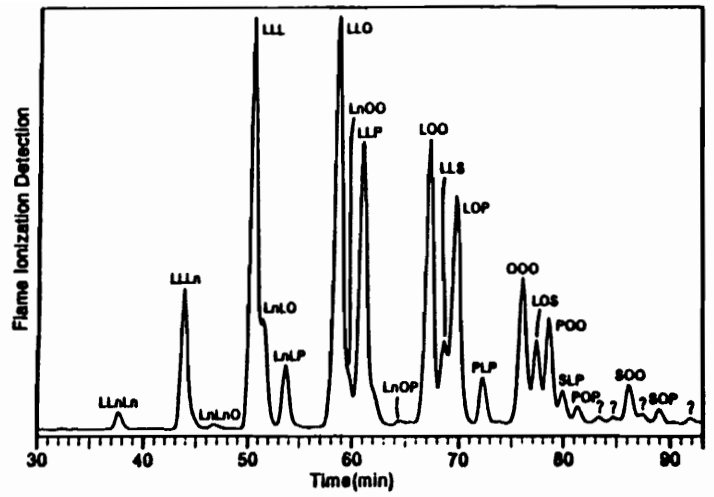


Figure B. 3. Tentative triglyceride composition of soybean oil.



(A)

Figure B. 4. (A) AOCS Official Method Ce 5b-89 soybean oil triglyceride reference chromatogram.



(B)

Figure B. 4. (B) Soybean oil reference triglyceride chromatogram (Neff, et. al, 1992)

Appendix C

Standard Curves for Triolein, Trilinolein, and *trans*-2-decenal.

Table C. 1. Linear regression curve for *tran*-2-decenal.

Trans-2-decenal Standard Curve						
ppm	Peak Area					
12.5	55272					
12.5	54535					
12.5	53854					
25	109228					
25	117058					
25	102480					
50	181531					
50	209159					
50	204387					
SUMMARY OUTPUT						
Regression Statistics						
Multiple R	0.98997806					
R Square	0.98005657					
Adjusted R Squ	0.97720751					
Standard Error	9568.06189					
Observations	9					
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	3.1492E+10	3.1492E+10	343.992735	3.2857E-07	
Residual	7	640834658	91547808.3			
Total	8	3.2133E+10				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	10168.5	6765.64144	1.5029617	0.17655201	-5829.6884	26166.6884
X Variable 1	3794.2381	204.573768	18.5470411	3.2857E-07	3310.49835	4277.97784

Upper 95%	*Lower 95.0%*	*Upper 95.0%*
26166.6884	-5829.6884	26166.6884
4277.97784	3310.49835	4277.97784

Table C. 2. Standard curve for *trans*-2-decenal.

t-2-decenal Standard Curve	
ppm	Peak Area
1	4422
6.25	27636
12.5	55272
25	110544
43.75	193452
50	221088

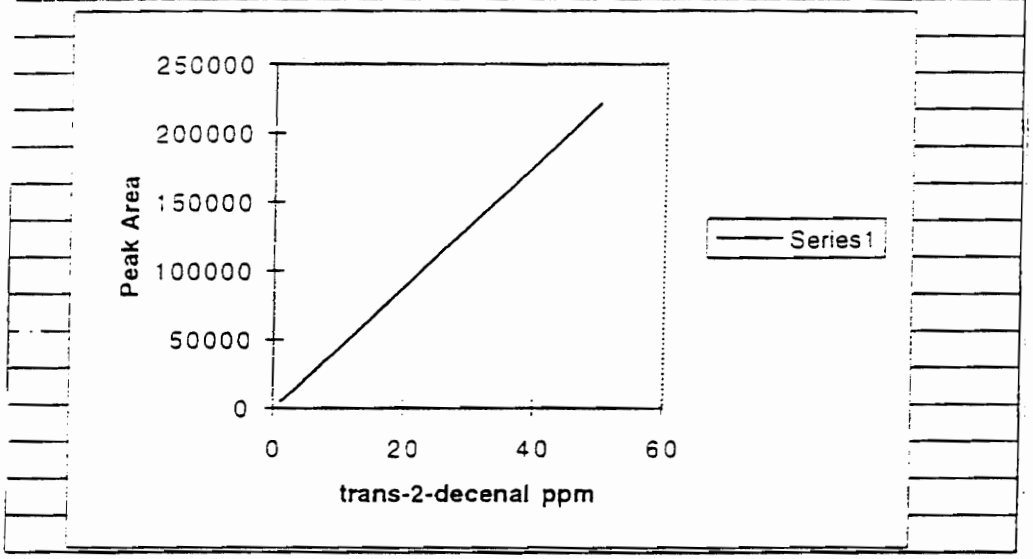


Table C. 3. Linear regression for triolein (OOO).

Linear Regression Curve for OOO						
mg	Peak Area					
5	1411381					
5	1463400					
10	2082629					
10	2237200					
30	8480916					
50	14156153					
50	13700000					
100	28273528					
SUMMARY OUTPUT						
Regression Statistics						
Multiple R	0.99946429					
R Square	0.99892886					
Adjusted R Squ	0.99875034					
Standard Error	1.17244438			*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Observations	8			2.54093358	-0.3462723	2.54093358
				3.6131E-06	3.3842E-06	3.6131E-06
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	7691.75225	7691.75225	5595.5243	3.842E-10	
Residual	6	8.24775499	1.37462583			
Total	7	7700				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	
Intercept	1.09733063	0.5899689	1.85998046	0.11222754	-0.3462723	
X Variable 1	3.4987E-06	4.6771E-08	74.8032372	3.842E-10	3.3842E-06	

Table C. 4. Linear regression for trilinolein (LLL).

Linear Regression Curve for LLL						
mg	Peak Area					
25	6554462					
25	6789473					
30	8028950					
30	8315397					
55	15859741					
55	15879992					
SUMMARY OUTPUT						
Regression Statistics						
Multiple R	0.99964038					
R Square	0.99928089					
Adjusted R Squ	0.99910112					
Standard Error	0.4310099			*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Observations	6			4.6778705	2.01058769	4.6778705
				3.376E-06	3.1336E-06	3.376E-06
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1032.59026	1032.59026	5558.44783	1.9397E-07	
Residual	4	0.74307813	0.18576953			
Total	5	1033.33333				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	
Intercept	3.3442291	0.48034036	6.96220717	0.00223704	2.01058769	
X Variable 1	3.2548E-06	4.3656E-08	74.5549987	1.9397E-07	3.1336E-06	

Table C. 5. Standard curve for triolein (OOO).

Triolein Standard Curve	
mg/10 ml	Peak Area
1	287478
5	1437390
25	7186952
50	14373905
75	21560857
100	28747810

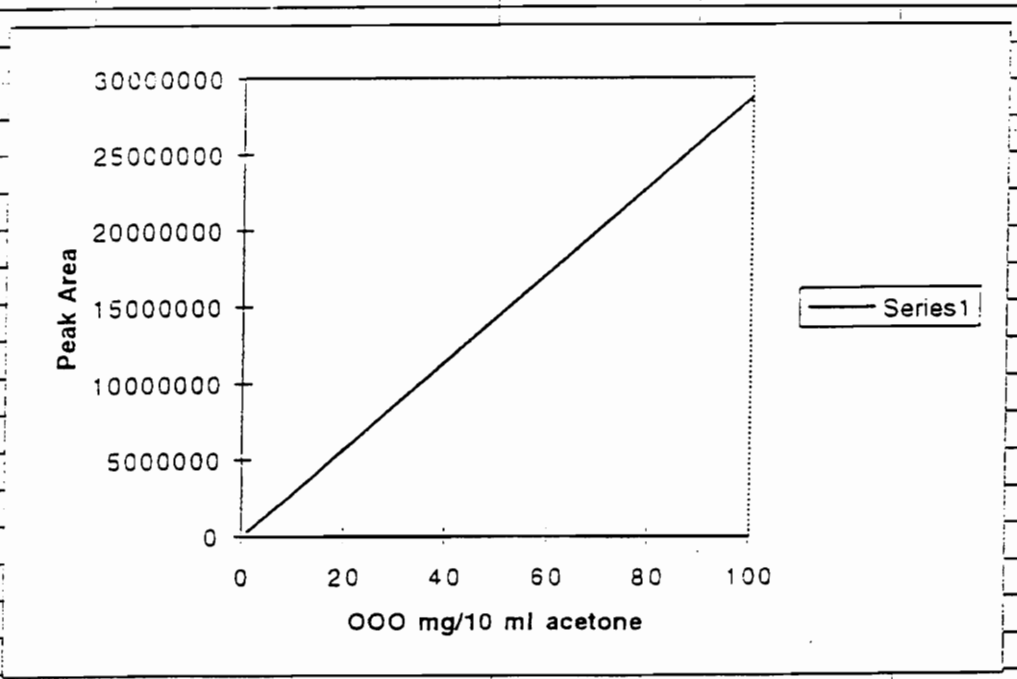


Table C. 6. Standard curve for trilinolein (LLL).

Trilinolein STD Curve	
mg/10 ml	Peak Area
1	287478
5	1437390
25	7186952
50	14373905
75	21560857
100	28747810

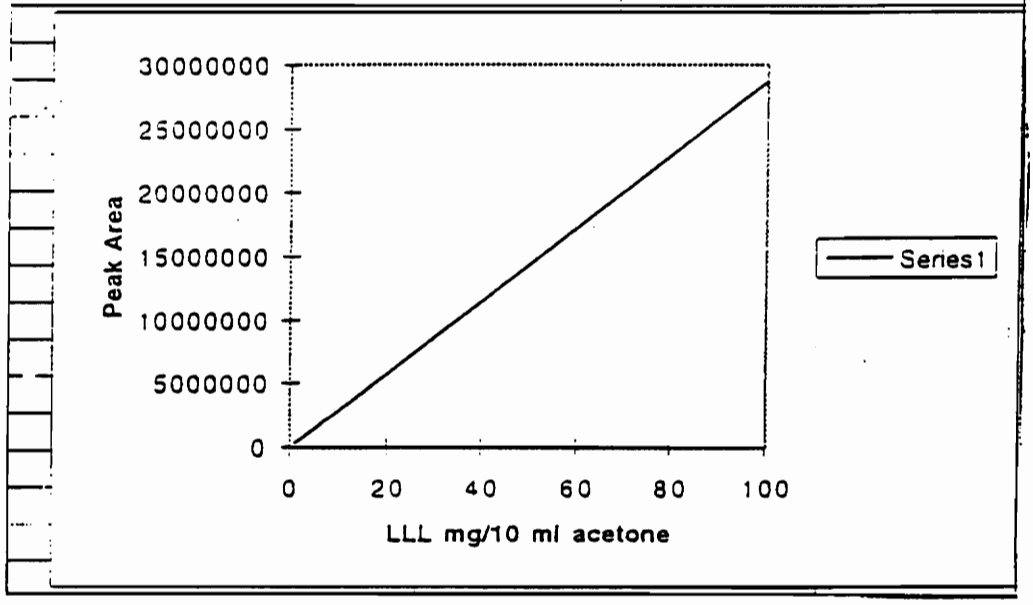


Table C. 7. Triolein (OOO) HPLC peak area comparison between the blend profile and fresh starting 100.0% high oleic acid sunflower oil.

Trial	Sample wt. (mg)	OOO Peak Area %	Std Curve OOO Peak Area	Std Curve (mg) value
a	80.70	83.23	18733343	66.26
b	80.80	83.28	18427493	65.18
c	85.00	83.07	19841672	70.20

Notes:

1. $\frac{\text{Std Curve (mg)}}{\text{Sample wt. (Mg)}}$ (100) = Std Curve % Composition

2. $\frac{\text{Std Curve \% Composition}}{\text{OOO Peak Area \%}}$ (100) = % Correlation

a = 66.26/80.70 (100) = 82.10%, 82.10/83.23 (100) = 98.64% Correlation

b= 65.18/80.80 (100) = 80.67%, 80.87/83.28 (100) = 96.87% Correlation

c= 70.20/85.00 (100) = 82.60%, 82.60/83.07 (100) = 99.40% Correlation

Table C. 8. Trilinolein (LLL) peak area comparison between the blend profile and fresh starting 100.00% high oleic acid sunflower oil.

Trial	Sample wt. (mg)	LLL Peak Area %	Std Curve LLL Peak Area	Std Curve (mg) Value
a	80.70	1.27	285107	1.07
b	80.80	1.22	270340	1.02
c	85.00	1.22	291122	1.09

Notes:

1. $\frac{\text{Std Curve (mg)}}{\text{Sample wt. (Mg)}}$ (100) = Std Curve % Composition

2. $\frac{\text{Std Curve \% Composition}}{\text{OOO Peak Area \%}}$ (100) = % Correlation

a= 1.07/80.70 (100) = 1.32, 1.32/1.27 (100) = 103.94% Correlation

b= 1.02/80.80 (100) = 1.26, 1.26/1.27 (100) = 103.28% Correlation

c= 1.09/85.00 (100) = 1.28, 1.28/1.22 (100) = 105.11 % Correlation

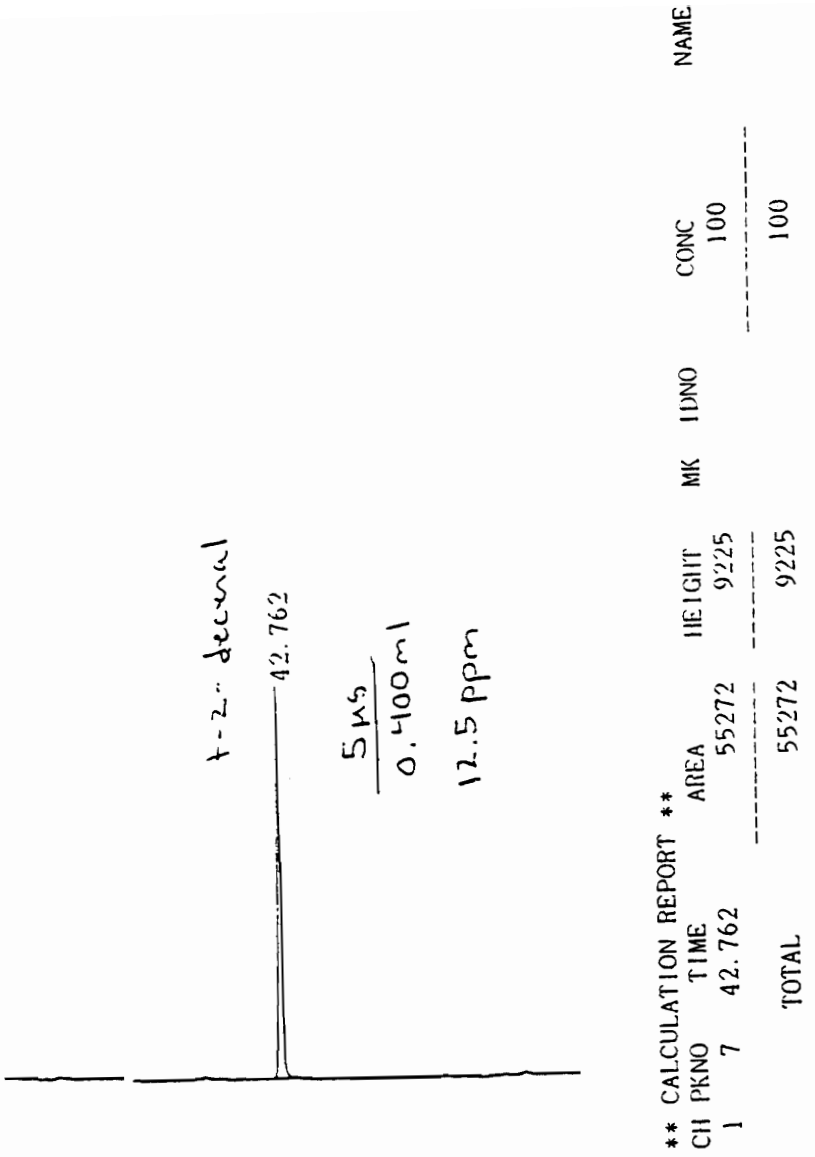


Figure C. 1. GC volatile chromatogram of *trans*-2-decenal at 12.5 ppm.

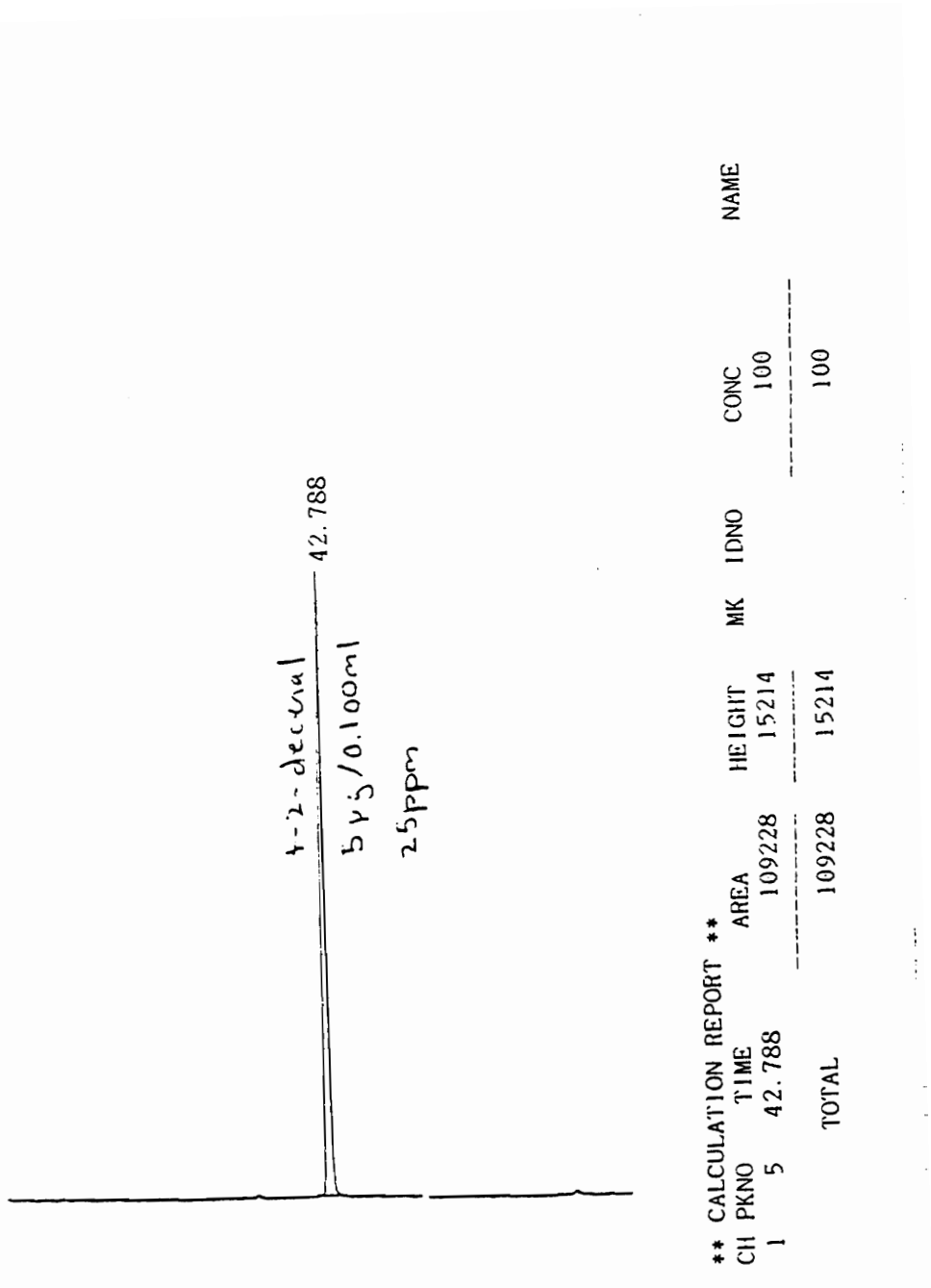
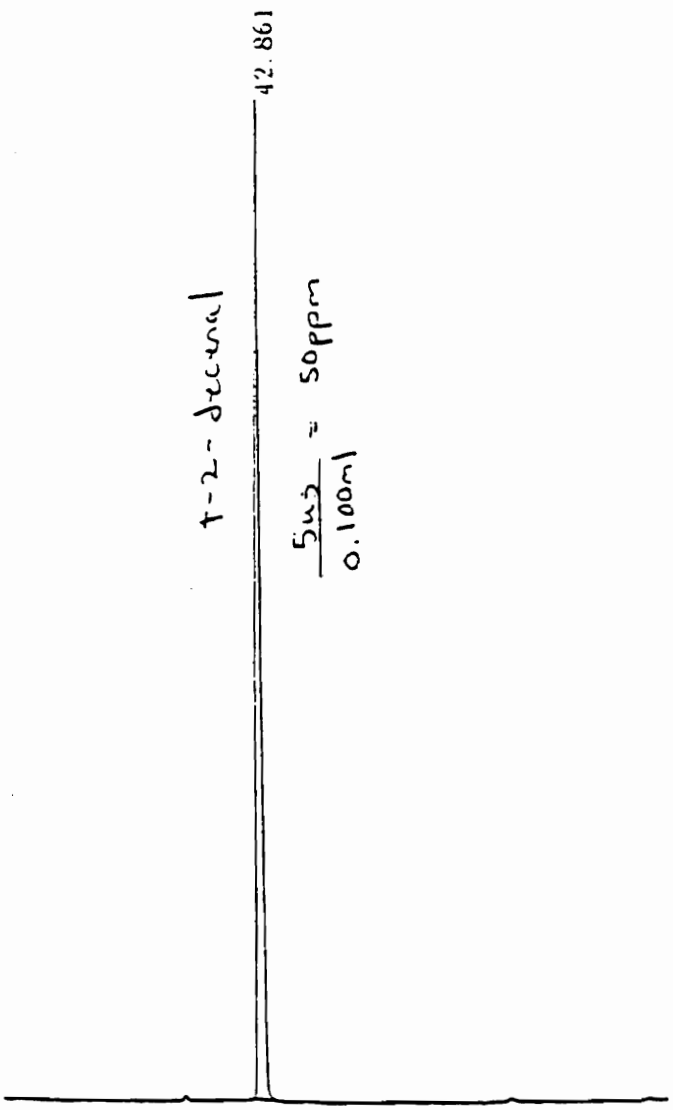


Figure C. 2. GC volatile chromatogram of *trans*-2-decenal at 25.0 ppm.



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	10	42.861	209159	23505			100	
		TOTAL	209159	23505			100	

Figure C. 3. GC volatile chromatogram of *trans*-2-decenal at 50.0 ppm.

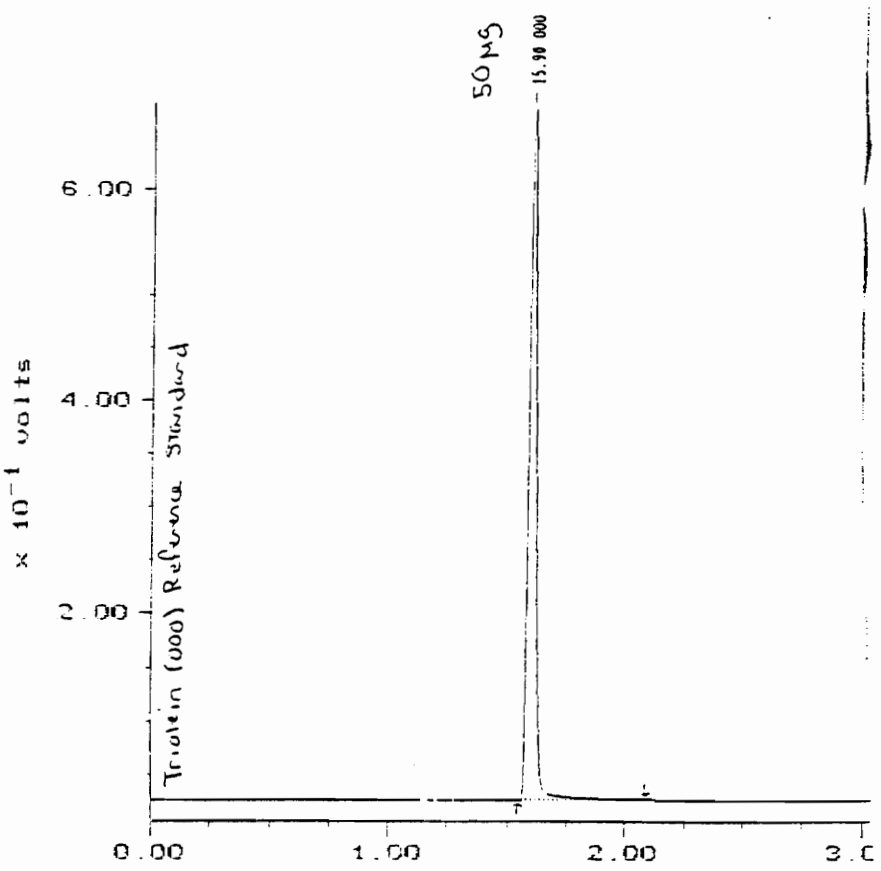


Figure C. 4. HPLC chromatogram of triolein (OOO) at 50.0mg/ 10.0 ml acetone.

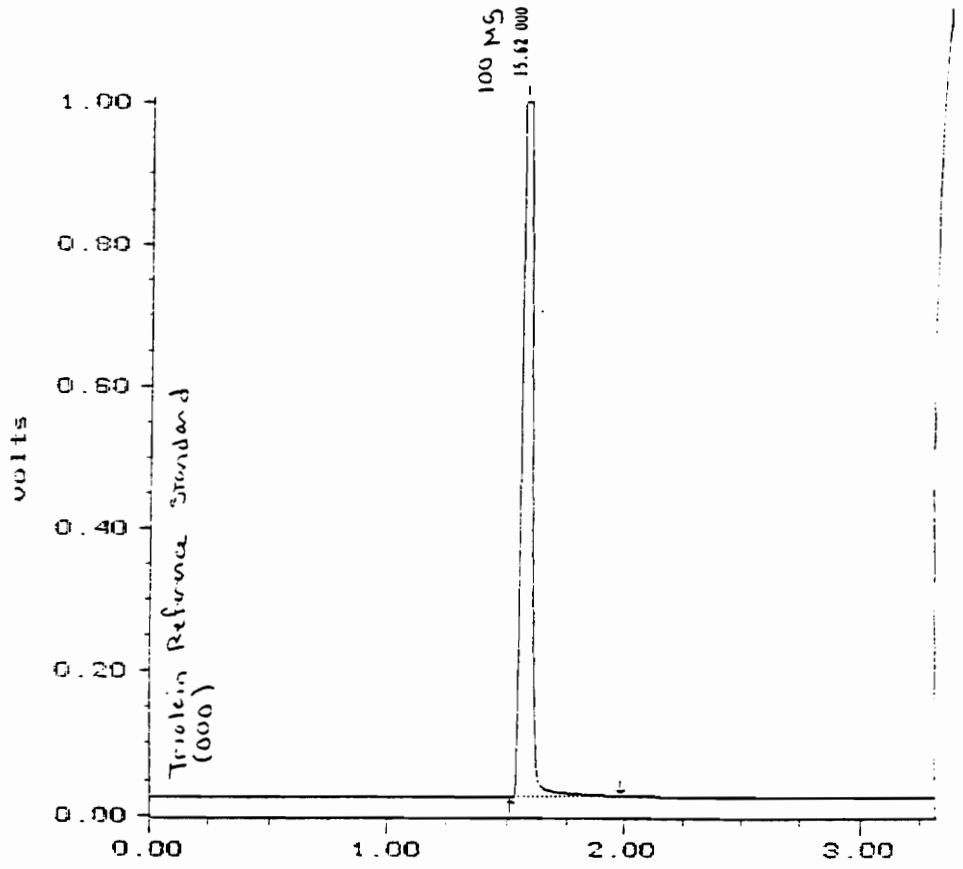


Figure C. 5. HPLC chromatogram of triolein (OOO) at 100.0 mg/ 10.0 ml acetone.

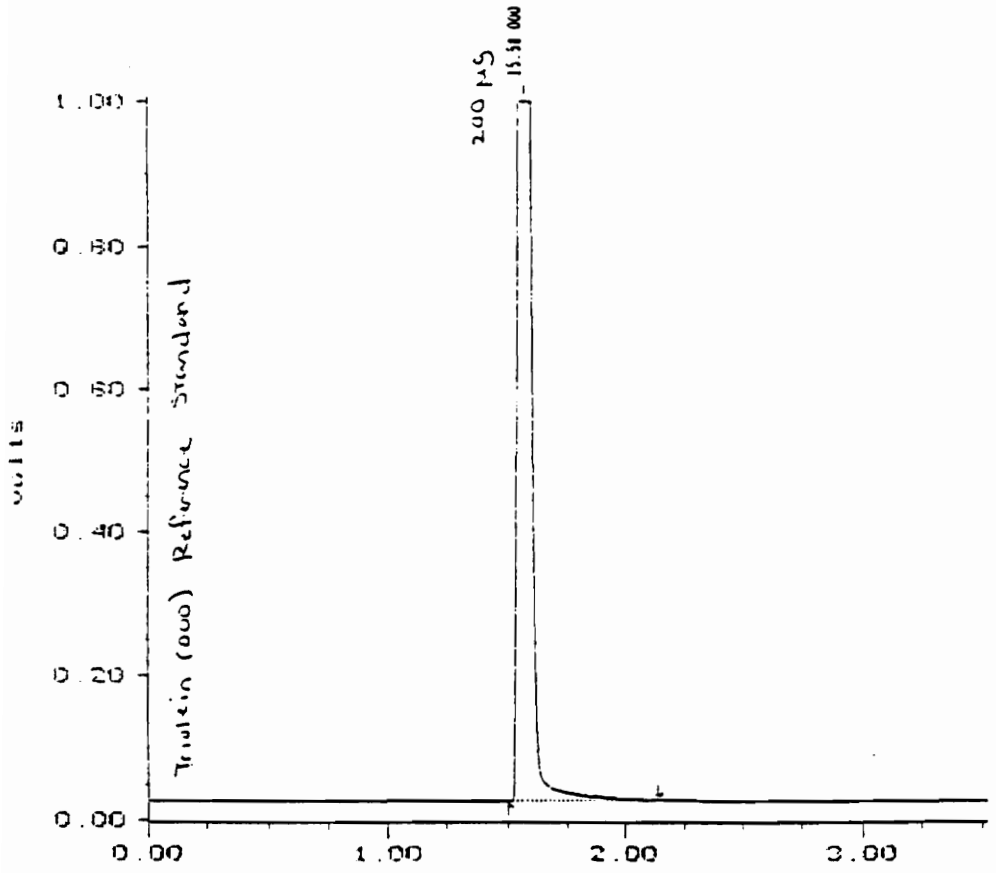


Figure C. 6. HPLC chromatogram of triolein (OOO) at 200.0 mg/ 10.0 ml acetone.

Appendix D

Simultaneous Confidence Intervals for Vegetable Oil Components.

Table D. 1. Simultaneous confidence intervals for palmitic acid before heating.

i	Y_i	M_i	K_i
1	2.90	9.50	9.11
2	3.50	9.50	9.11
3	4.30	9.50	9.11
4	5.90	9.50	9.11
5	8.50	9.50	9.11
6	9.00	9.50	9.11
7	(9.50)	9.00	(8.61)

Notes:

1. i = Blend
2. Y_i = Mean average of component
3. M_i = The largest mean for all blends
4. K_i = Simultaneous confidience intervals
5. ($Y_i > K_i$) represents the blend or blends with the largest means.

* Applies to Tables D.1 through D.14.

Table D. 2. Simultaneous confidence intervals for palmitic acid after 3000 minutes of heating at 180°C.

i	Y_i	M_i	K_i
1	2.90	10.60	10.26
2	3.60	10.60	10.26
3	4.70	10.60	10.26
4	6.70	10.60	10.26
5	9.00	10.60	10.26
6	9.70	10.60	10.26
7	(10.60)	9.70	(9.36)

Table D. 3. Simultaneous confidence intervals for stearic acid before heating.

i	Y_i	M_i	K_i
1	3.99	4.16	4.07
2	3.89	4.16	4.07
3	3.92	4.16	4.07
4	(4.06)	4.14	(4.05)
5	(4.14)	4.16	(4.07)
6	4.02	4.16	4.07
7	4.04	4.16	4.07

Table D. 4. Simultaneous confidence intervals for stearic acid after heating for 3000 minutes at 180°C.

i	Y_i	M_i	K_i
1	4.00	4.30	4.23
2	4.00	4.30	4.23
3	4.10	4.30	4.23
4	4.10	4.30	4.23
5	(4.30)	4.10	(4.03)
6	(4.30)	4.10	(4.03)
7	(4.30)	4.10	(4.03)

Table D. 5. Simultaneous confidence intervals for oleic before heating.

i	Y_i	M_i	K_i
1	(82.80)	76.70	(74.58)
2	76.70	82.80	80.68
3	68.10	82.80	80.68
4	56.20	82.80	80.68
5	33.50	82.80	80.68
6	28.90	82.80	80.68
7	23.50	82.80	80.68

Table D. 6. Simultaneous confidence intervals for oleic acid after 3000 minutes of heating at 180°C.

i	Y_i	M_i	K_i
1	(83.50)	77.60	(75.12)
2	77.60	83.50	81.04
3	69.50	83.50	81.04
4	52.40	83.50	81.04
5	35.10	83.50	81.04
6	30.00	83.50	81.04
7	24.90	83.50	81.04

Table D. 7. Simultaneous confidence intervals for linoleic acid before heating.

i	Y_i	M_i	K_i
1	9.78	55.97	54.31
2	14.27	55.97	54.31
3	20.87	55.97	54.31
4	30.43	55.97	54.31
5	47.56	55.97	54.31
6	51.52	55.97	54.31
7	(55.97)	51.52	(49.86)

Table D. 8. Simultaneous confidence intervals for linoleic acid after 3000 minutes at 180°C.

i	Y_i	M_i	K_i
1	8.70	54.10	53.00
2	13.20	54.10	53.00
3	19.80	54.10	53.00
4	32.90	54.10	53.00
5	46.20	54.10	53.00
6	50.10	54.10	53.00
7	(54.10)	50.10	(53.50)

Table D. 9. Simultaneous confidence intervals for linolenic acid before heating.

i	Y_i	M_i	K_i
1	0	7.00	6.72
2	0.80	7.00	6.72
3	1.70	7.00	6.72
4	3.06	7.00	6.72
5	5.80	7.00	6.72
6	6.30	7.00	6.72
7	(7.00)	6.30	(5.72)

Table D. 10. Simultaneous confidence intervals for linolenic acid after 3000 minutes of heating at 180°C.

i	Y_i	M_i	K_i
1	0	6.10	5.74
2	0.46	6.10	5.74
3	1.40	6.10	5.74
4	3.20	6.10	5.74
5	5.10	6.10	5.74
6	5.60	6.10	5.74
7	(6.10)	5.60	(5.24)

Table D. 11. Simultaneous confidence intervals for triolein before heating.

i	Y_i	M_i	K_i
1	(83.70)	80.40	(75.95)
2	(80.40)	83.70	(79.25)
3	70.90	83.70	79.25
4	50.10	83.70	79.25
5	16.50	83.70	79.25
6	8.80	83.70	79.25
7	1.50	83.70	79.25

Table D. 12. Simultaneous confidence intervals for triolein after heating.

i	Y_i	M_i	K_i
1	(84.90)	81.70	(77.87)
2	(81.70)	84.90	(81.07)
3	74.50	84.90	81.07
4	50.90	84.90	81.07
5	19.10	84.90	81.07
6	11.00	84.90	81.07
7	1.20	84.90	81.07

Table D. 13. Simultaneous confidence intervals ofr trilinolein before heating.

i	Y_i	M_i	K_i
1	1.20	29.70	28.19
2	2.60	29.70	28.19
3	6.30	29.70	28.19
4	14.30	29.70	28.19
5	25.90	29.70	28.19
6	(28.90)	29.70	(28.19)
7	(29.70)	28.90	(27.39)

Table D. 14. Simultaneous confidence intervals for trilinolein after 3000 minutes of heating at 180°C.

i	Yi	Mi	Ki
1	0.80	28.70	27.40
2	2.00	28.70	27.40
3	4.70	28.70	27.40
4	13.20	28.70	27.40
5	24.00	28.70	27.40
6	26.80	28.70	27.40
7	(28.70)	26.80	(25.50)

Blend		Component increase or decrease						
	7					+	+	+
	6				+	+	+	+
	5				+	+	+	+
(+)	4	+	+		+		+	+
	3				+	+	+	+
	2				+	+	+	+
	1				+	+	+	+
		L	Ln	LLL	000	O	P	S
	1	−	−	−				
	2	−	−	−				
(−)	3	−	−	−				
	4			−		−		
	5	−	−	−				
	6	−	−	−				
	7	−	−	−	−			
Σ Values:		-5	-5	-7	6	6	7	7

1	2	3	4	5	6	7	8	9	10	11	12	13	14
-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7

(Ranking Scale)

Figure D. 1. Relative stability ranking of components found in thermally oxidized high oleic acid sunflower and soybean oil blends. A (+) value indicates an increase after 3000 minutes of heating at 180°C and a (-) value indicates a decrease. A higher ranking value indicates a greater degreee of thermal oxidative stability.

Appendix E

Peroxide Trends During and After Heating.

Table E. 1. Peroxide values from time zero to 72 hours.

Blend	0:0	5:300	24:300	29:600	48:600	53:900	72:900
1a	1.35	1.90	2.25	2.38	1.57	1.08	1.38
1b	1.07	1.72	1.42	1.31	1.36	1.08	1.37
1c	0.43	1.13	1.53	1.55	1.86	1.62	2.50
1d	0.53	1.44	1.93	2.38	2.44	2.48	2.57
	(0.84)	(1.55)	(1.78)	(1.90)	(1.81)	(1.56)	(1.95)
2a	0.81	1.30	1.67	0.97	0.89	1.44	0.89
2b	1.87	2.30	2.74	2.93	3.68	1.50	4.33
2c	0.86	3.73	1.37	2.83	2.85	2.41	2.00
2d	0.84	2.70	2.20	4.14	4.07	4.76	4.39
	(1.09)	(2.51)	(2.00)	(2.72)	(2.87)	(2.53)	(2.90)
3a	0.60	1.88	2.17	1.99	2.43	1.72	1.82
3b	1.22	1.93	2.27	2.43	1.85	1.16	1.61
3c	1.52	2.33	2.43	1.82	1.82	2.39	2.03
3d	1.69	2.13	3.57	7.09	7.16	7.16	4.01
	(1.26)	(2.07)	(2.61)	(3.33)	(3.31)	(3.11)	(2.37)
4a	1.54	2.54	1.63	1.50	1.74	2.30	3.04
4b	0.87	1.60	1.87	2.16	1.95	3.12	3.45
4c	1.72	2.55	2.90	3.57	3.25	4.38	5.13
4d	2.14	3.21	3.66	4.50	4.05	4.98	6.30
	(1.57)	(2.47)	(2.51)	(2.93)	(2.75)	(3.70)	(4.48)
5a	0.74	1.08	1.09	1.63	1.53	2.15	1.62
5b	0.67	1.97	1.58	1.89	2.01	1.81	2.12
5c	0.69	1.79	1.46	1.83	2.10	1.81	2.17
5d	1.62	3.65	3.11	2.56	3.10	5.77	7.06
	(0.93)	(2.12)	(1.81)	(1.98)	(2.18)	(2.88)	(3.24)
6a	0.48	1.43	1.58	1.24	2.00	1.91	2.05
6b	0.97	2.37	2.56	2.56	1.69	2.77	5.05
6c	2.28	3.70	4.19	4.30	4.77	5.31	5.01
6d	1.71	4.07	3.43	2.70	2.67	4.70	7.80
	(1.36)	(2.89)	(2.94)	(2.70)	(2.78)	(3.67)	(4.98)
7a	0.35	1.24	1.94	1.62	2.08	1.93	2.17
7b	0.60	2.15	1.97	4.07	4.14	5.92	3.31
7c	1.20	2.74	2.67	3.40	3.98	5.58	6.91
7d	0.28	1.31	2.30	1.50	1.82	1.97	2.15
	(0.61)	(1.86)	(2.22)	(2.65)	(3.00)	(3.85)	(3.63)

Notes:

1. ( ) = The average of the 4 trials/blend.
2. Each individual value was based on 3 peroxide values.
3. The first number in each column represents the hours of use, where the second number represents the minutes of actual heating at 180°C.

Table E. 2. Peroxide values (PV) after 3000 minutes of heating at 180°C.

(PV = *u*eqv. O_2/g vegetable oil blend)

Blend	Trial A	Trial B	Trial C	Trial D
1	6.07	6.18	6.37	5.05
2	6.09	6.37	6.40	6.10
3	8.64	8.81	8.47	9.19
4	10.26	10.71	10.95	9.70
5	14.88	15.07	16.34	15.10
6	15.37	15.32	15.83	14.85
7	20.86	19.75	19.59	18.84

(Blend)	(HOSO:PUSB)
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Appendix F

FAME Data and Representative Chromatograms.

Table F. 1. GC/FAME analysis of fresh vegetable oil blends: % weight based on 50.0 mg of sample.

Blend	16:0	18:0	18:1	18:2	18:3
1a	2.87	3.97	82.17	10.12	0
1b	2.86	3.86	82.61	9.76	0
1c	2.90	3.91	83.73	9.45	0
	(2.88)	(3.91)	(82.84)	(9.78)	(0)
2a	3.49	3.89	76.96	14.13	0.83
2b	3.43	3.89	76.68	14.42	0.83
2c	3.45	3.89	76.45	14.27	0.80
	(3.46)	(3.89)	(76.70)	(14.27)	(0.82)
3a	4.20	3.88	67.70	20.87	1.70
3b	4.33	3.93	68.06	20.78	1.69
3c	4.32	3.96	68.48	20.97	1.68
	(4.28)	(3.92)	(68.08)	(20.87)	(1.69)
4a	5.93	4.13	56.27	30.56	3.09
4b	5.89	4.18	56.32	30.50	3.10
4c	5.93	4.16	55.96	30.23	3.03
	(5.92)	(4.16)	(56.18)	(30.43)	(3.07)
5a	8.60	4.13	33.45	47.55	5.72
5b	8.53	4.14	33.69	47.83	5.80
5c	8.31	4.15	33.43	47.31	5.79
	(8.48)	(4.14)	(33.52)	(47.56)	(5.77)
6a	9.19	4.04	28.98	51.53	6.25
6b	8.94	4.01	28.92	51.53	6.33
6c	9.06	4.02	28.90	51.52	6.29
	(9.06)	(4.02)	(28.90)	(51.52)	(6.29)
7a	9.02	4.11	23.67	56.13	7.06
7b	9.85	4.00	23.41	55.86	6.88
7c	9.61	4.01	23.46	55.91	7.01
	(9.49)	(4.04)	(23.51)	(55.97)	(6.98)

( ) = Mean averages

* See Table 4. for vegetable oil blend levels.

Table F. 2. GC/FAME analysis of vegetable oils heated for 3000 minutes at 180oC: % weight based on 50.0 mg of sample.

Blend	16:0	18:0	18:1	18:2	18:3
1a	2.83	4.00	83.06	8.75	0
1b	3.06	4.06	84.34	8.53	0
1c	2.95	3.97	83.44	8.76	0
1d	2.77	3.94	83.06	8.90	0
	(2.90)	(3.99)	(83.47)	(8.73)	(0)
2a	3.62	4.05	77.36	13.07	0.61
2b	3.68	4.06	77.69	13.11	0.63
2c	3.41	3.93	76.78	13.57	0.59
2d	3.54	4.06	78.42	13.12	0
	(3.56)	(4.02)	(77.56)	(13.22)	(0.46)
3a	4.80	4.08	69.98	19.70	1.43
3b	4.63	4.07	69.00	19.64	1.45
3c	4.65	4.00	69.09	20.05	1.47
3d	4.74	4.07	70.00	19.77	1.42
	(4.70)	(4.06)	(69.52)	(19.79)	(1.44)
4a	6.86	4.16	52.45	32.71	3.21
4b	6.75	4.18	52.52	32.73	3.20
4c	6.35	4.04	52.03	32.79	3.25
4d	6.78	4.10	52.45	33.45	3.22
	(6.68)	(4.12)	(52.36)	(32.92)	(3.22)
5a	9.25	4.30	35.22	46.16	5.08
5b	9.09	4.28	34.86	45.56	5.11
5c	8.85	4.26	35.42	46.41	5.06
5d	8.99	4.23	35.04	46.63	5.11
	(9.04)	(4.27)	(35.13)	(46.19)	(5.09)
6a	10.12	4.24	30.06	50.05	5.53
6b	9.35	4.27	29.70	49.60	5.67
6c	9.60	4.27	30.12	50.32	5.63
6d	9.57	4.25	30.02	50.36	5.61
	(9.66)	(4.26)	(30.02)	(50.08)	(5.61)
7a	10.93	4.32	24.75	53.32	6.03
7b	10.77	4.37	24.91	53.88	6.07
7c	10.45	4.30	25.02	54.88	6.16
7d	10.25	4.31	24.91	54.37	6.15
	(10.60)	(4.32)	(24.90)	(54.11)	(6.10)

( ) = Mean averages

* See Table 4. for vegetable oil blend levels.

Table F. 3. Blend code key for GC/FAME analysis.

Blend # Assignment	(HOSO:PUSB) Ratio
1	100:0
2	90:10
3	75:25
4	50:50
5	25:75
6	10:90
7	0:100

Notes:

1. See Appendix N or Appendix B, Table B.4 for fatty acid nomenclature.
2. HOSO = High oleic acid sunflower oil.
3. PUSB = Polyunsaturated soybean oil.

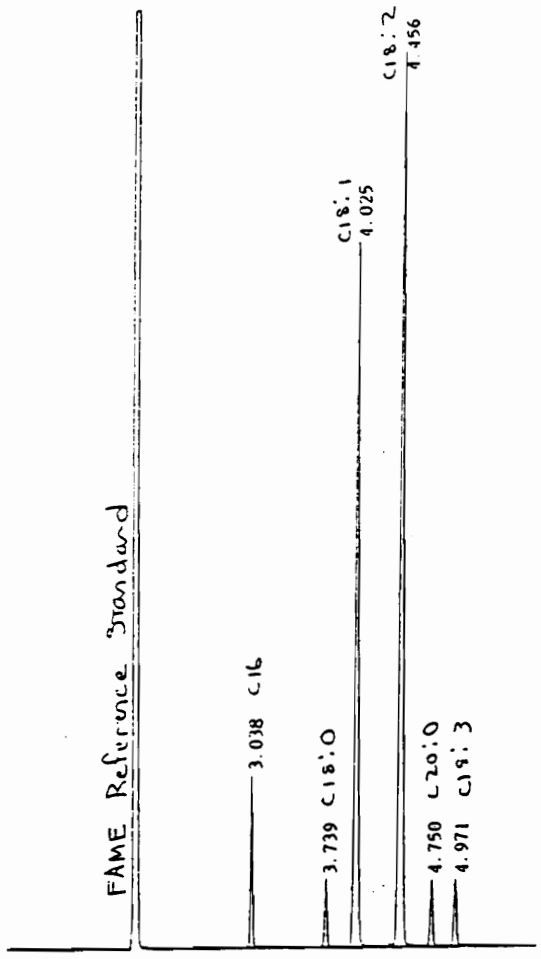


Figure F. 1. GC/FAME reference standard.

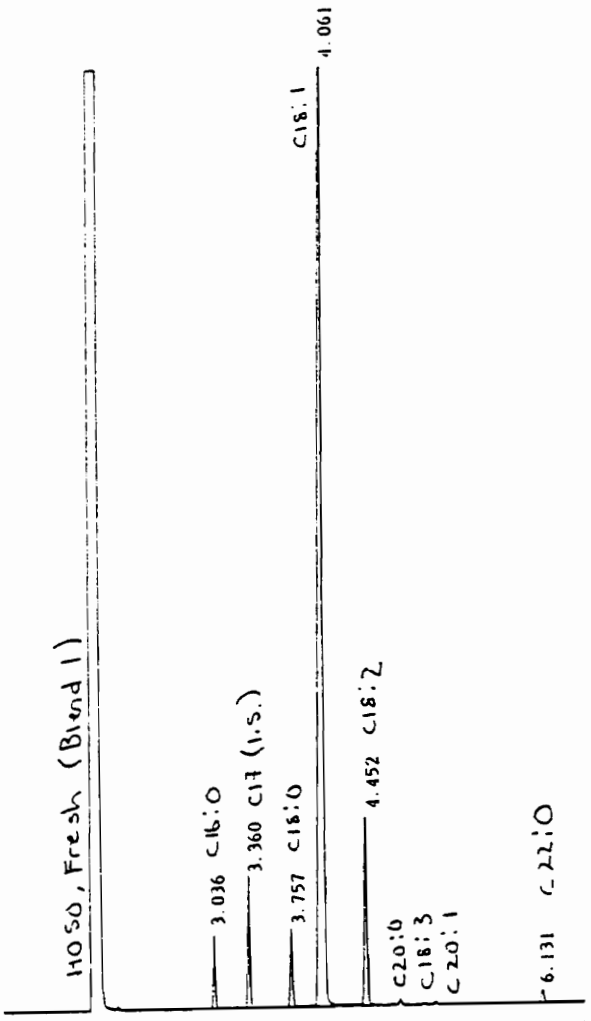


Figure F. 2. GC/FAME analysis of fresh blend one.

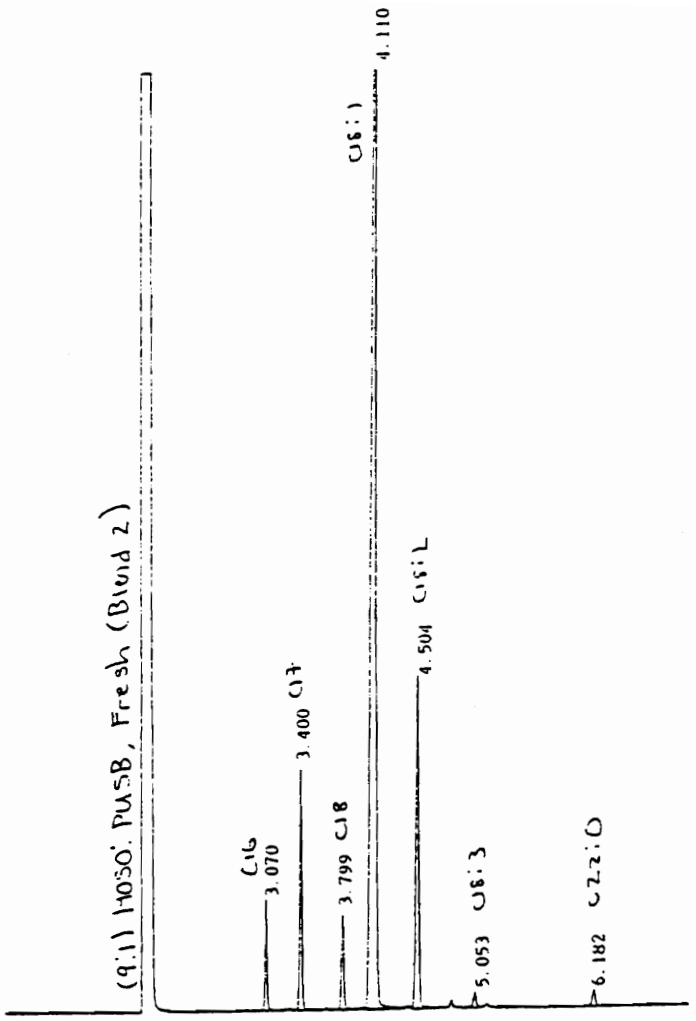


Figure F. 3. GC/FAME analysis of fresh blend two.

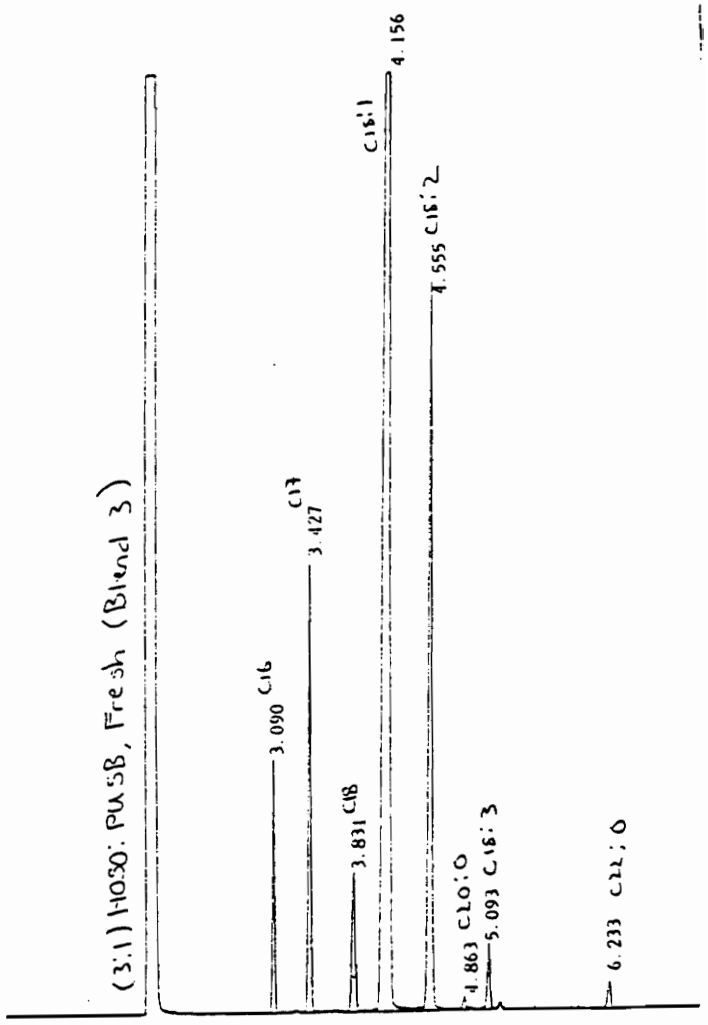


Figure F. 4. GC/FAME analysis of fresh blend three.

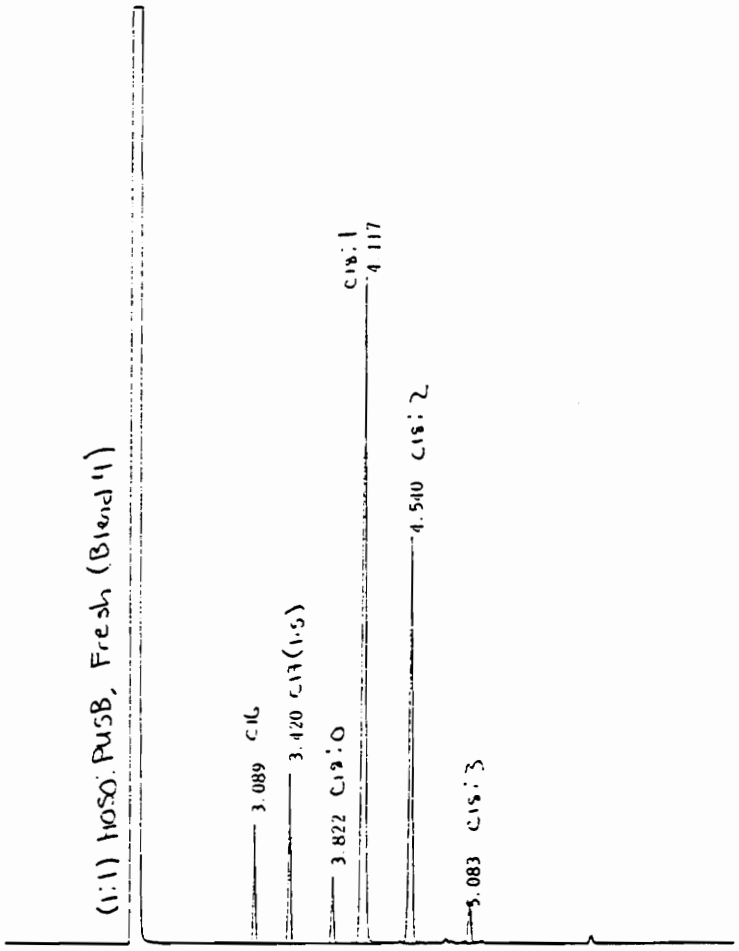


Figure F. 5. GC/FAME analysis of fresh blend four.

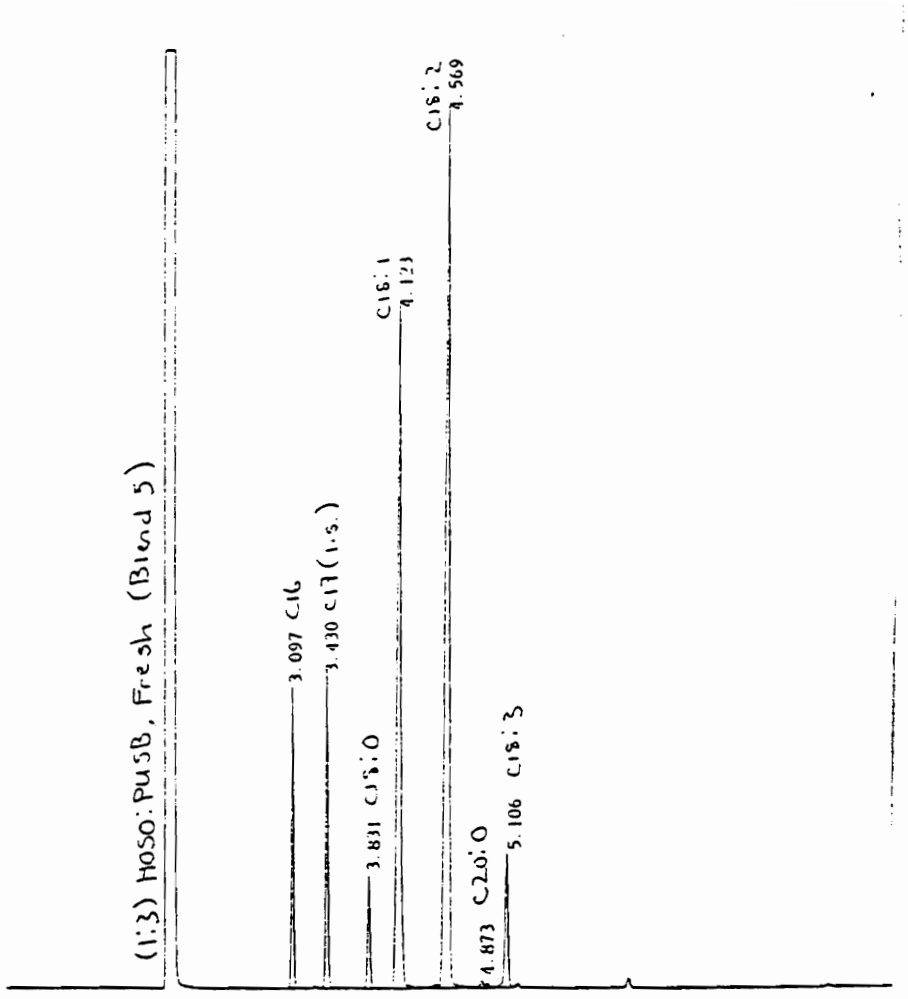


Figure F. 6. GC/FAME analysis of fresh blend five.

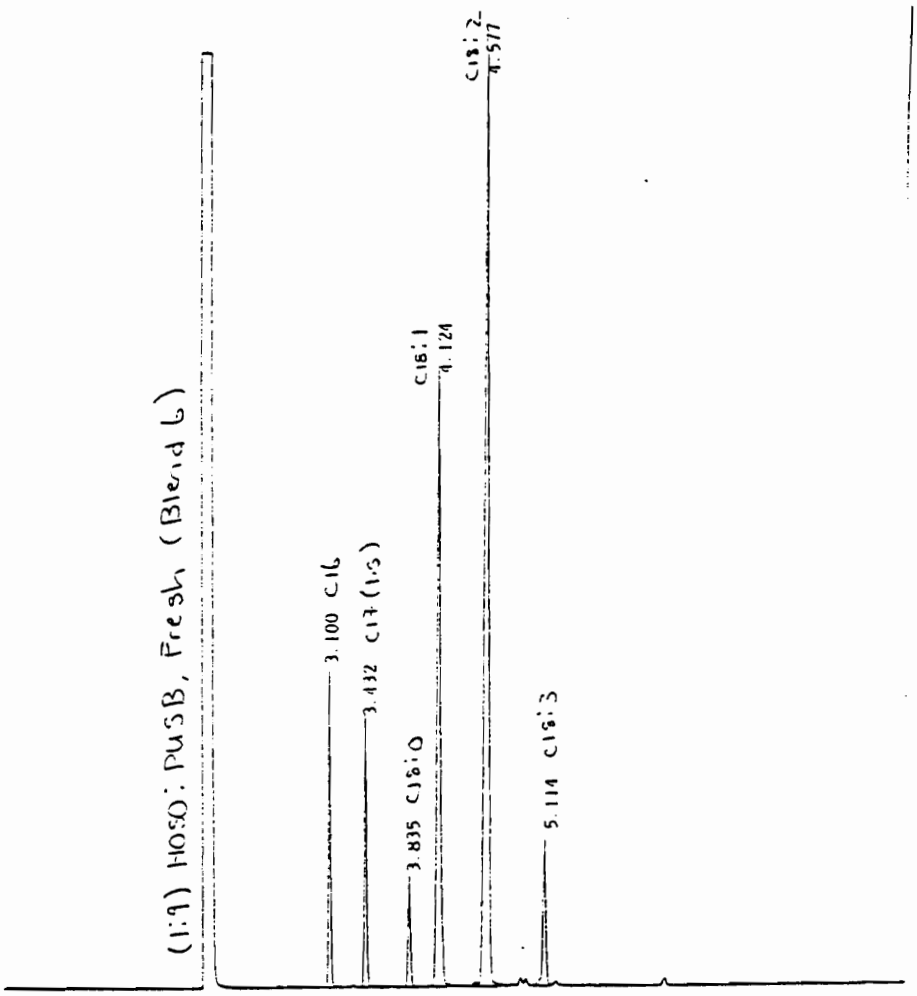


Figure F. 7. GC/FAME analysis of fresh blend six.

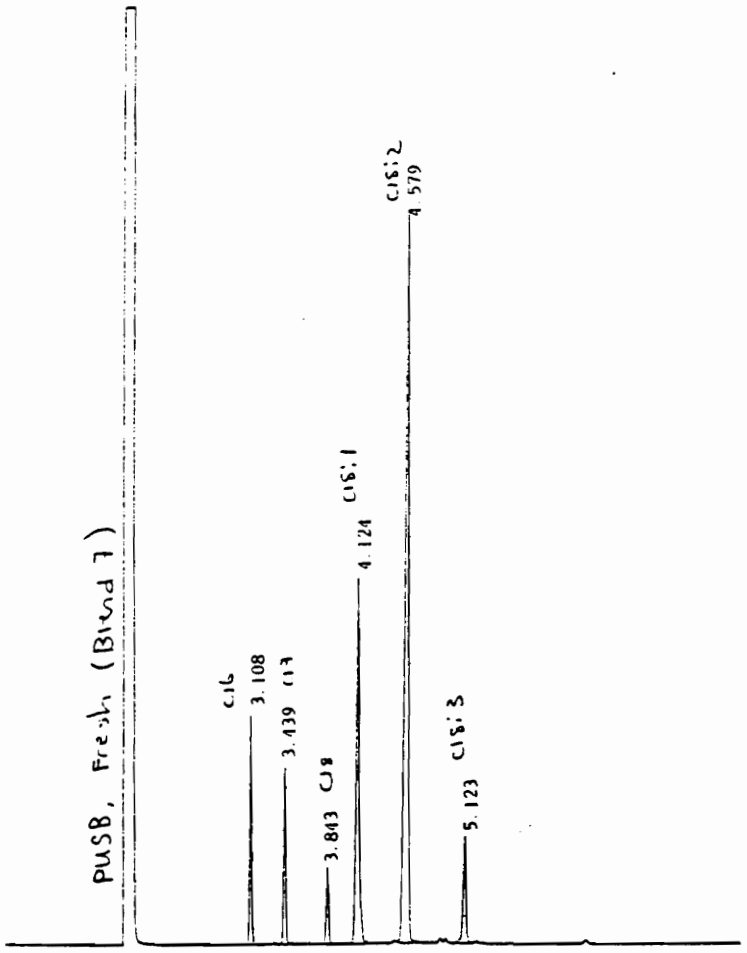


Figure F. 8. GC/FAME analysis of fresh blend seven.

Appendix G

Triglyceride Data and Representative Chromatograms.

Table G. 1. HPLC/Triglyceride analysis of fresh vegetable oil blends: % weight based on 85.0 mg of sample.

Blend	LLL	OOO	DB 6-9	DB 3-5	DB 1-3
1a	1.21	83.76	0	8.10	91.90
1b	1.22	83.28	0	8.02	91.69
1c	1.01	84.47	0.19	7.07	92.74
1d	1.39	83.20	0.23	8.15	91.41
	(1.21)	(83.68)	(0.10)	(7.83)	(91.91)
2a	2.70	80.05	0	12.16	87.85
2b	2.49	80.85	0.32	11.29	88.40
2c	2.45	80.55	0.32	11.13	88.30
2d	2.60	80.17	0.34	11.53	87.91
	(2.56)	(80.40)	(0.24)	(11.53)	(88.11)
3a	7.07	66.73	0.96	24.97	73.88
3b	6.34	69.58	1.00	22.17	76.84
3c	5.47	73.54	0.78	18.98	80.24
3d	5.66	73.67	0.97	19.24	79.38
	(6.13)	(70.88)	(0.93)	(21.34)	(77.58)
4a	12.10	55.98	2.10	36.98	60.91
4b	15.58	45.35	3.09	47.36	49.45
4c	15.85	46.93	3.03	46.45	50.52
4d	13.65	52.17	2.51	40.92	56.58
	(14.29)	(50.11)	(2.68)	(42.93)	(54.36)
5a	24.60	24.62	5.24	70.14	24.62
5b	26.40	16.02	6.15	76.33	17.49
5c	26.64	16.75	5.95	75.82	18.22
5d	26.16	16.63	6.14	75.56	18.28
	(25.95)	(18.50)	(5.87)	(74.46)	(19.65)
6a	28.99	9.35	6.66	82.58	10.73
6b	28.99	8.47	6.84	83.08	10.05
6c	28.98	8.57	6.84	83.10	10.04
6d	28.82	8.98	7.01	82.58	10.41
	(28.94)	(8.84)	(6.84)	(82.83)	(10.31)
7a	28.32	1.32	8.10	86.69	5.21
7b	30.00	1.72	7.22	87.47	5.28
7c	31.03	1.56	7.45	87.79	4.67
7d	29.41	1.30	7.68	87.39	4.94
	(29.69)	(1.47)	(7.61)	(87.33)	(5.02)

( ) = Mean averages

* See Table 4. for vegetable oil blend levels.

Table G. 2. HPLC/Triglyceride analysis of vegetable oil blends heated for 900 minutes at 180°C: % weight based on 85.0 mg of sample.

Blend	LLL	OOO	DB 6-9	DB 3-5	DB 1-3
1a	0.86	85.06	0.23	6.65	93.13
1b	0.86	85.06	0.23	6.65	93.13
1c	0.89	84.43	0.27	6.78	92.95
1d	0.82	85.15	0.30	6.38	93.33
	(0.86)	(84.92)	(0.26)	(6.61)	(93.13)
2a	2.28	80.55	0.41	11.05	88.52
2b	2.46	79.90	0.39	11.46	87.94
2c	2.25	81.35	0.39	10.51	88.85
2d	2.17	81.31	0.43	10.34	89.02
	(2.29)	(80.78)	(0.40)	(10.84)	(88.58)
3a	5.63	72.47	0.86	20.14	79.00
3b	5.17	74.74	0.79	18.31	80.89
3c	4.77	75.65	0.73	17.12	81.95
3d	4.98	75.15	0.74	17.75	81.51
	(5.14)	(74.50)	(0.78)	(18.33)	(80.84)
4a	13.72	51.49	2.50	41.77	55.73
4b	15.18	48.07	2.71	45.31	51.88
4c	15.56	46.18	2.96	47.01	50.01
4d	15.48	45.46	3.15	47.36	49.42
	(14.98)	(47.80)	(2.83)	(45.36)	(51.76)
5a	23.85	24.89	5.22	69.87	24.89
5b	27.27	15.93	5.73	76.89	17.32
5c	26.12	17.30	5.64	75.36	18.93
5d	25.64	17.45	5.80	74.81	19.33
	(25.72)	(18.90)	(5.60)	(74.23)	(20.12)
6a	29.11	10.74	5.95	81.88	12.13
6b	28.51	9.03	6.51	82.76	10.67
6c	28.49	9.16	6.56	82.71	10.66
6d	28.45	9.20	6.64	82.59	10.70
	(28.64)	(9.53)	(6.41)	(82.50)	(11.04)
7a	29.40	1.80	7.40	88.96	3.62
7b	27.90	1.46	6.22	83.57	10.08
7c	30.78	1.09	7.16	88.43	4.36
7d	28.62	1.36	7.61	87.06	5.31
	(29.20)	(1.43)	(7.10)	(87.00)	(5.84)

( ) = Mean averages

* See Table 4. for vegetable oil blend levels.

Table G. 3. HPLC/Triglyceride analysis of vegetable oils heated for 3000 minutes at 180°C: % weight based on 85.0 mg of sample.

Blend	LLL	OOO	DB 6-9	DB 3-5	DB 1-3
1a	0.78	85.21	0.33	6.32	93.35
1b	0.90	84.89	0.35	6.86	92.78
1c	0.89	84.43	0.27	6.78	92.59
1d	0.82	85.15	0.30	6.38	93.33
	(0.85)	(84.92)	(0.31)	(6.58)	(93.01)
2a	2.02	81.24	0.51	10.16	89.07
2b	2.24	81.11	0.52	10.47	88.75
2c	1.70	82.81	0.59	9.56	89.85
2d	2.00	81.74	0.56	10.20	89.12
	(1.99)	(81.72)	(0.57)	(10.10)	(89.20)
3a	5.03	73.68	0.88	18.36	80.52
3b	4.76	74.05	0.84	18.40	80.76
3c	4.49	75.55	0.52	17.24	81.82
3d	4.63	75.55	0.53	17.82	81.22
	(4.73)	(74.52)	(0.69)	(17.95)	(81.08)
4a	13.47	49.51	2.62	42.97	54.09
4b	13.17	50.94	2.56	42.16	55.25
4c	12.96	51.68	2.79	41.38	55.70
4d	13.03	51.34	2.60	41.90	55.44
	(13.16)	(50.87)	(2.64)	(42.10)	(53.12)
5a	23.63	18.85	5.61	72.99	20.99
5b	23.81	19.17	5.17	73.43	21.10
5c	24.13	19.18	5.11	73.63	20.94
5d	24.58	19.41	5.12	73.45	21.18
	(24.04)	(19.15)	(5.25)	(73.37)	(21.05)
6a	26.24	11.23	6.42	80.51	12.75
6b	27.02	10.78	7.02	81.38	12.09
6c	26.96	11.03	6.41	80.96	12.30
6d	26.83	11.15	6.34	81.01	12.34
	(26.76)	(11.05)	(6.55)	(80.96)	(12.37)
7a	28.26	1.47	7.16	86.31	6.15
7b	28.28	1.29	6.91	86.59	6.15
7c	29.35	1.09	6.32	87.81	5.75
7d	28.95	0.99	6.77	86.94	5.75
	(28.71)	(1.21)	(6.79)	(86.91)	(0.35)

( ) = Mean averages

* See Table 4. for vegetable oil blend levels.

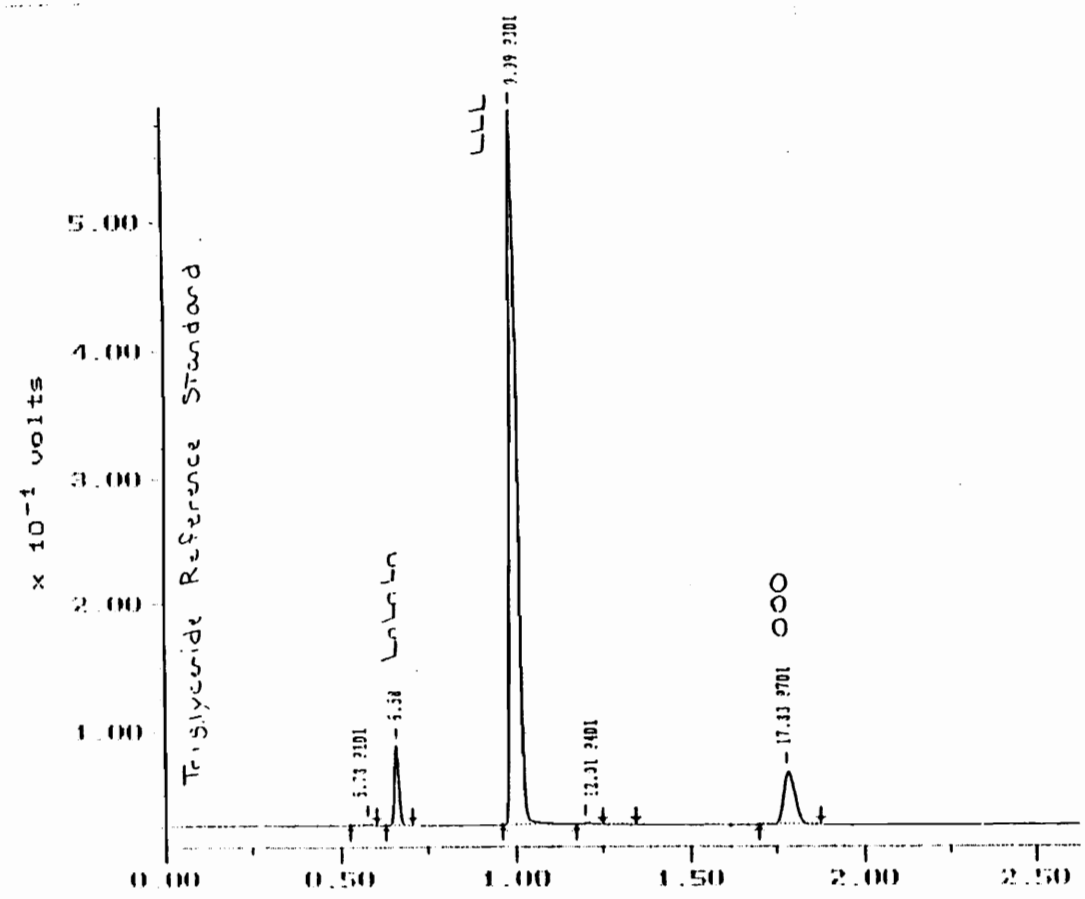


Figure G. 1. HPLC/Triglyceride reference chromatogram.

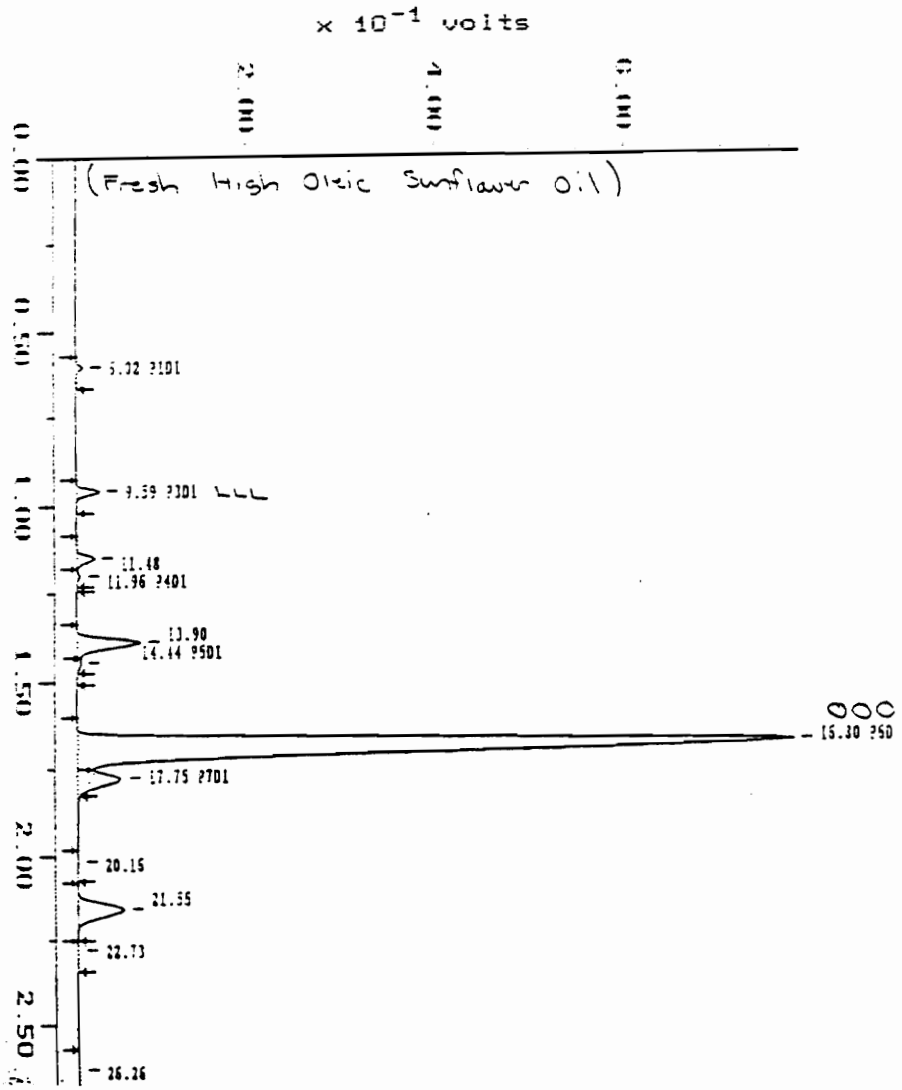


Figure G. 2. HPLC/Triglyceride chromatogram of fresh high oleic sunflower oil.

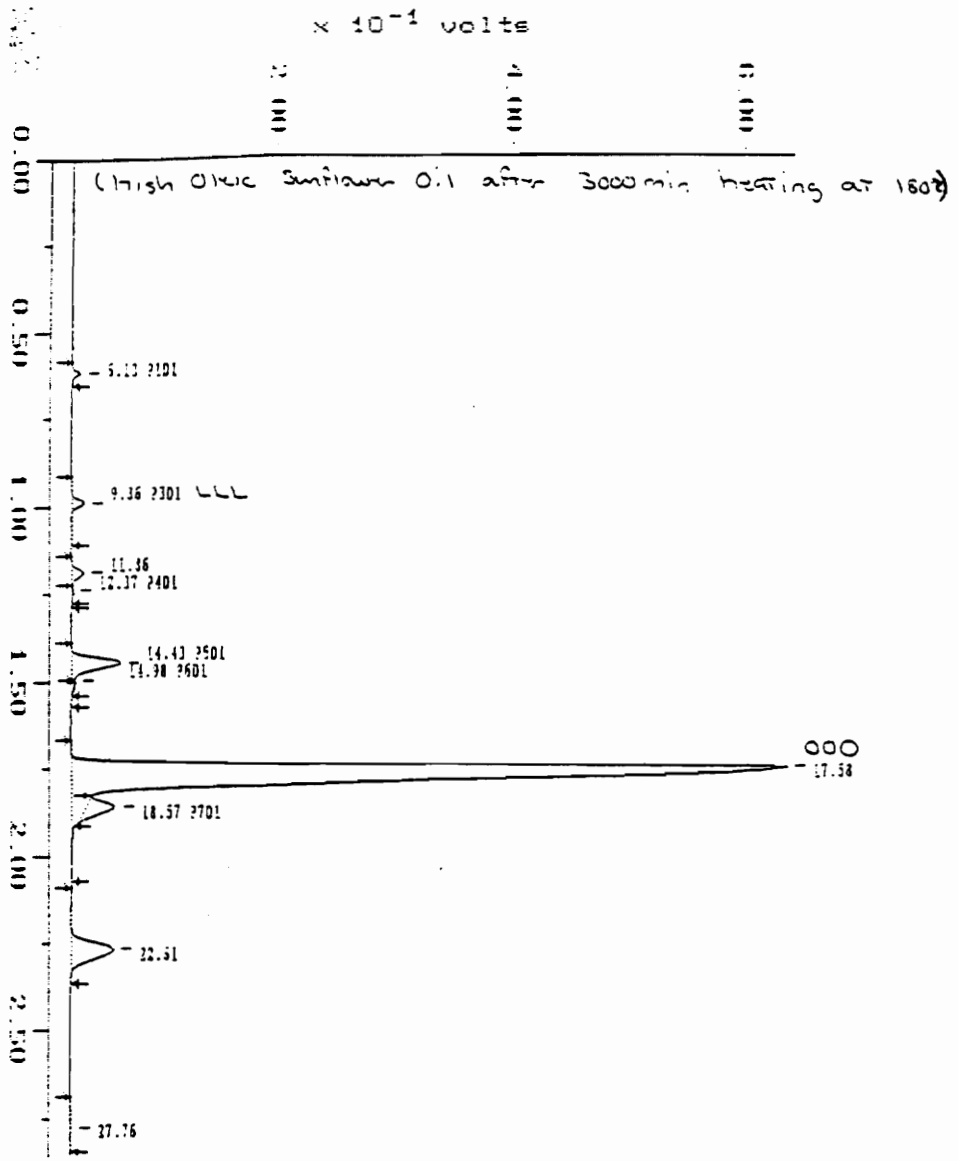


Figure G. 3. HPLC/Triglyceride chromatogram of high oleic sunflower oil heated for 3000 minutes at 180°C.

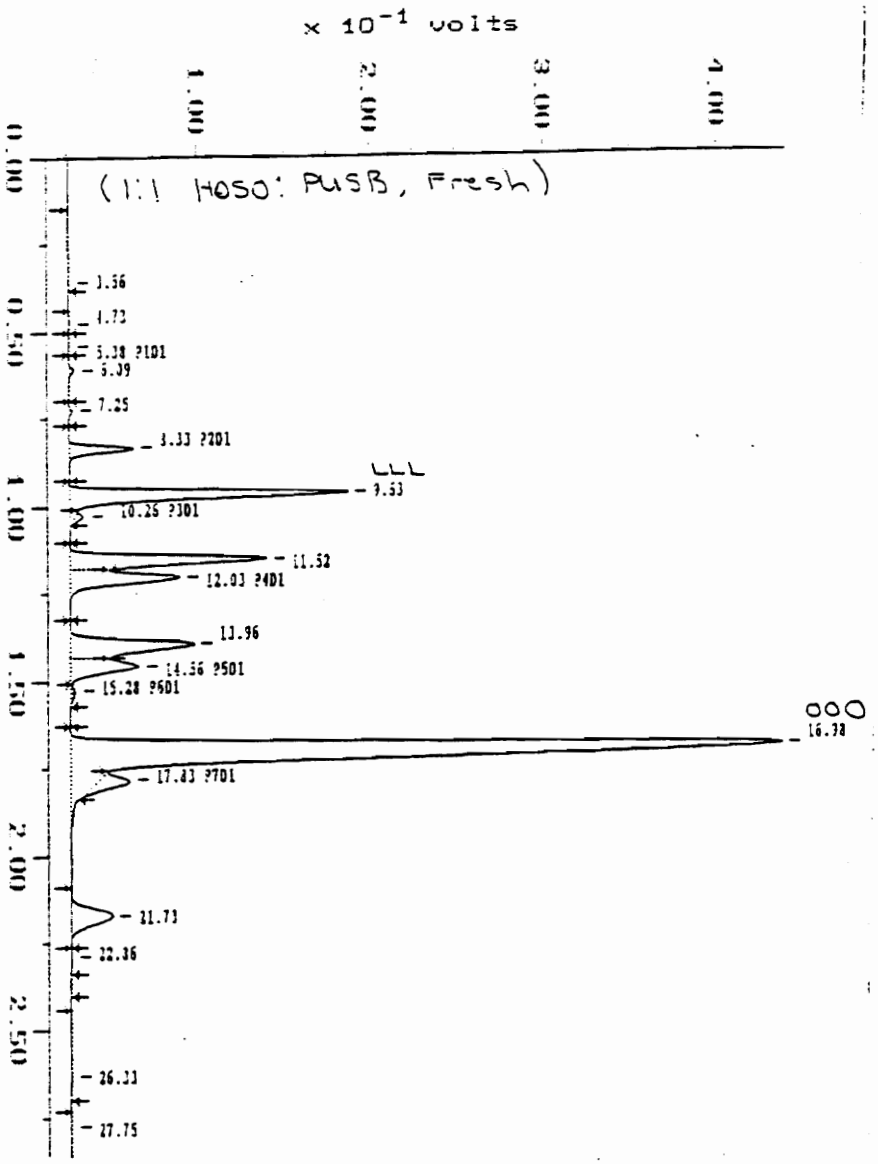


Figure G. 4. HPLC/Triglyceride chromatogram of fresh (1:1) high oleic sunflower oil to soybean oil.

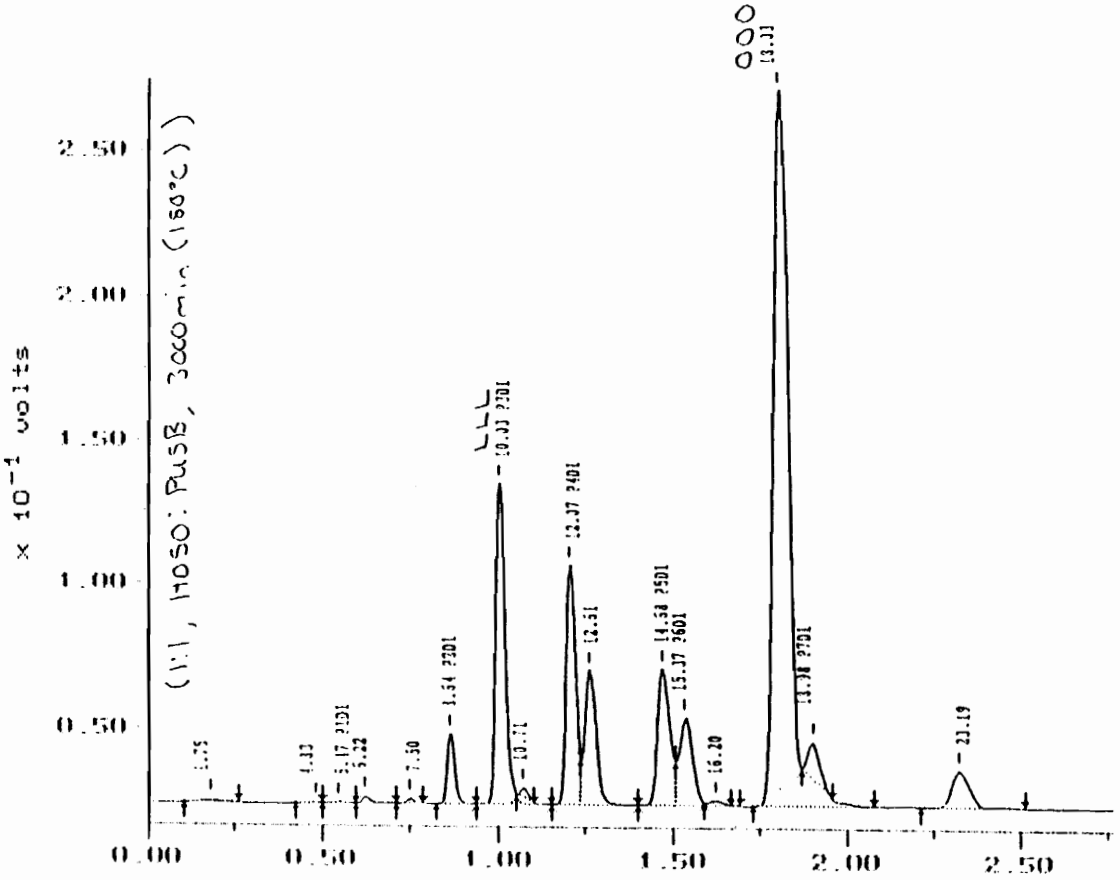


Figure G. 5. HPLC/Triglyceride chromatogram of (1:1) high oleic to soybean oil heated for 3000 minutes at 180°C.

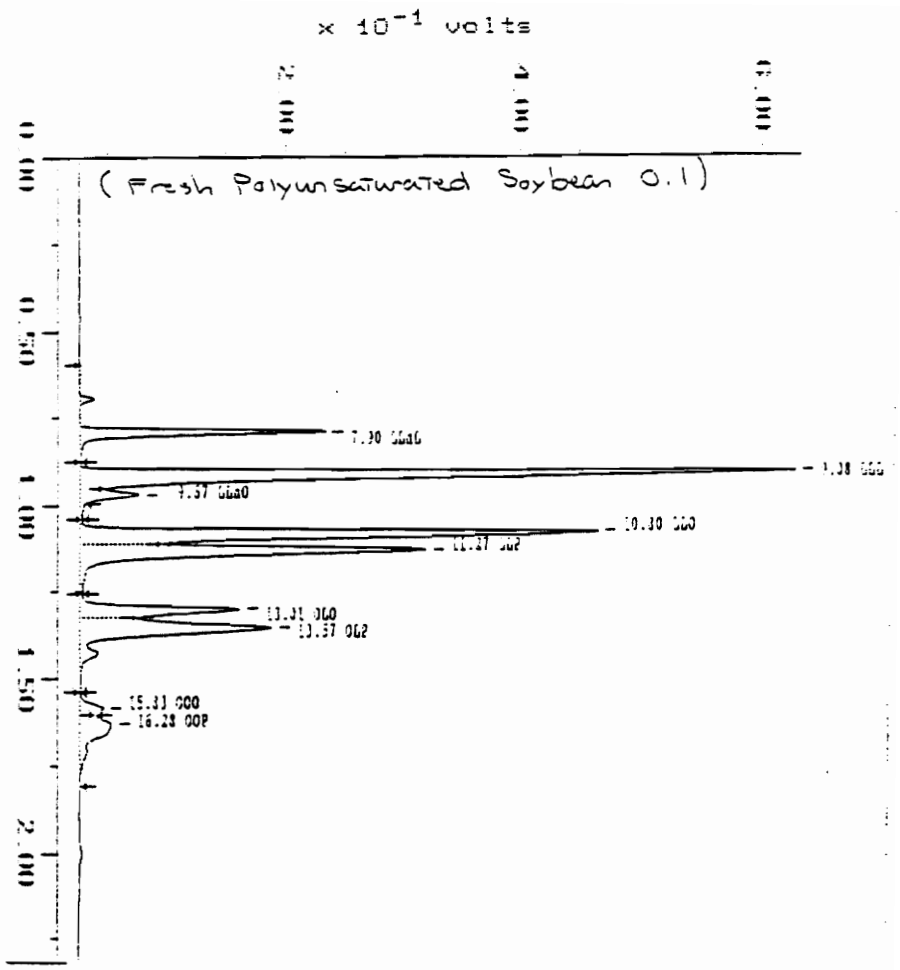


Figure G. 6. HPLC/Triglyceride chromatogram of fresh soybean oil.

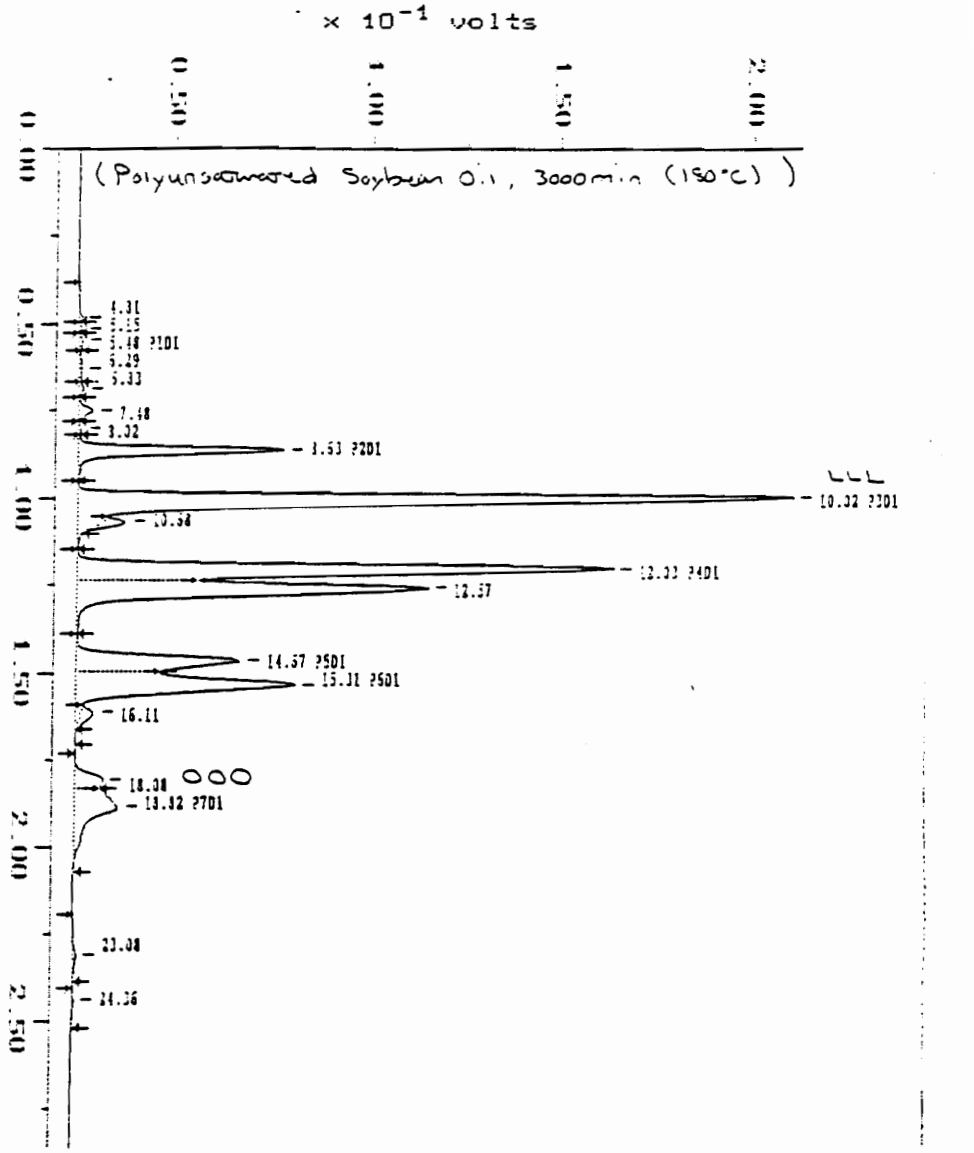


Figure G. 7. HPLC/Triglyceride chromatogram of soybean oil heated for 3000 minutes at 180°C.

Appendix H

Major Volatile Data and Representative Chromatograms.

Table H. 1. Major GC/Volatile Analysis: (cont.)

Sample	C_7H_8	$C_6H_{12}O$	$C_7H_{14}O$	$C_7H_{12}O$	$C_9H_{18}O$	$C_7H_{10}O$	$C_{10}H_{16}O$
2aD1	12.11	2.42	0	0	0	0	0
2aD2	8.58	0	2.18	19.91	11.26	7.39	2.21
2aD3	6.32	4.30	0	22.23	18.73	1.52	2.32
2aD4	3.03	3.98	4.23	26.70	11.48	6.33	1.42
2bD1	26.98	0	0	0	0	0	0
2bD2	10.25	13.09	5.51	22.00	8.00	5.72	1.59
2bD3	4.47	0.90	2.62	28.17	15.60	9.68	3.95
2bD4	3.63	3.77	3.47	25.02	16.09	8.01	2.72
2cD1	41.18	0	0	0	0	0	8.23
2cD2	5.50	8.22	4.72	19.86	7.73	5.52	5.01
2cD3	4.92	7.31	3.36	20.90	13.07	7.44	3.54
2cD4	2.93	5.90	4.08	27.00	13.53	7.82	3.03
2dD1	49.73	0	0	0	0	0	0
2dD2	4.67	5.69	4.48	24.30	10.39	7.17	2.56
2dD3	6.01	5.87	4.62	27.07	11.14	7.87	2.36
2dD4	3.03	4.68	3.20	18.86	15.06	7.99	2.96
1aD1	17.84	3.34	0	0	0	0	0
1aD2	2.95	0.82	2.25	14.20	14.90	1.27	2.33
1aD3	2.80	2.28	2.65	18.05	22.03	1.50	3.79
1aD4	6.39	5.59	3.28	16.73	16.21	5.03	2.34
1bD1	51.16	0	0	0	0	0	0
1bD2	4.44	0	1.48	11.94	14.80	0	2.33
1bD3	9.32	6.00	2.88	31.74	16.85	9.64	3.23
1bD4	6.00	9.65	8.20	32.20	18.45	0	0
1cD1	30.43	0	0	0	3.39	0	0
1cD2	10.97	13.53	5.16	18.38	12.86	14.30	2.17
1cD3	7.90	11.70	7.29	24.35	15.58	1.57	2.33
1cD4	3.68	2.67	3.18	23.88	25.08	2.60	3.20
1dD1	28.83	0	0	0	0	0	0
1dD2	7.33	12.12	4.86	20.51	13.22	1.52	2.63
1dD3	6.21	8.30	6.95	23.01	15.07	1.44	2.33
1dD4	3.43	2.50	3.15	20.63	19.62	1.90	2.71

Notes:

1. C_7H_8 = Toluene
2. $C_6H_{12}O$ = Hexanal
3. $C_7H_{14}O$ = Heptenal
4. $C_7H_{12}O$ = (Z)-2-Heptenal
5. $C_9H_{18}O$ = Nonanal
6. $C_7H_{10}O$ = (E,E)-2,4-Heptadienal
7. $C_{10}H_{16}O$ = (E,Z)-2,4-Decadienal
8. 1st # and Letter = Blend Series
9. D1 = Day one
10. D2 = Day two
11. D3 = Day three
12. D4 = Day four

Table H. 1. Major GC/Volatile Analysis: (cont.)

Sample	C_7H_8	$C_8H_{12}O$	$C_7H_{14}O$	$C_7H_{12}O$	$C_8H_{18}O$	$C_7H_{10}O$	$C_{10}H_{16}O$
5cD1	9.22	0	0	0	0	0	0
5cD2	25.11	3.08	0	0	16.93	0	0
5cD3	6.21	4.55	2.04	29.43	2.68	17.59	4.04
5cD4	3.93	5.30	1.31	29.09	2.50	18.12	3.94
5dD1	69.04	0	0	0	0	0	0
5dD2	3.16	3.85	3.31	36.78	4.15	17.15	3.23
5dD3	4.03	7.73	4.00	33.66	3.15	14.14	2.31
5dD4	3.15	3.46	2.36	36.28	4.10	21.17	3.21
4aD1	28.61	0	0	0	0	0	0
4aD2	11.02	3.88	3.62	33.17	10.98	13.31	10.34
4aD3	6.36	6.24	4.49	28.08	8.74	11.47	2.75
4aD4	4.07	0	1.61	19.12	13.52	25.81	6.89
4bD1	28.23	3.46	0	0	0	0	0
4bD2	11.18	11.29	3.76	26.00	4.12	11.50	2.13
4bD3	5.15	5.87	2.83	30.12	6.38	18.46	3.52
4bD4	1.65	0.76	0.88	20.68	11.00	24.34	5.85
4cD1	62.15	0	0	0	0	0	0
4cD2	7.00	10.14	4.30	27.16	5.41	11.87	3.30
4cD3	5.12	12.98	4.93	29.69	4.36	11.60	1.79
4cD4	3.89	6.25	3.45	39.54	6.20	18.17	2.45
4dD1	25.90	0	0	0	0	0	0
4dD2	10.39	13.71	4.40	24.10	4.82	10.70	2.91
4dD3	5.12	6.03	5.15	34.86	5.49	15.00	2.78
4dD4	2.90	4.80	3.00	35.09	6.02	17.23	2.60
3aD1	22.37	0	0	0	0	0	0
3aD2	7.34	4.85	2.84	26.71	0	11.00	0
3aD3	5.35	0	0	29.91	10.37	13.12	0
3aD4	4.26	4.49	2.66	26.58	9.62	11.33	2.30
3bD1	10.57	0	0	0	0	0	15.05
3bD2	10.67	6.67	3.97	21.20	7.37	5.89	0
3bD3	7.16	10.40	3.29	28.26	7.60	10.83	3.82
3bD4	2.58	7.85	1.66	29.92	11.33	15.58	3.15
3cD1	37.33	0	0	0	0	0	0
3cD2	8.06	2.33	4.52	31.23	5.64	14.17	3.00
3cD3	6.69	11.78	4.26	27.15	0.96	12.12	2.36
3cD4	2.32	0.74	12.55	24.74	16.92	13.21	7.90
3dD1	26.39	7.24	0	0	0	0	0
3dD2	6.18	6.95	4.04	24.16	6.98	9.49	2.47
3dD3	3.31	1.72	2.18	26.00	13.14	13.67	4.00
3dD4	2.59	3.76	2.60	29.87	10.48	13.03	2.85

Table H. 1.

Major GC/Volatile Analysis:
(% Peak Area based on Extract Volumes between 3 and 6 µl)

Sample	C_7H_8	$C_8H_{12}O$	$C_7H_{14}O$	$C_7H_{12}O$	$C_8H_{18}O$	$C_7H_{10}O$	$C_{10}H_{18}O$
7aD1	8.44	0	6.72	0	0	0	0.73
7aD2	3.01	4.30	0.48	31.97	1.82	11.69	2.11
7aD3	2.69	0	0	10.80	4.56	24.76	10.65
7aD4	1.25	0.82	0.28	22.01	3.61	2.19	6.45
7bD1	7.42	7.64	0	0	0	0	0
7bD2	6.48	4.04	2.97	17.02	6.04	5.94	1.49
7bD3	1.45	1.29	0.71	27.62	2.97	19.46	5.71
7bD4	2.59	2.75	0	32.92	3.20	20.43	5.37
7cD1	38.12	0	0	0	0	0	0
7cD2	11.81	36.72	2.52	15.04	0	5.86	0
7cD3	4.54	5.71	2.60	36.43	0.66	19.53	4.48
7cD4	0	2.05	0	41.52	2.61	27.51	4.99
7dD1	15.00	0	0	0	0	0	0
7dD2	7.88	6.08	3.93	29.19	1.79	12.72	3.00
7dD3	1.59	0	0	14.19	24.34	23.02	10.99
7dD4	1.49	0.78	1.31	18.94	4.56	24.47	9.30
6aD1	24.34	0	0	0	0	0	0
6aD2	7.75	9.36	2.64	19.63	1.73	8.19	6.18
6aD3	9.85	5.76	0	35.06	0	0	0
6aD4	0	0	0	18.33	5.82	24.30	9.53
6bD1	26.26	0	0	0	0	0	0
6bD2	6.73	8.20	6.50	27.99	2.57	12.46	2.96
6bD3	6.02	10.53	4.37	32.85	2.20	14.62	2.74
6bD4	4.28	8.90	3.46	34.71	2.07	14.90	3.47
6cD1	24.85	1.46	0	0	0	0	0
6cD2	7.25	8.55	3.14	26.28	2.33	11.31	2.39
6cD3	5.85	3.14	3.20	32.14	3.12	17.92	8.78
6cD4	5.06	7.77	2.74	36.03	2.18	16.26	2.75
6dD1	78.00	0	0	0	0	0	0
6dD2	7.61	11.21	4.08	29.68	2.28	12.71	2.20
6dD3	4.11	4.86	3.06	37.64	3.08	17.81	3.26
6dD4	2.67	1.51	1.26	27.55	4.69	26.91	4.69
5aD1	21.80	0	0	0	0	0	0
5aD2	1.92	0.54	0	17.31	1.43	6.67	5.25
5aD3	2.48	3.95	1.54	31.01	3.32	16.08	4.63
5aD4	6.07	13.34	4.24	29.73	2.25	10.44	1.39
5bD1	14.77	0	0	0	0	0	0
5bD2	8.37	7.50	0	34.89	4.39	16.98	3.99
5bD3	6.11	16.57	3.40	21.97	1.47	9.25	19.55
5bD4	3.84	1.91	1.15	22.80	23.35	3.87	5.60

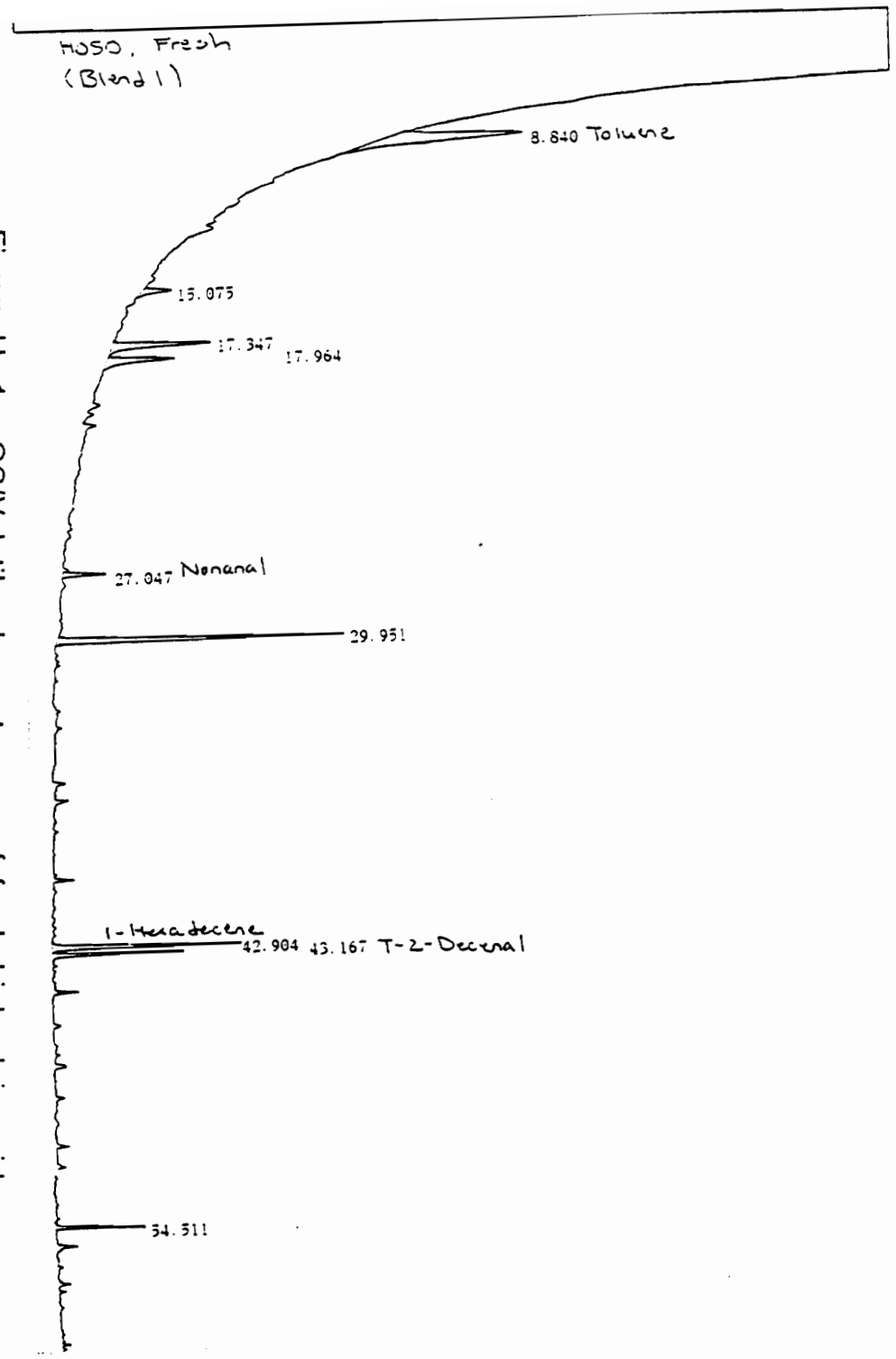


Figure H. 1. GC/Volatile chromatogram of fresh high oleic acid sunflower oil.

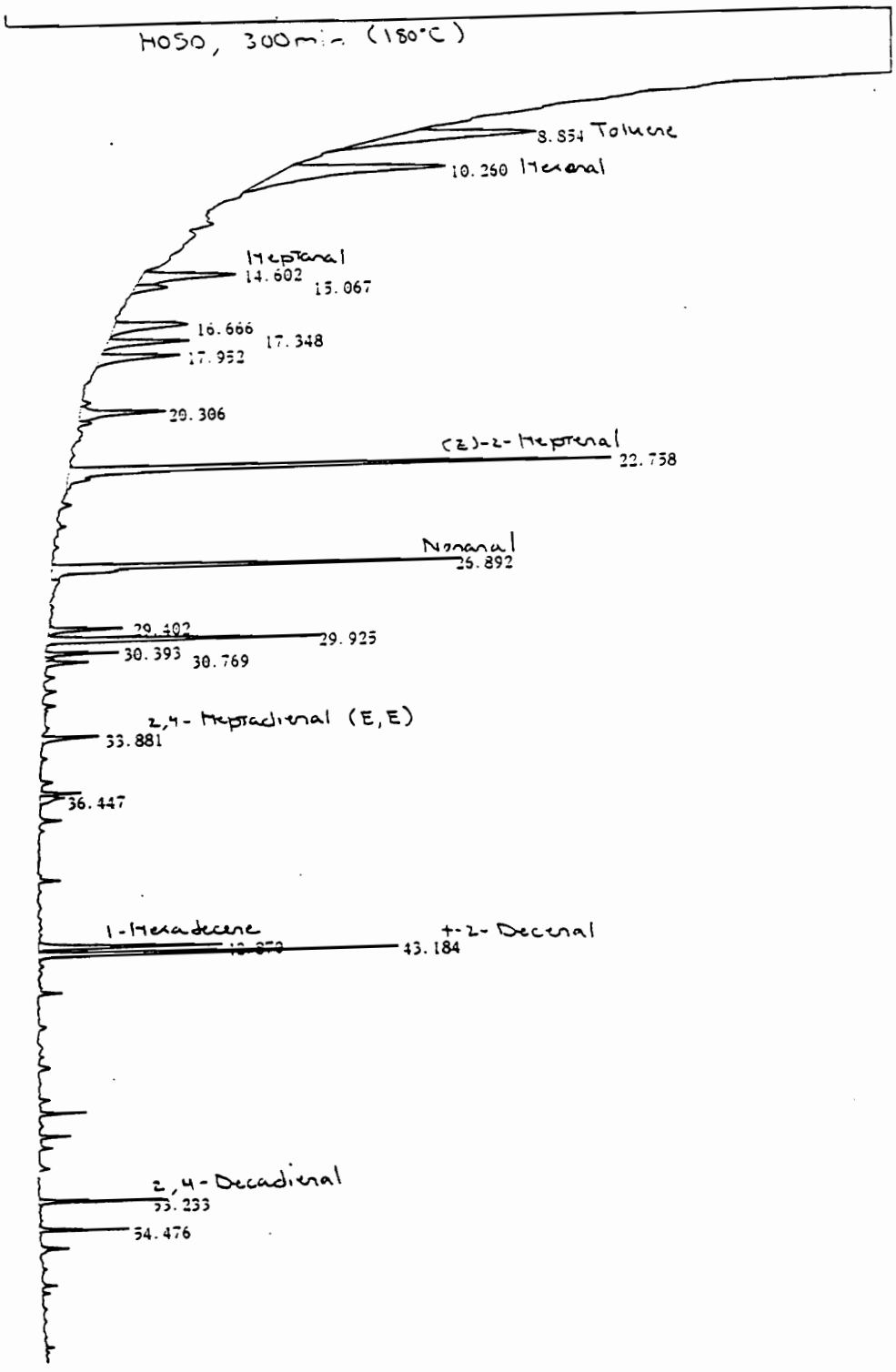


Figure H. 2. GC/Volatile chromatogram of high oleic acid sunflower oil heated at 180°C for 300 minutes.

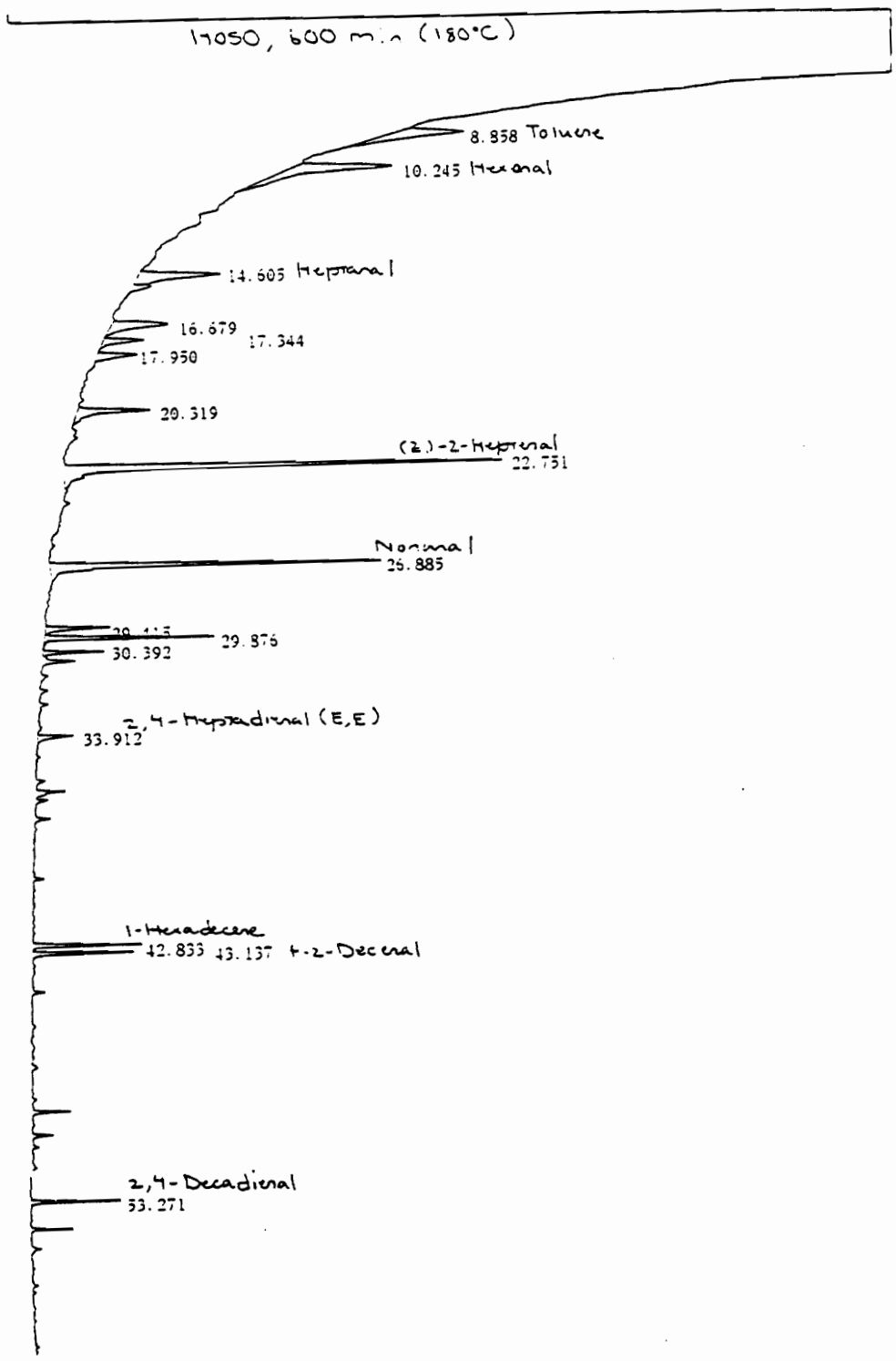


Figure H. 3. GC/Volatile chromatogram of high oleic acid sunflower oil heated at 180°C for 600 minutes.

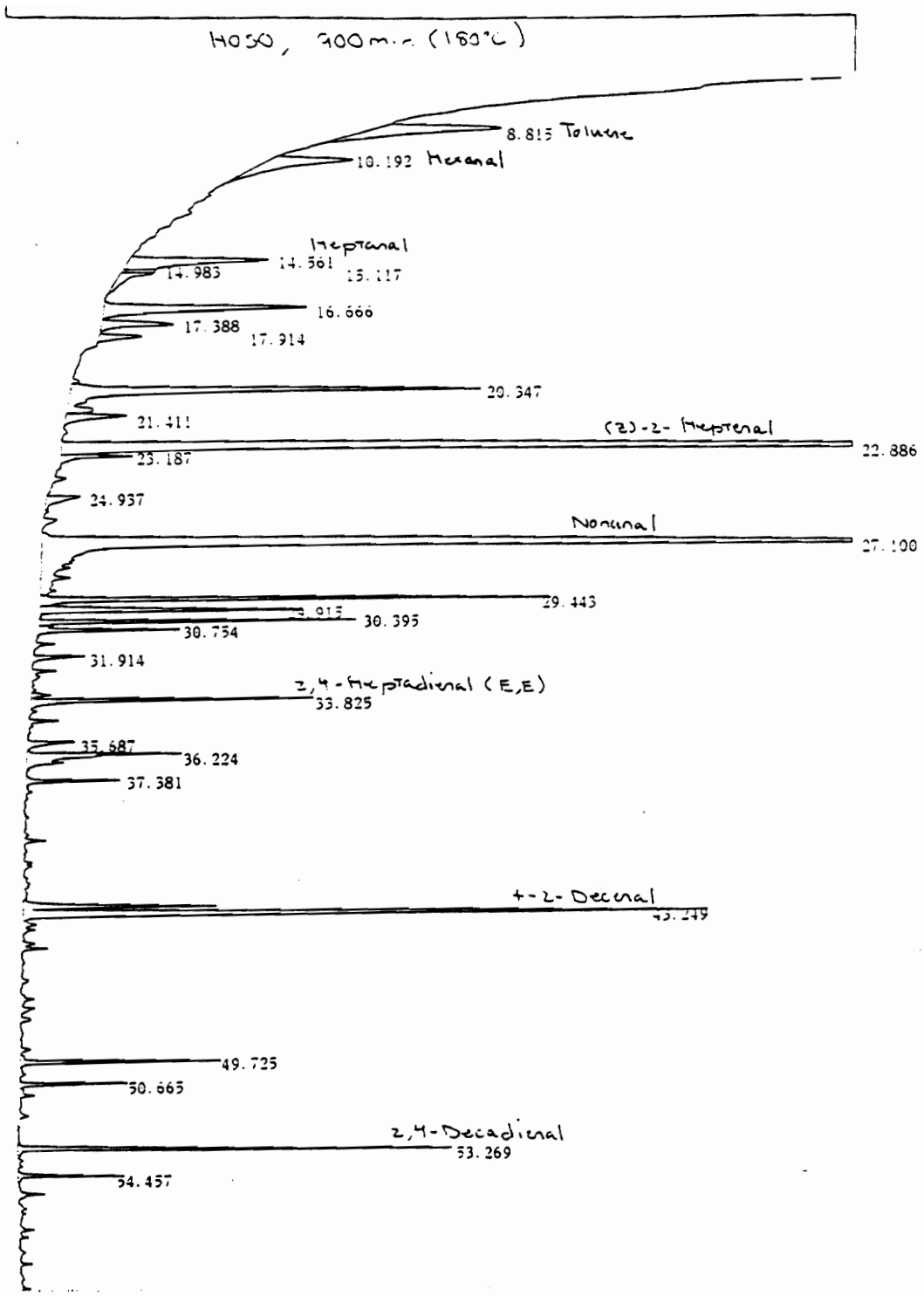


Figure H. 4. GC/Volatile chromatogram of high oleic acid sunflower oil heated at 180°C for 900 minutes.

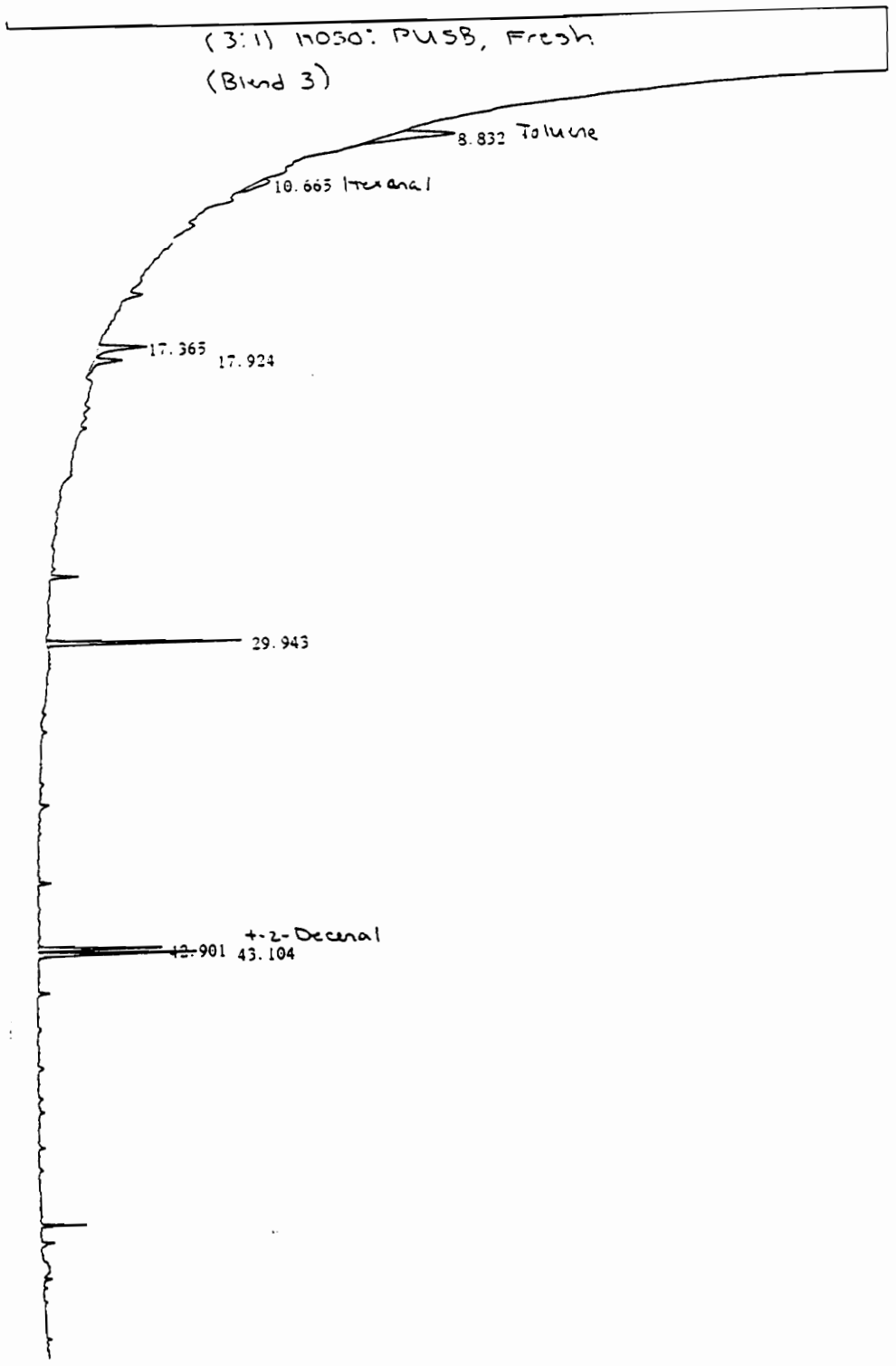


Figure H. 5. GC/Volatile chromatogram of fresh (3:1) high oleic acid sunflower to soybean oil.

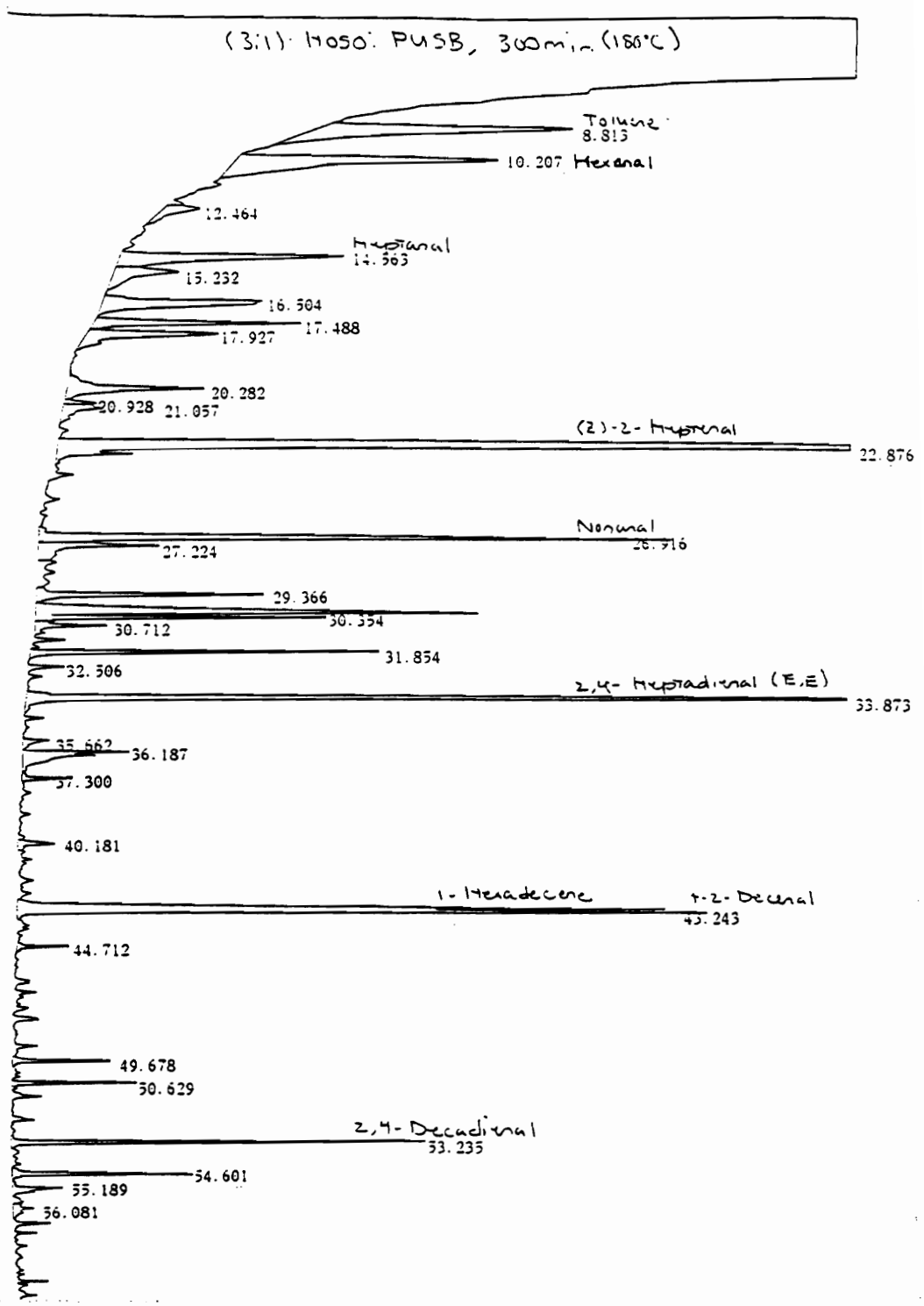


Figure H. 6. GC/Volatile chromatogram of (3:1) high oleic acid sunflower to soybean oil heated at 180°C for 300 minutes.

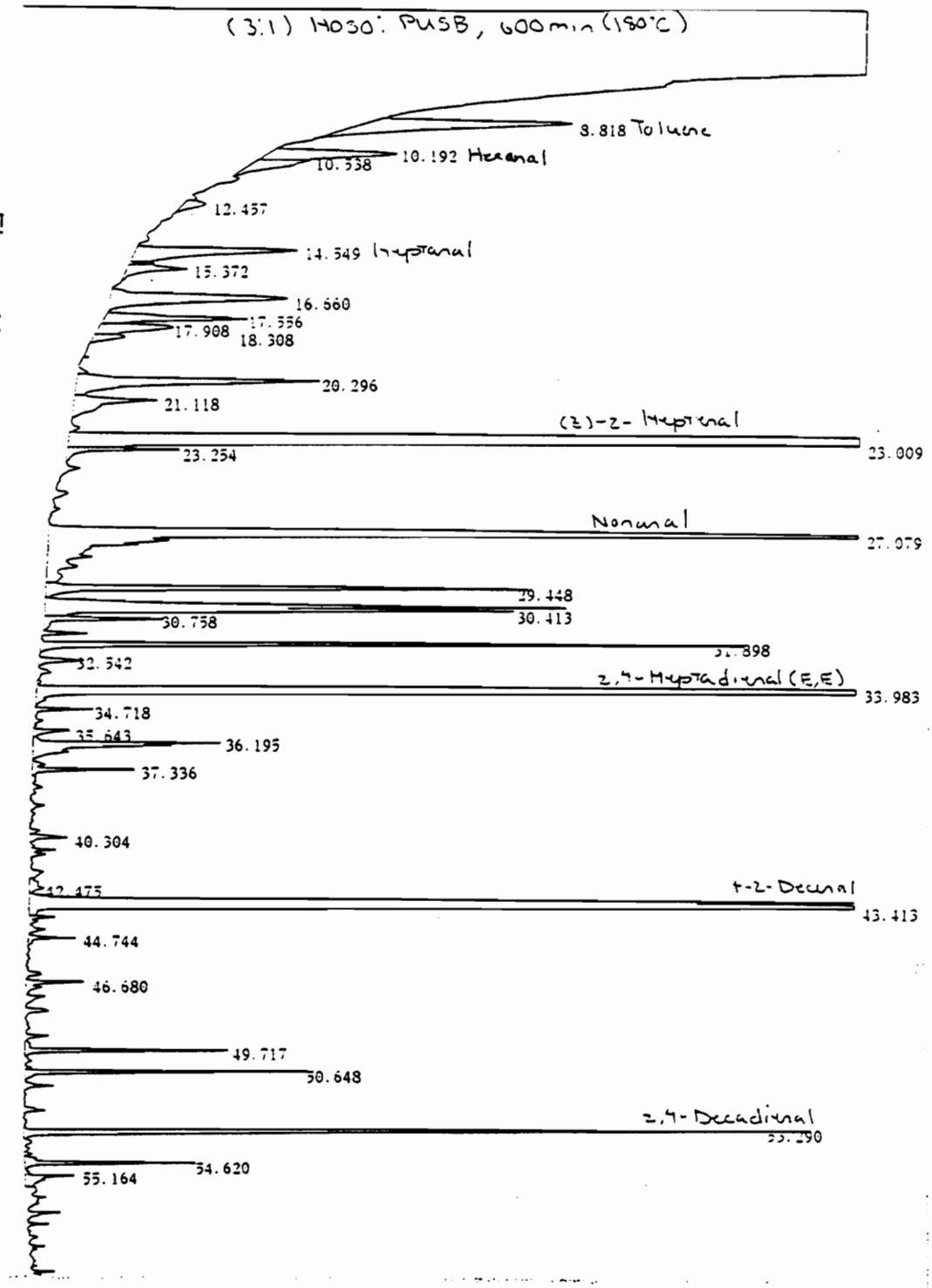


Figure H. 7. GC/Volatile chromatogram of (3:1) high oleic acid sunflower to soybean oil heated at 180°C for 600 minutes.

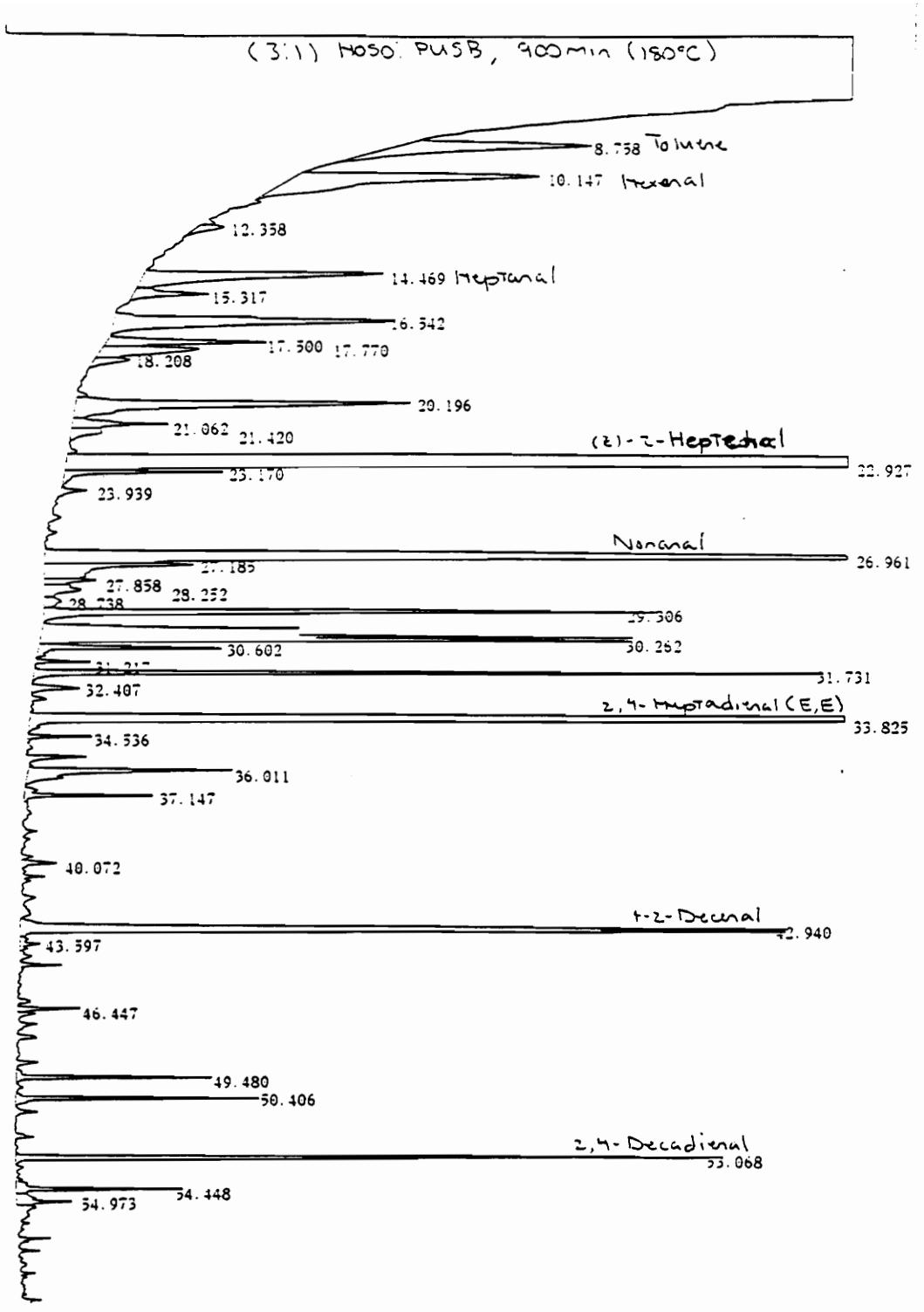


Figure H. 8. GC/Volatile chromatogram of (3:1) high oleic acid sunflower to soybean oil heated at 180°C for 900 minutes.

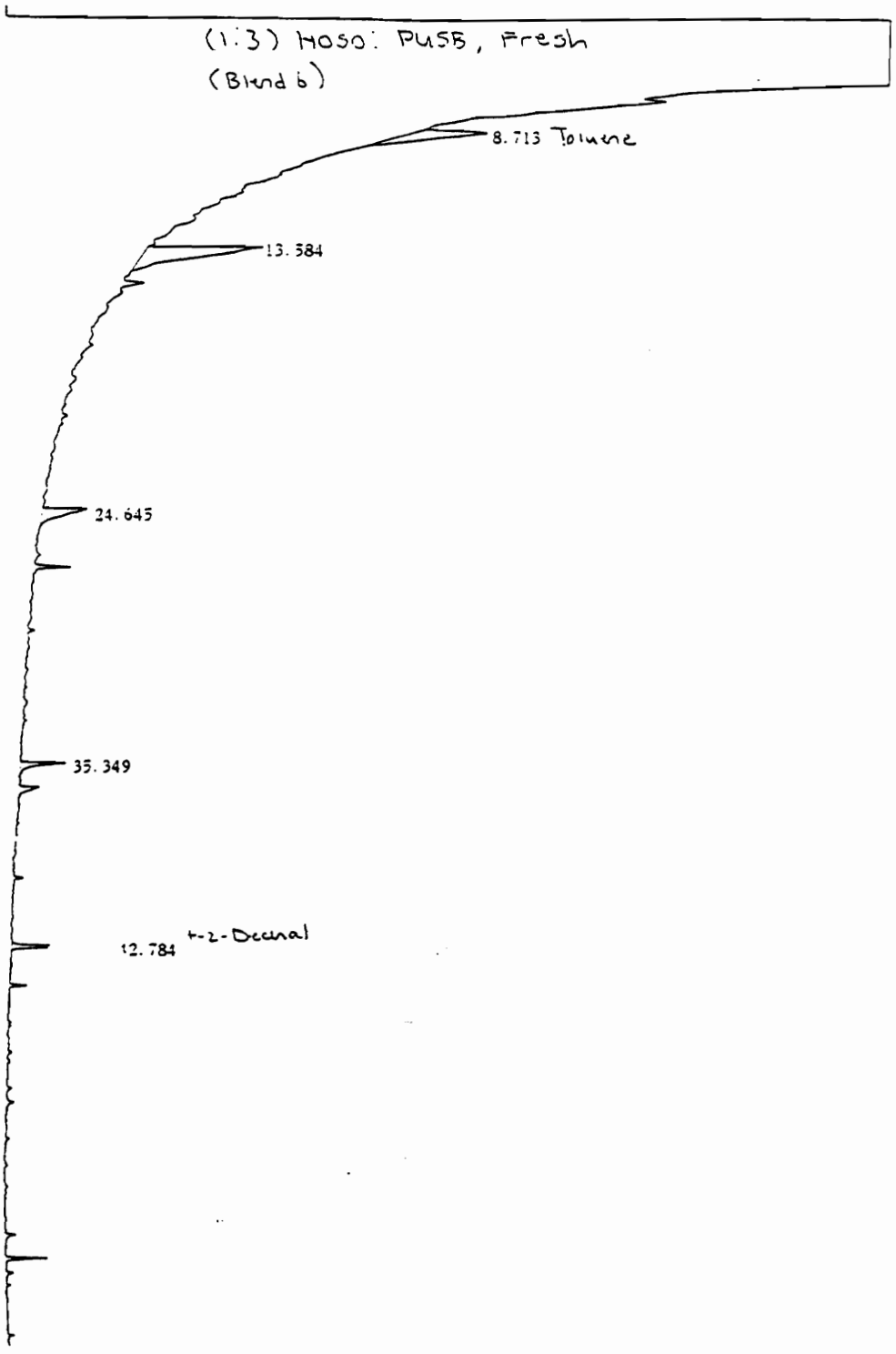


Figure H. 9. GC/Volatile chromatogram of fresh (1:3) high oleic acid sunflower to soybean oil.

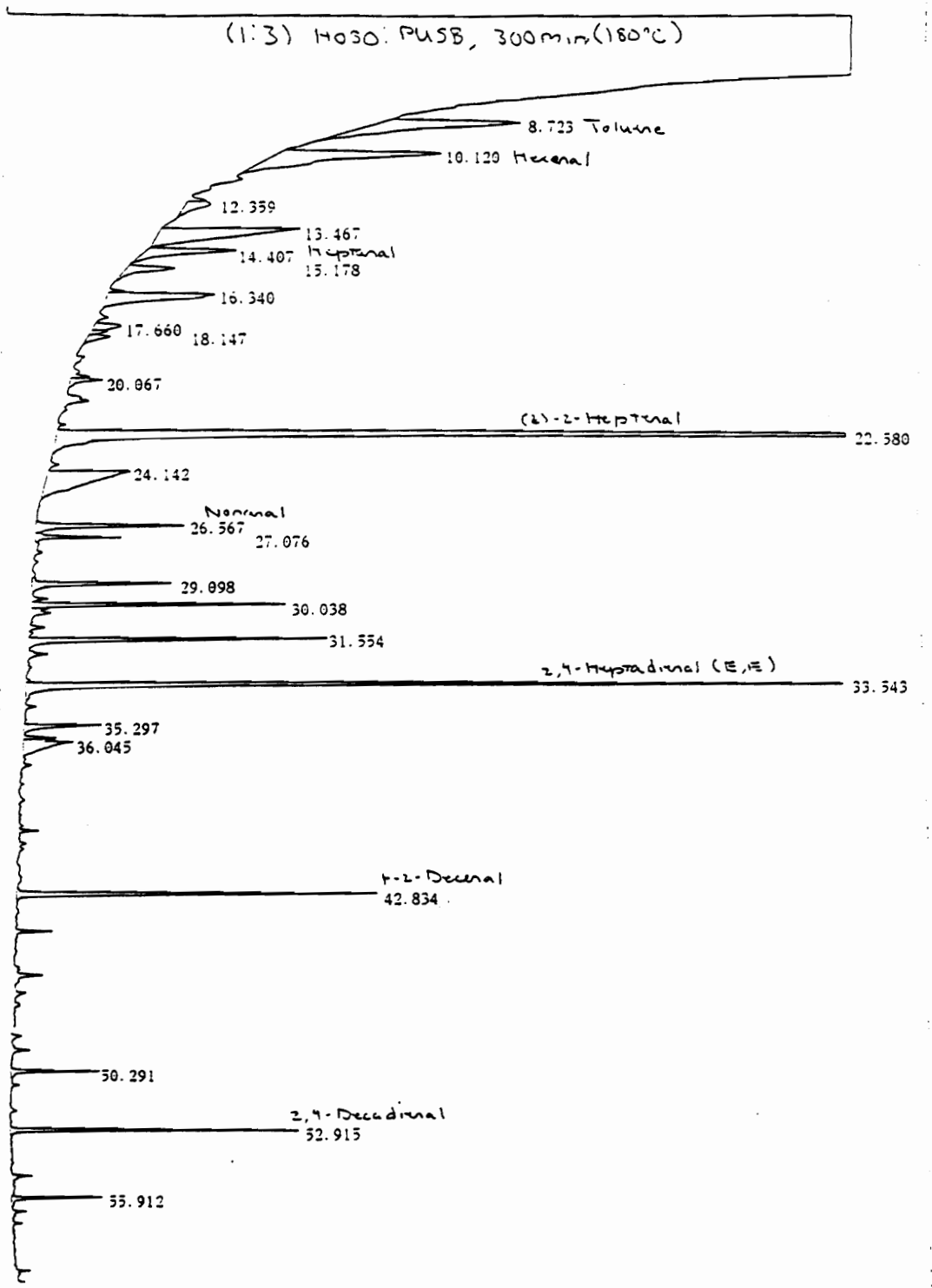


Figure H. 10. GC/Volatile chromatogram of (1:3) high oleic acid sunflower to soybean oil heated at 180°C for 300 minutes.

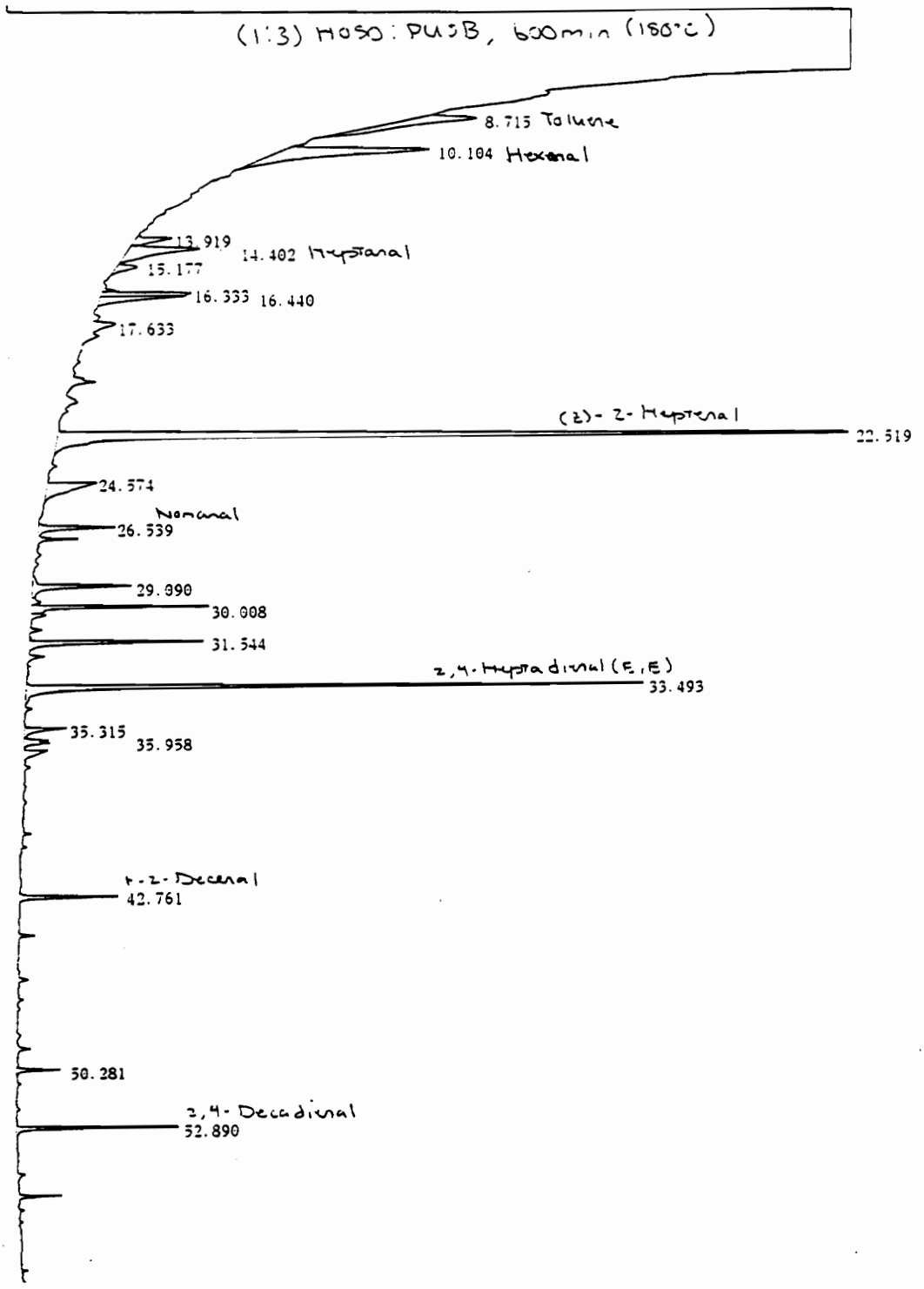


Figure H. 11. GC/Volatile chromatogram of (1:3) high oleic acid sunflower to soybean oil heated at 180°C for 600 minutes.

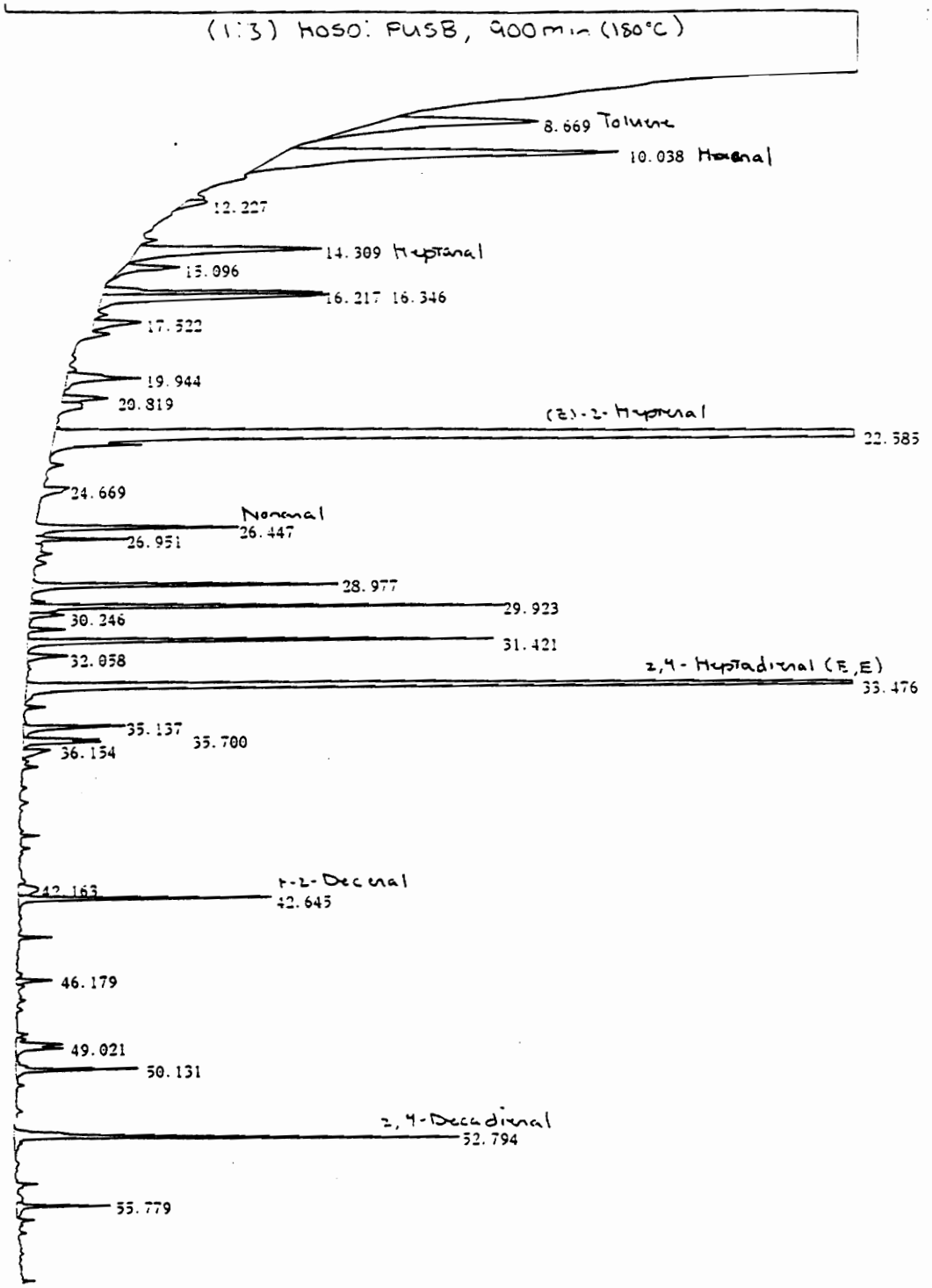


Figure H. 12. GC/Volatile chromatogram of (1:3) high oleic acid sunflower to soybean oil heated at 180°C for 900 minutes.

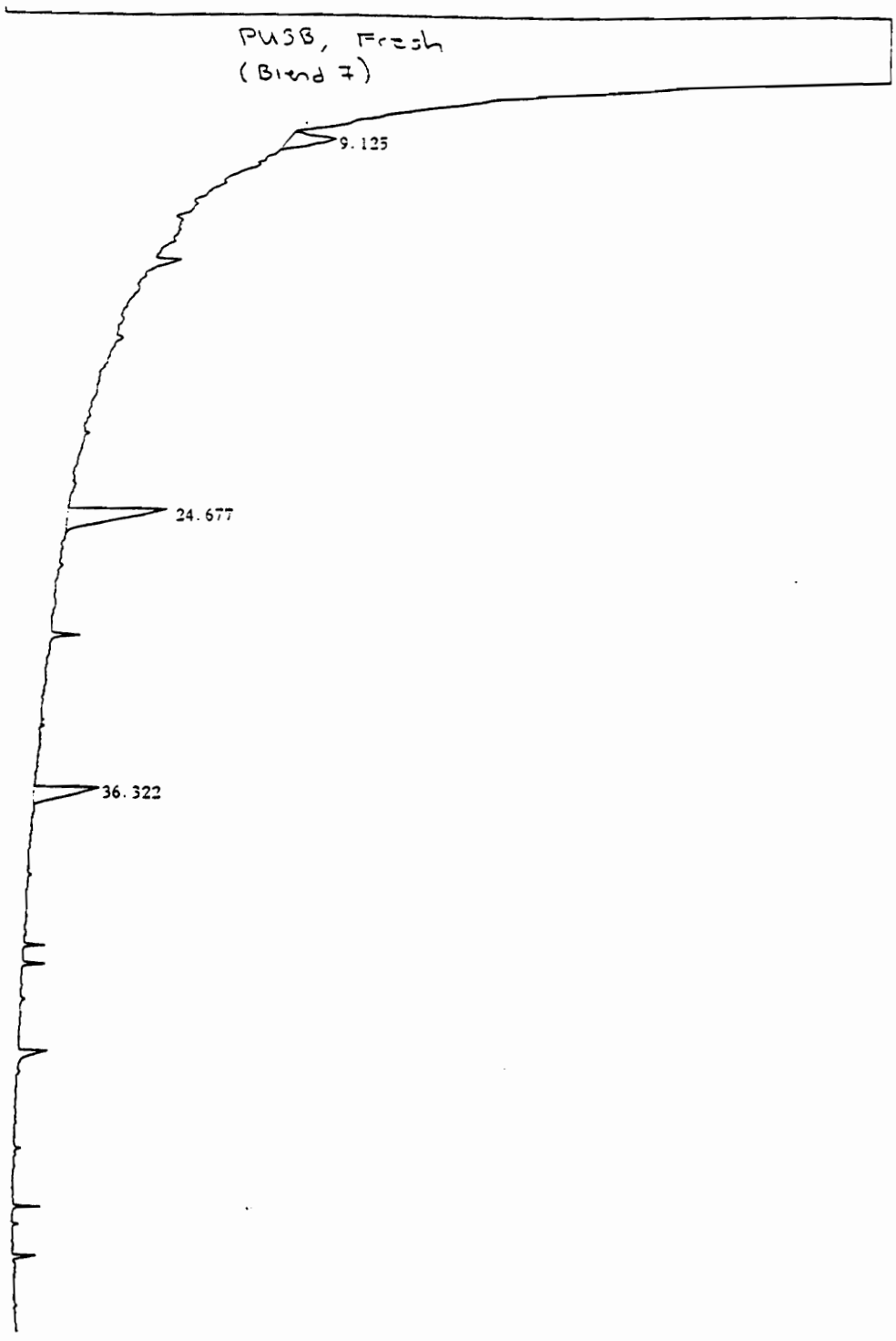


Figure H. 13. GC/Volatile chromatogram of fresh soybean oil.

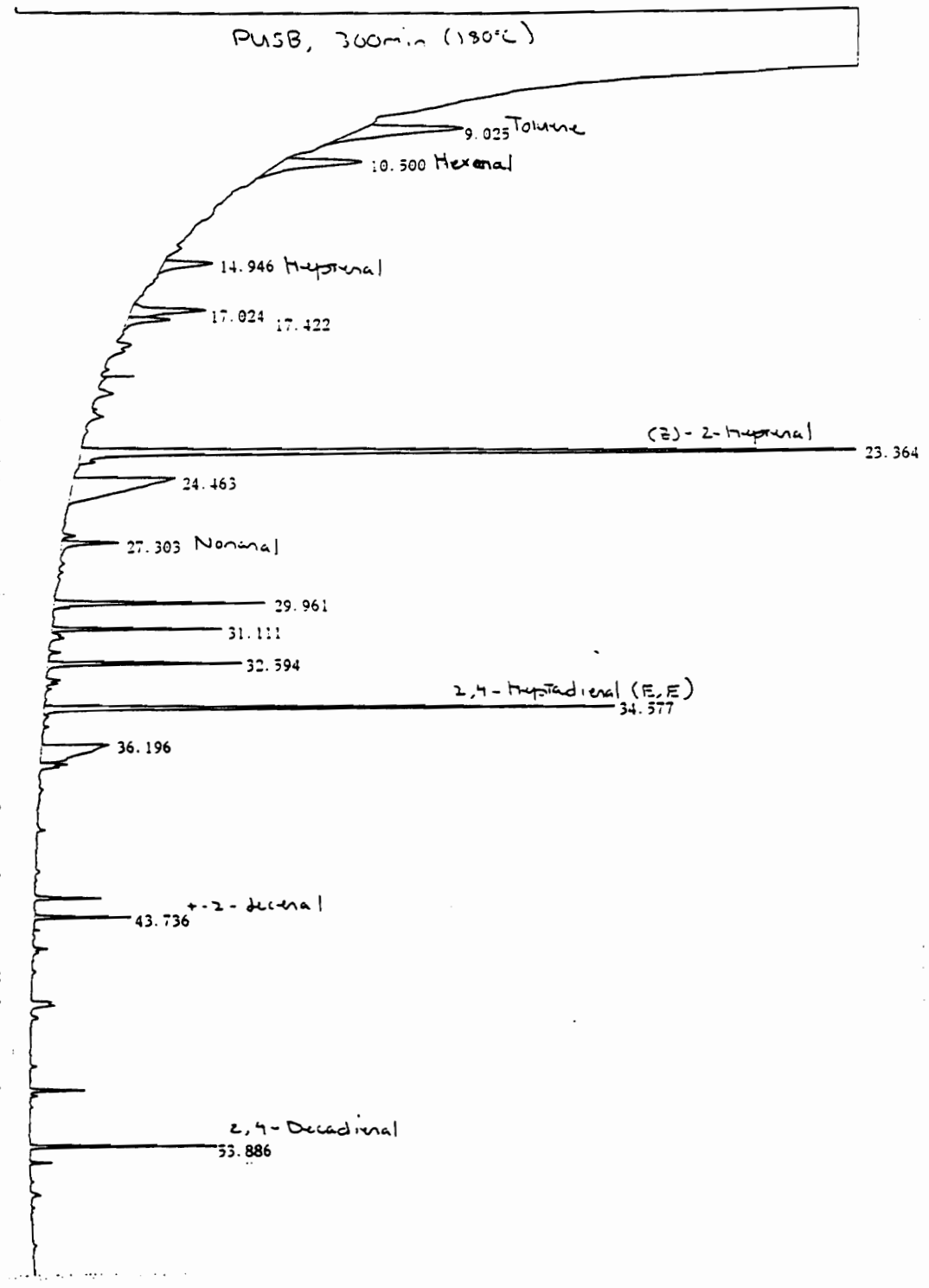


Figure H. 14. GC/Volatile chromatogram of soybean oil heated at 180°C for 300 minutes.

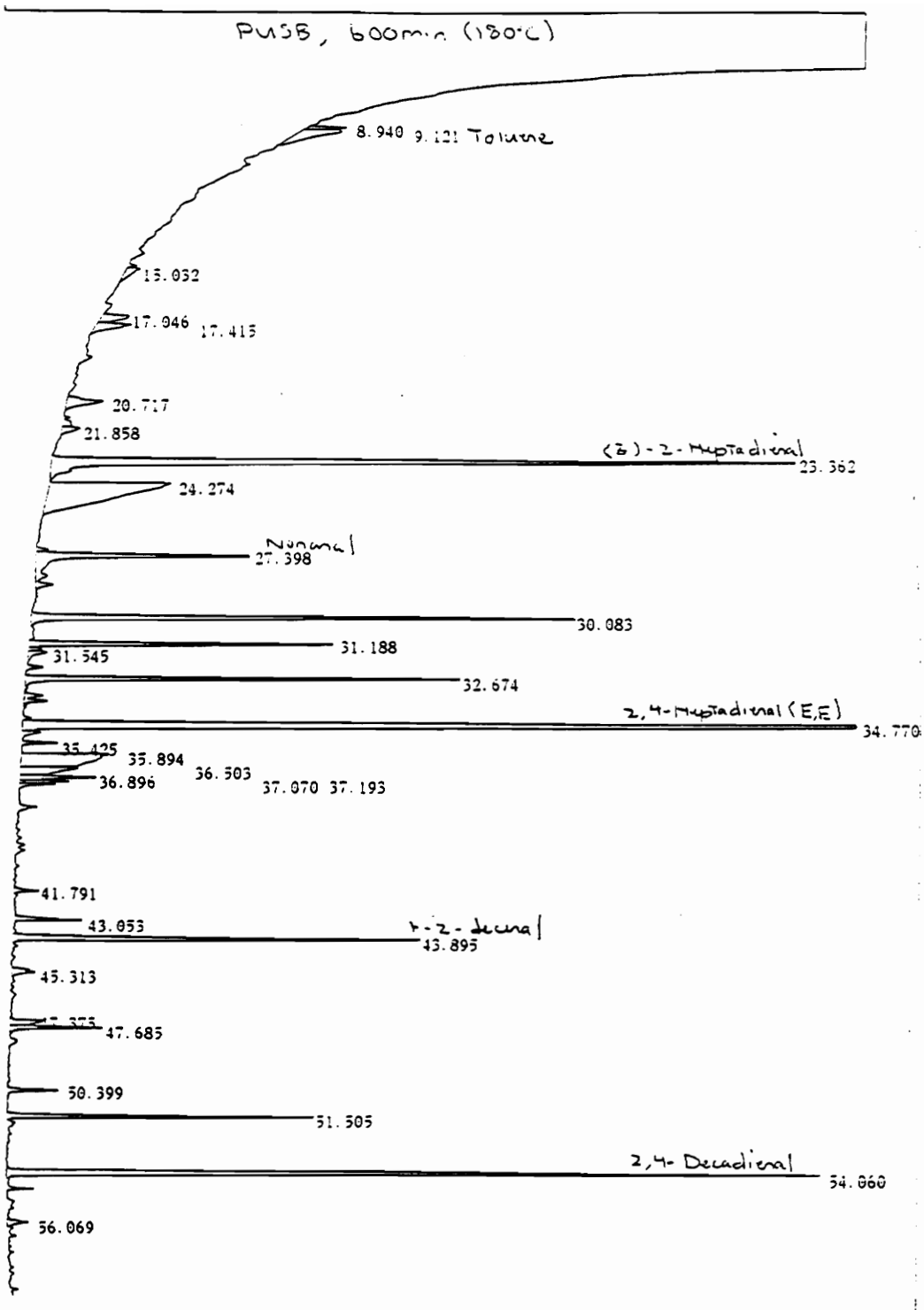


Figure H. 15. GC/Volatile chromatogram of soybean oil heated at 180°C for 600 minutes.

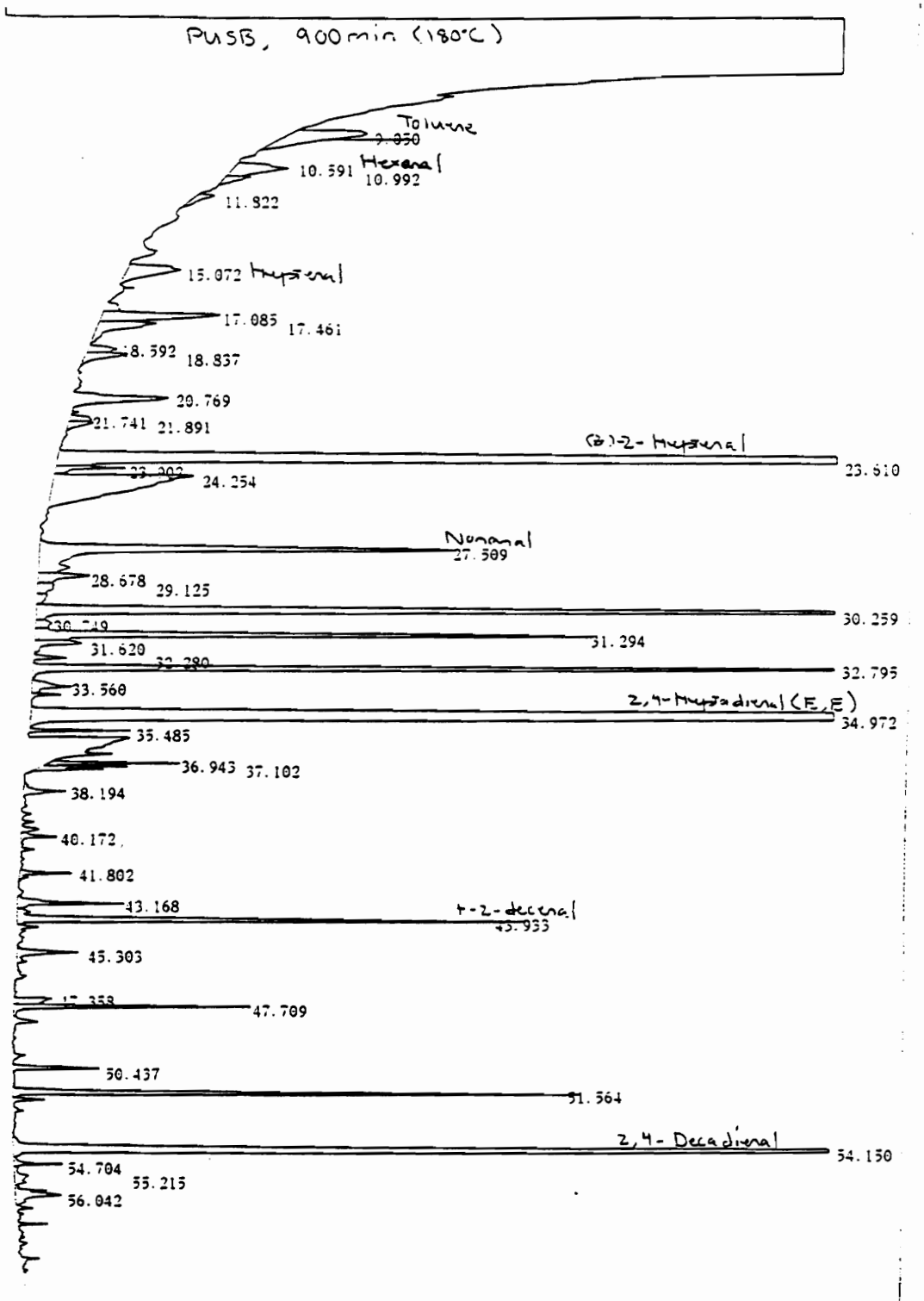


Figure H. 16. GC/Volatile chromatogram of soybean oil heated at 180°C for 900 minutes.

Appendix I

GC/MS Data, Chromatograms, and Spectra

Table I. 1. GC/MS peak areas of (1:1) high oleic sunflower to soybean oil heated for 900 minutes at 180°C.

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	13.590	VV	0.174	134909759	13.490	13.766
2	13.886	VV	0.276	221097125	13.766	14.517
3	15.684	VV	0.257	472492480	15.281	16.799
4	17.621	VV	0.220	543808940	17.294	18.032
5	18.147	VV	0.229	79942590	18.032	18.688
6	21.967	VV	0.418	183304276	21.851	22.835
7	23.126	PV	0.209	125282781	22.835	23.461
8	23.749	VV	0.213	119337759	23.461	23.989
9	25.552	VV	0.210	196449153	25.042	26.028
10	27.136	VV	0.229	90596492	26.944	27.788
11	29.666	VV	0.266	85984140	29.095	29.887
12	32.566	VV	0.251	501070656	32.177	33.022
13	33.289	VV	0.671	289484825	33.022	34.736
14	36.690	VV	0.273	427511878	36.276	37.674
15	39.451	VV	0.235	221061794	39.146	39.709
16	39.868	VV	0.177	125100235	39.709	40.100
17	41.929	PV	0.191	65198652	41.658	42.158
18	44.012	VV	0.207	192978907	43.738	44.455
19	45.083	VV	0.403	104745388	44.989	45.838
20	46.071	VV	0.246	195943540	45.838	46.673
21	52.451	VV	0.175	78909592	52.101	52.688
22	53.281	VV	0.329	761125401	52.688	53.746
23	54.393	VV	0.167	54290587	54.111	54.615
24	58.710	VV	0.195	58319415	58.364	59.150
25	59.508	VV	0.184	140381993	59.150	59.740
26	61.846	VV	0.231	436921414	61.233	62.092
27	64.422	VV	0.213	82617735	64.201	64.791
28	68.154	VV	0.389	66603589	67.416	68.472
29	72.982	VV	0.291	53958367	72.487	73.405
30	76.313	VV	0.255	53660065	75.940	76.689

File : C:\HPCHEM\1\DATA\TAW\DATA\S5.D
Operator : Troy Wilkerson
Acquired : 25 Jul 96 1:41 pm using AcqMethod VOL
Instrument : 5972 - In
Sample Name: Vegetable Volatiles
Misc Info :
Vial Number: 1

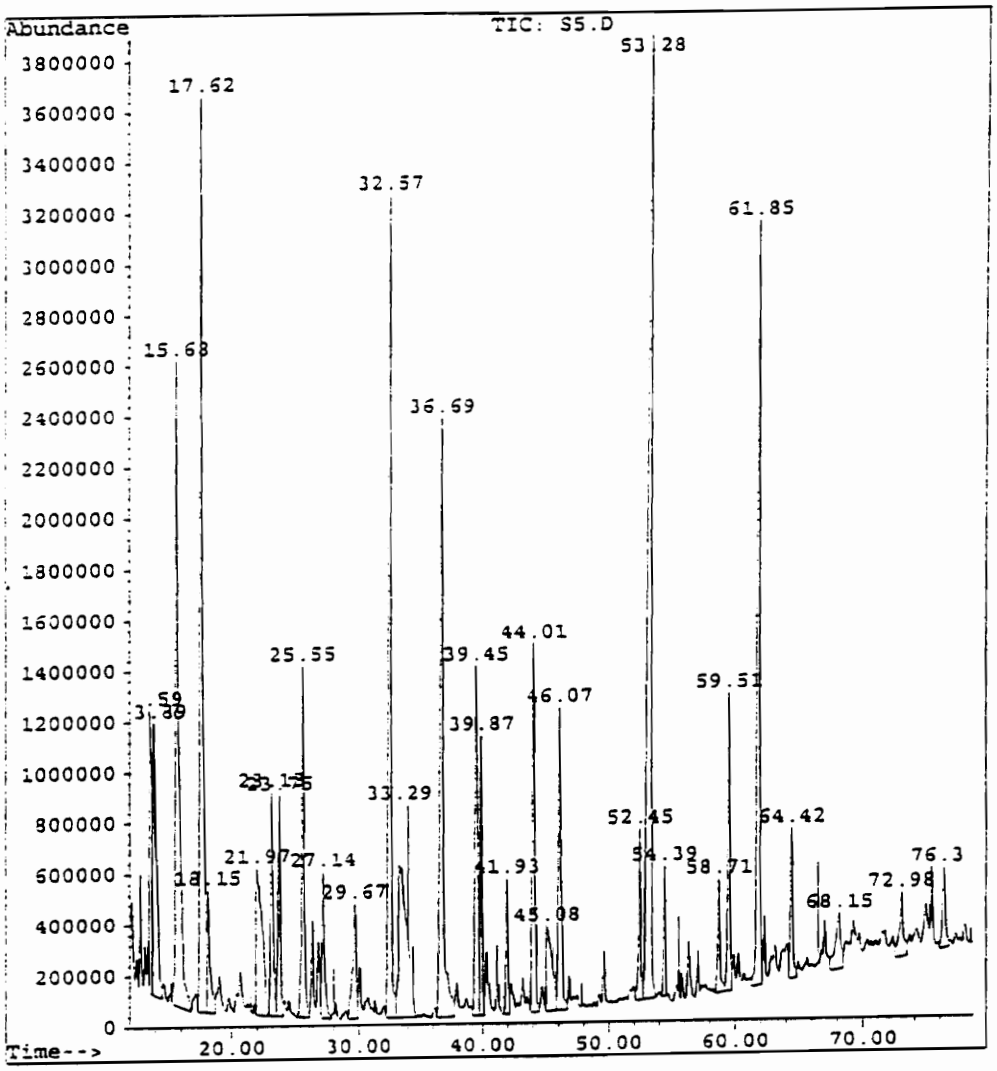
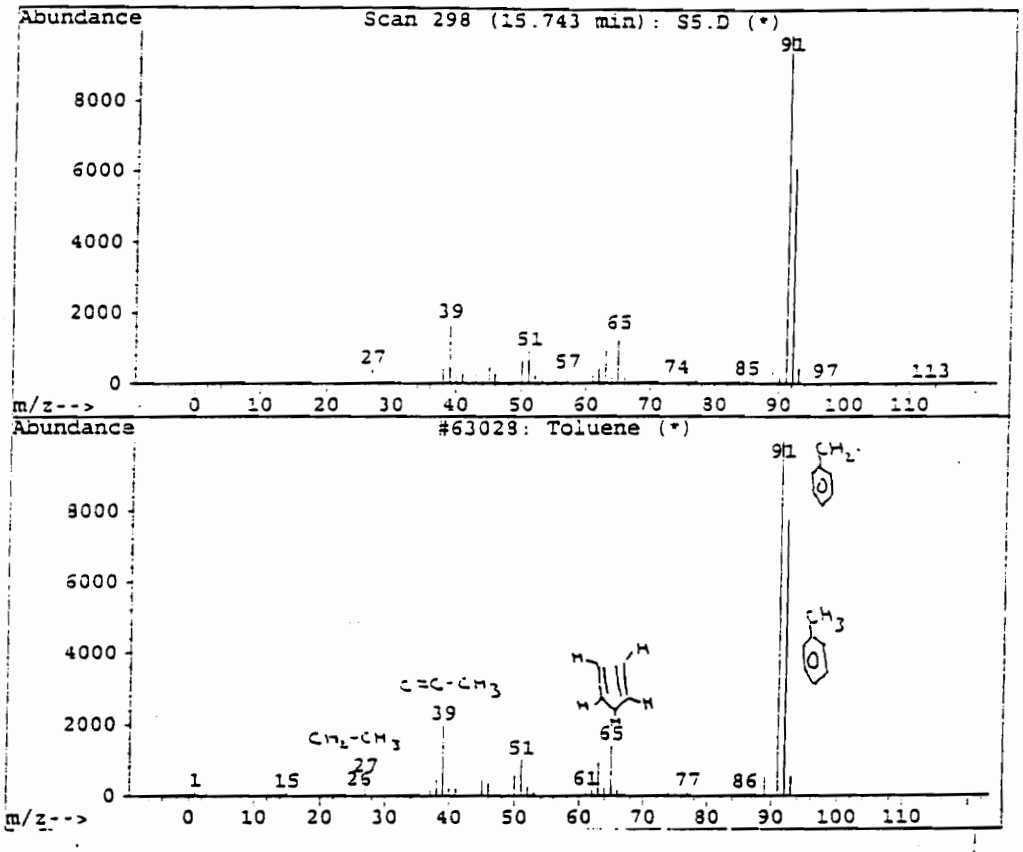


Figure I. 1. GC/MS chromatogram of (1:1) high oleic acid sunflower to soybean oil heated for 900 minutes at 180°C.

Table I. 2. Volatile compounds identified by GC/MS after extraction from (1:1) high oleic acid sunflower oil to soybean oil following 900 minute of heating at 180°C.

Volatile Compound	Retention Time	% Peak Area
Toluene	15.68	6.38
Hexanal	17.63	7.32
Undecane	18.15	1.08
Heptanal	23.13	1.69
2-Pentylfuran	25.55	2.65
1-Dodecene	26.30	0.60
1-pentanol	26.70	0.58
Tridecane	30.04	0.41
(Z)-2-Heptenal	32.56	6.75
Nonanal	36.69	5.76
(E)-2-Octenal	39.45	2.98
1-Octen-3-ol	39.86	1.68
Pentadecane	43.20	0.26
(E,E)-2,4-Heptadienal	44.01	2.60
Benzaldehyde	46.07	2.64
Hexadecane	49.62	0.35
1-Hexadecene	52.46	1.06
(E,E)-2,4-Decadienal	61.85	5.89
Benzyl Alcohol	64.42	1.11

Library Searched : C:\DATABASE\NBS75K.L
Quality : 97
ID : Toluene

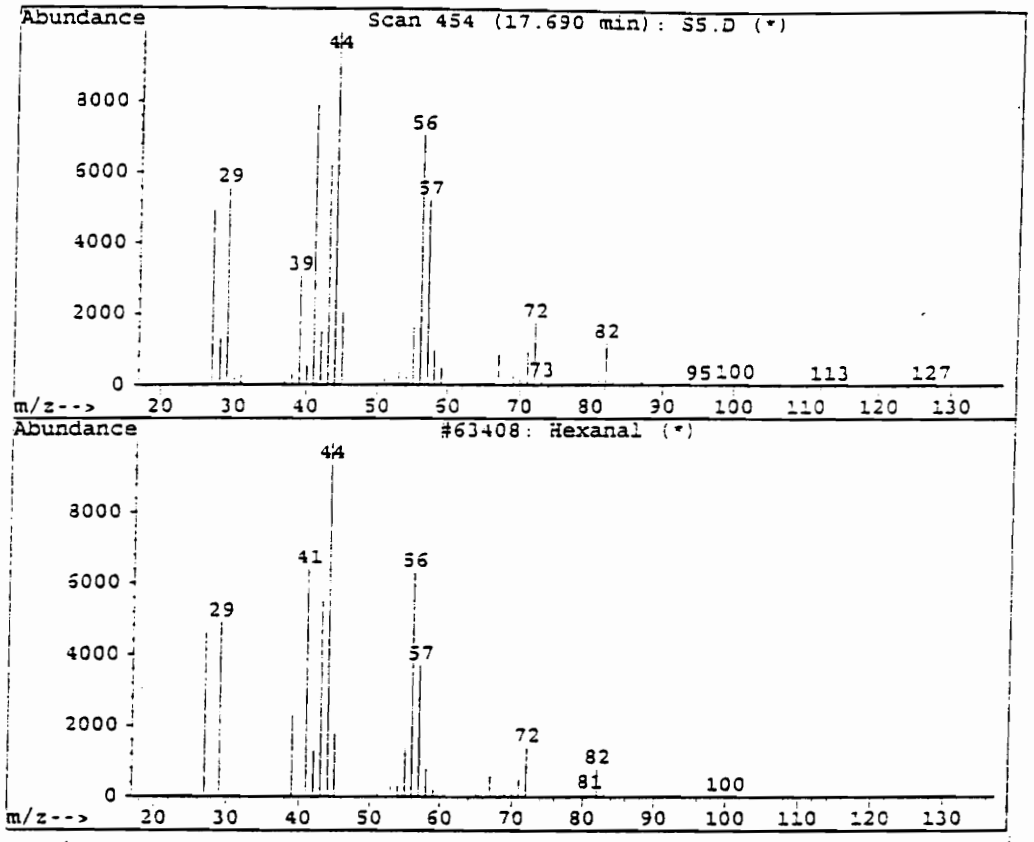


Toluene

Match Quality : 97
Entry Number : 63028
CAS Number : 000108-88-3
Molecular Weight : 92.06
Molecular Formula: C7H8
Retention Index : 0
Company ID : NIST 1992
Melting Point :
Boiling Point :
Misc Information :
QI=75 Amino Acids; Metals; Carbohydrates; Misc. Natural products
tty acids and Lipids;

Figure I. 2. GC/MS spectrum of toluene.

Library Searched : C:\DATABASE\NBS75K.L
Quality : 97
ID : Hexanal 2

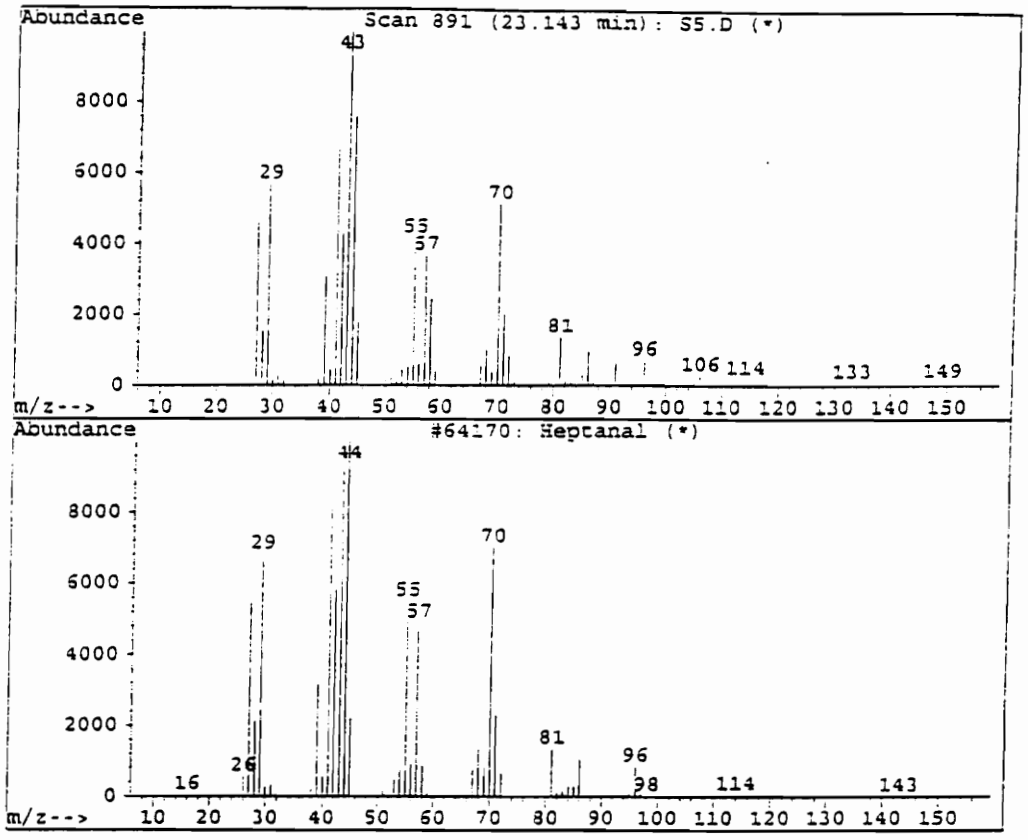


Hexanal

Match Quality : 97
Entry Number : 63408
CAS Number : 000066-25-1
Molecular Weight : 100.00
Molecular Formula: C6H12O
Retention Index : 0
Company ID : NIST 1992
Melting Point :
Boiling Point :
Misc Information :
QI=72 Derivatives; Metals; Carbohydrates; Misc. Natural products; Fatty acids and Lipids;

Figure I. 3. GC/MS spectrum of hexanal.

Library Searched : C:\DATABASE\NBS75K.L
Quality : 87
ID : Heptanal 3



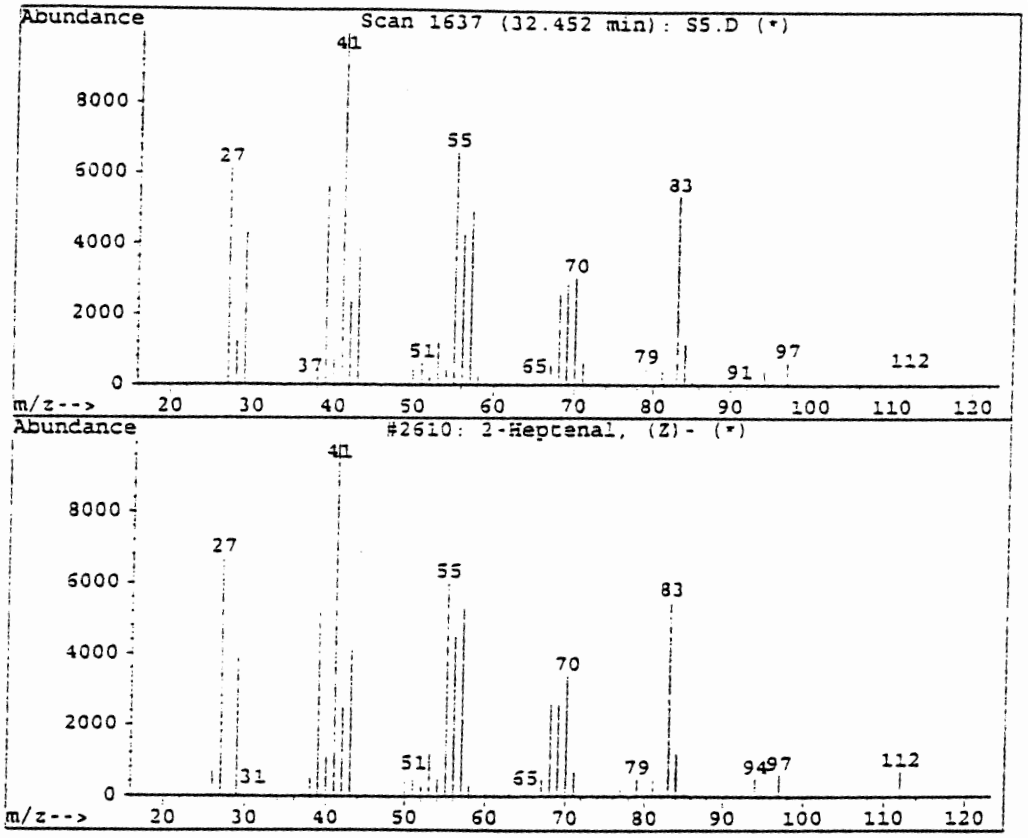
Heptanal

Match Quality	:	87
Entry Number	:	64170
CAS Number	:	000111-71-7
Molecular Weight	:	114.00
Molecular Formula	:	C7H14O
Retention Index	:	0
Company ID	:	NIST 1992
Melting Point	:	
Boiling Point	:	
Misc Information	:	

QI=61 Derivatives; Metals; Carbohydrates; Misc. Natural products;

Figure I. 4. GC/MS spectrum of heptanal.

Library Searched : C:\DATABASE\NBS75K.L
Quality : 96
ID : 2-Heptenal, (Z)-



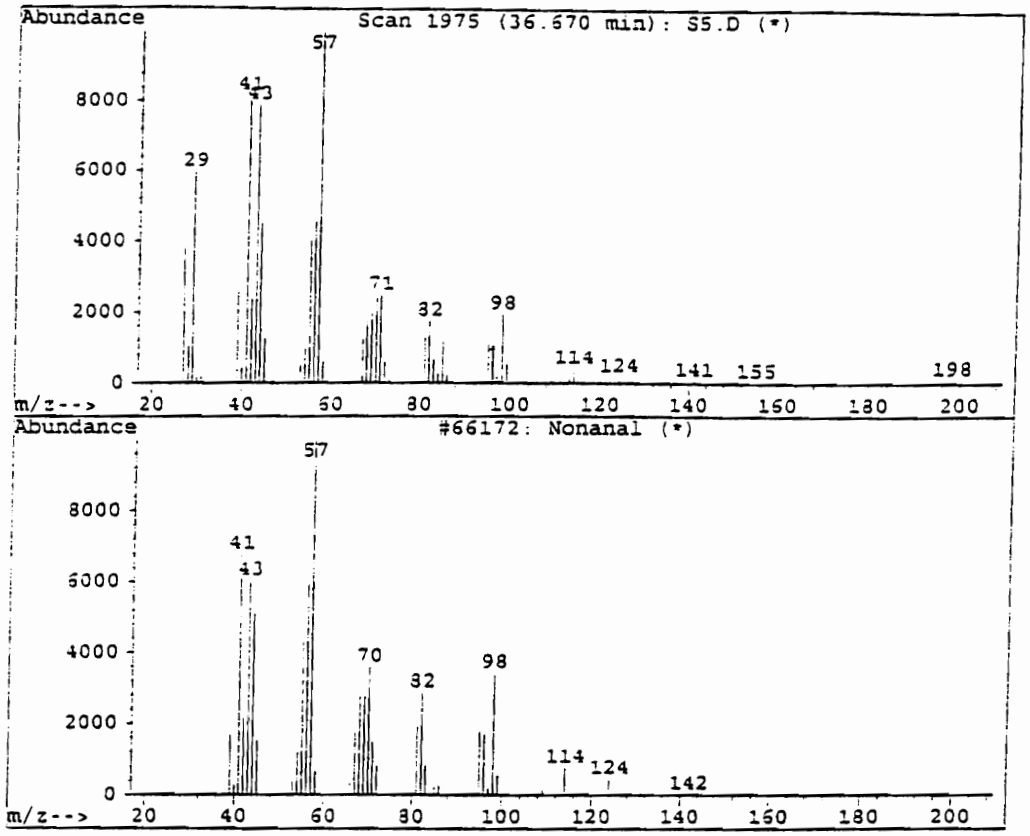
2-Heptenal, (Z)-

Match Quality	:	96
Entry Number	:	2610
CAS Number	:	057266-86-1
Molecular Weight	:	112.00
Molecular Formula	:	C7H12O
Retention Index	:	0
Company ID	:	NIST 1992
Melting Point	:	
Boiling Point	:	
Misc Information	:	

QI=78 Steroids;

Figure I. 5. GC/MS spectrum of (Z)-2-heptenal.

Library Searched : C:\DATABASE\NBS75K.L
Quality : 80
ID : Nonanal 12

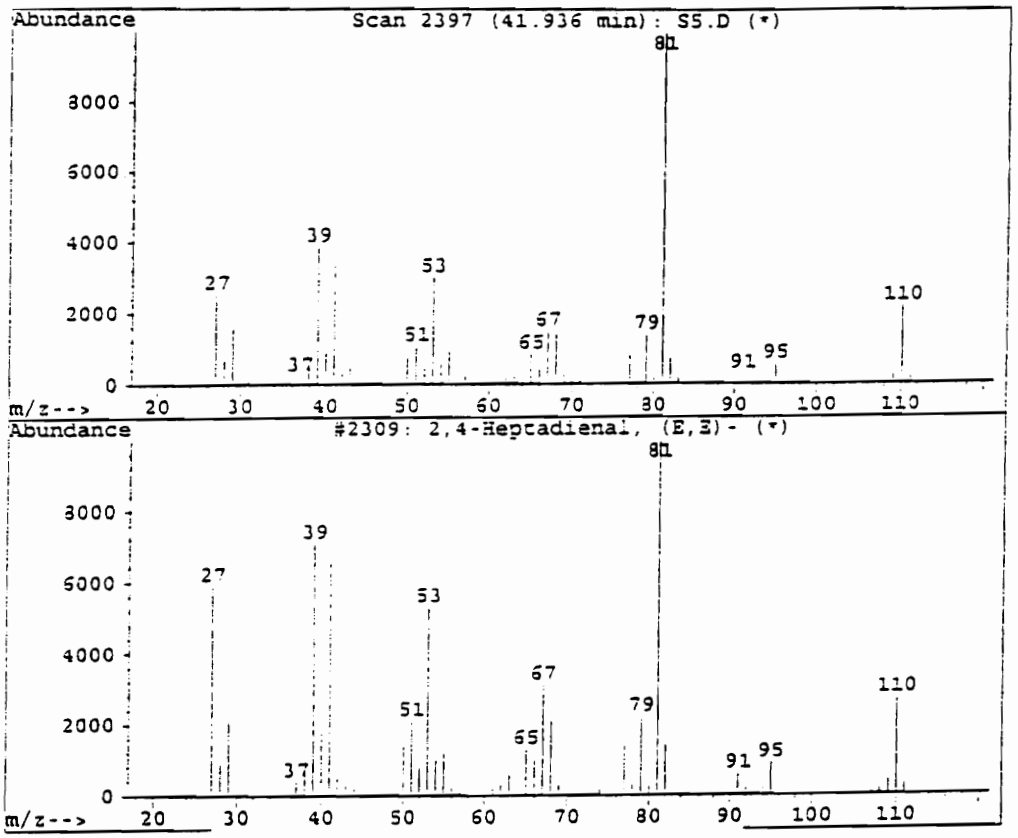


Nonanal

Match Quality : 80
Entry Number : 66172
CAS Number : 000124-19-6
Molecular Weight : 142.00
Molecular Formula: C9H18O
Retention Index : 0
Company ID : NIST 1992
Melting Point :
Boiling Point :
Misc Information :
QI=62 Derivatives; Metals; Carbohydrates; Misc. Natural products; Fatty acids and Lipids;

Figure I. 6. GC/MS spectrum of nonanal.

Library Searched : C:\DATABASE\NBS75K.L
Quality : 93
ID : 2,4-Heptadienal, (E,E)- (*)



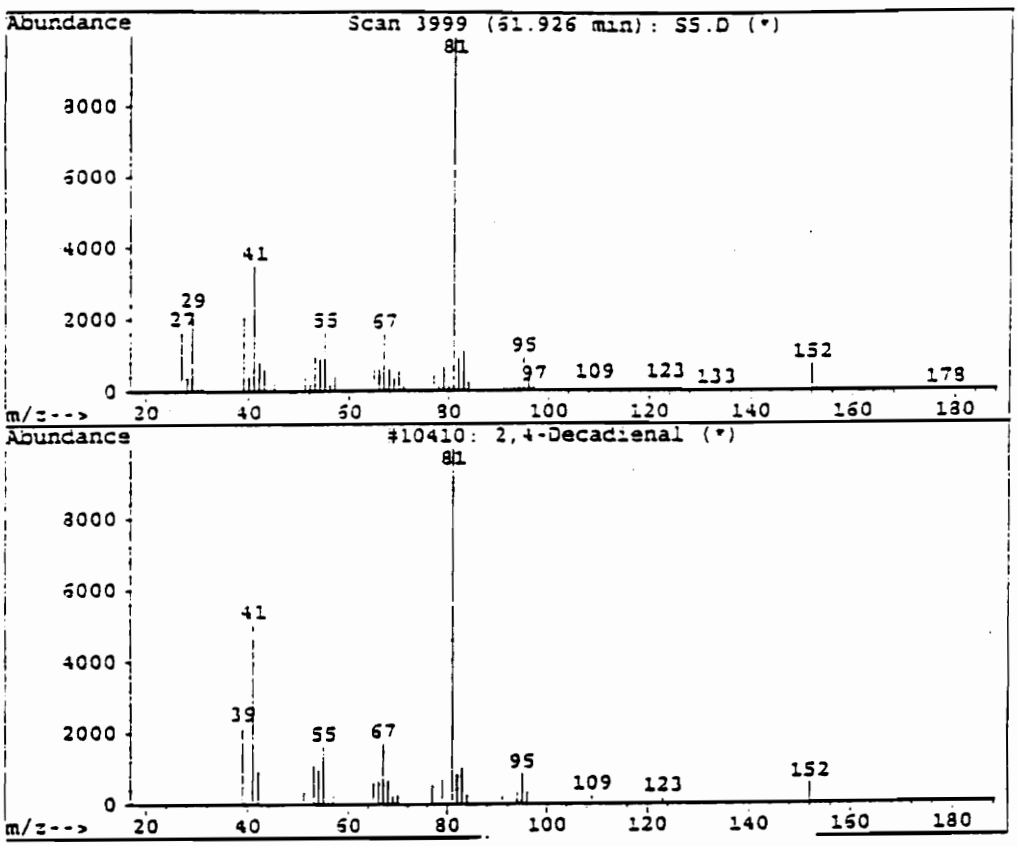
2,4-Heptadienal, (E,E)-

Match Quality	:	90
Entry Number	:	63862
CAS Number	:	004313-03-5
Molecular Weight	:	110.00
Molecular Formula	:	C7H10O
Retention Index	:	0
Company ID	:	NIST 1992
Melting Point	:	
Boiling Point	:	
Misc Information	:	

QI=62 Drugs; Metals; Misc. Natural products;

Figure I. 7. GC/MS spectrum of 2,4-heptadienal.

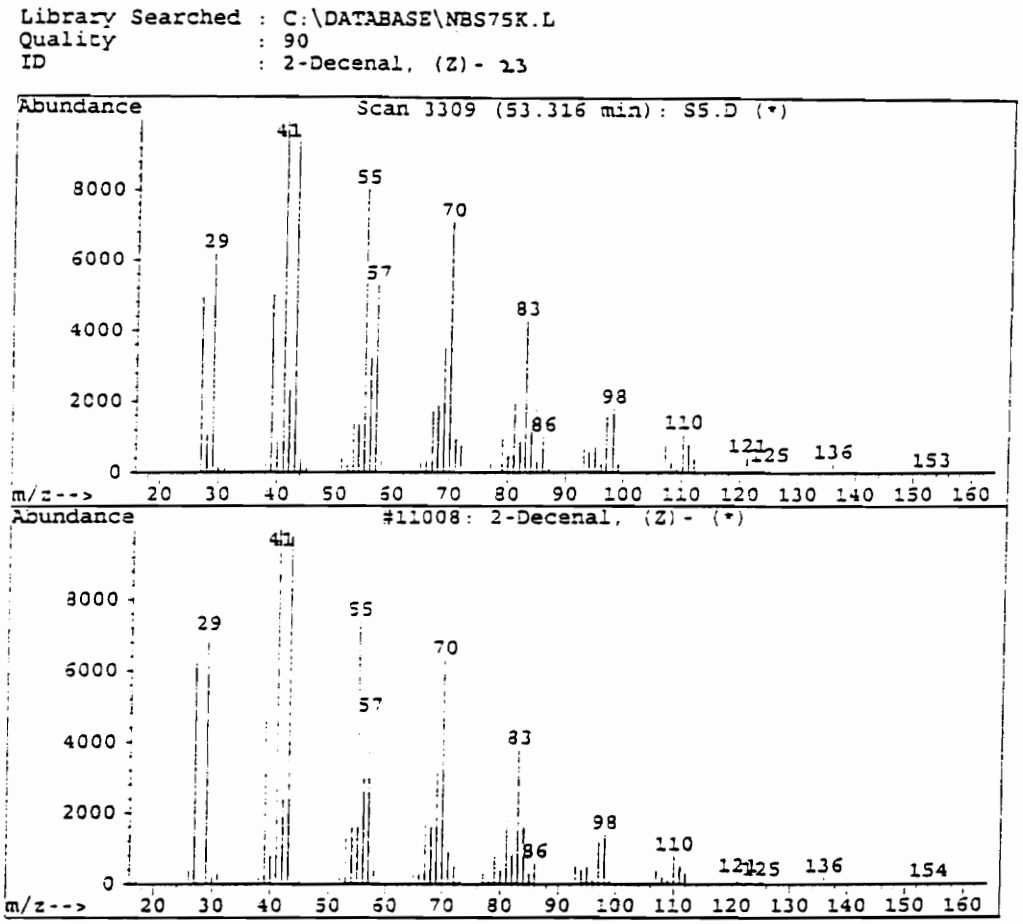
Library Searched : C:\DATABASE\NBS75K.L
Quality : 94
ID : 2,4-Decadienal



2,4-Decadienal

Match Quality : 94
Entry Number : 10410
CAS Number : 002363-88-4
Molecular Weight : 152.12
Molecular Formula: C10H16O
Retention Index : 0
Company ID : NIST 1992
Melting Point :
Boiling Point :
Misc Information :
QI=58 Steroids; Alkaloids; Misc. Natural products;

Figure I. 8. GC/MS spectrum of 2,4-decadienal.



2-Decenal, (Z)-

Match Quality	:	90
Entry Number	:	11008
CAS Number	:	002497-25-8
Molecular Weight	:	154.14
Molecular Formula	:	C10H18O
Retention Index	:	0
Company ID	:	NIST 1992
Melting Point	:	
Boiling Point	:	
Misc Information	:	

QI=68 Steroids;

Figure I. 9. GC/MS spectrum of (Z)-2-decenal.

Appendix J

Monoglyceride and Diglyceride Data and Representative Chromatograms.

Table J. 1. Monoglyceride and diglyceride mean averages after 3000 minutes of heating at 180°C: % weight per 85.0 mg of sample.

Blend	%Mono and Diglyceride
1	0
2	0.13
3	0.28
4	0.13
5	0.31
6	0.30
7	0.41

* Based on the average of 4 trials per blend.

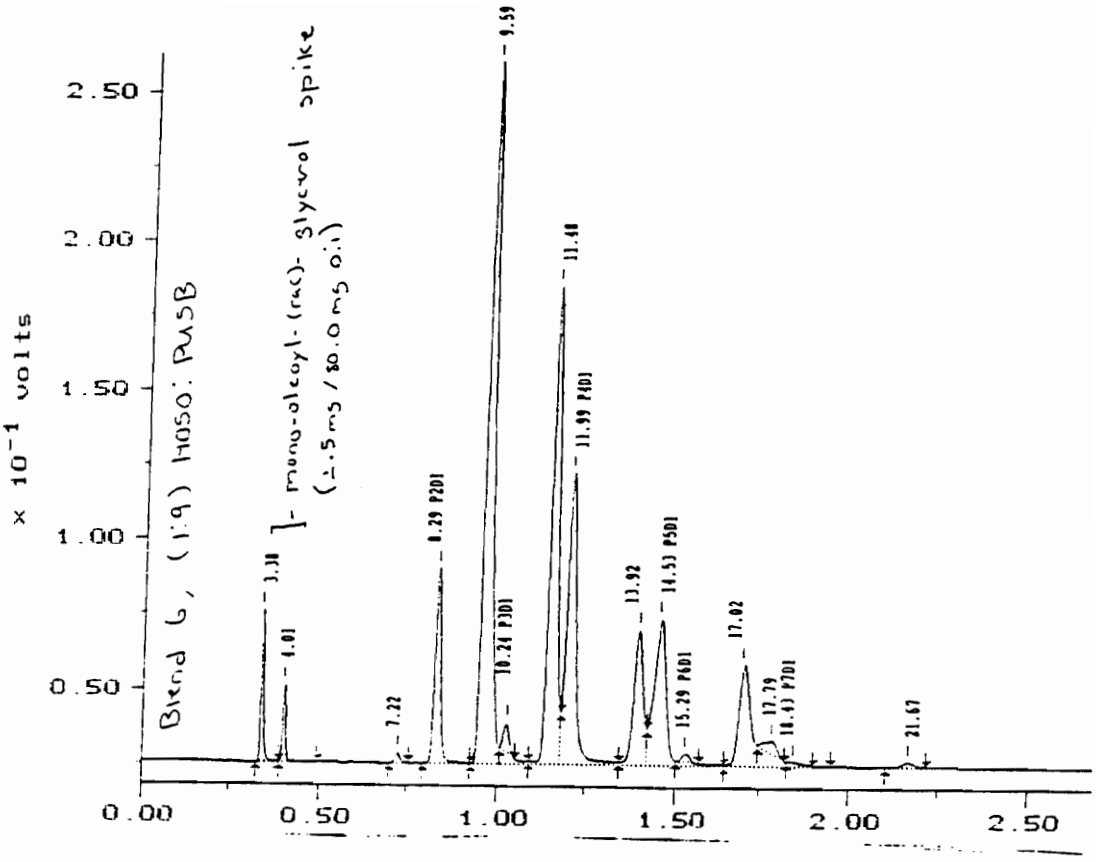


Figure J. 1. HPLC/Triglyceride chromatogram with rac-mono-oleolyglycerol spike: 2.5 mg/80.0 mg vegetable oil.

Appendix K

Peroxidation Reactions of Oleic, Linoleic, and Linolenic Acid.

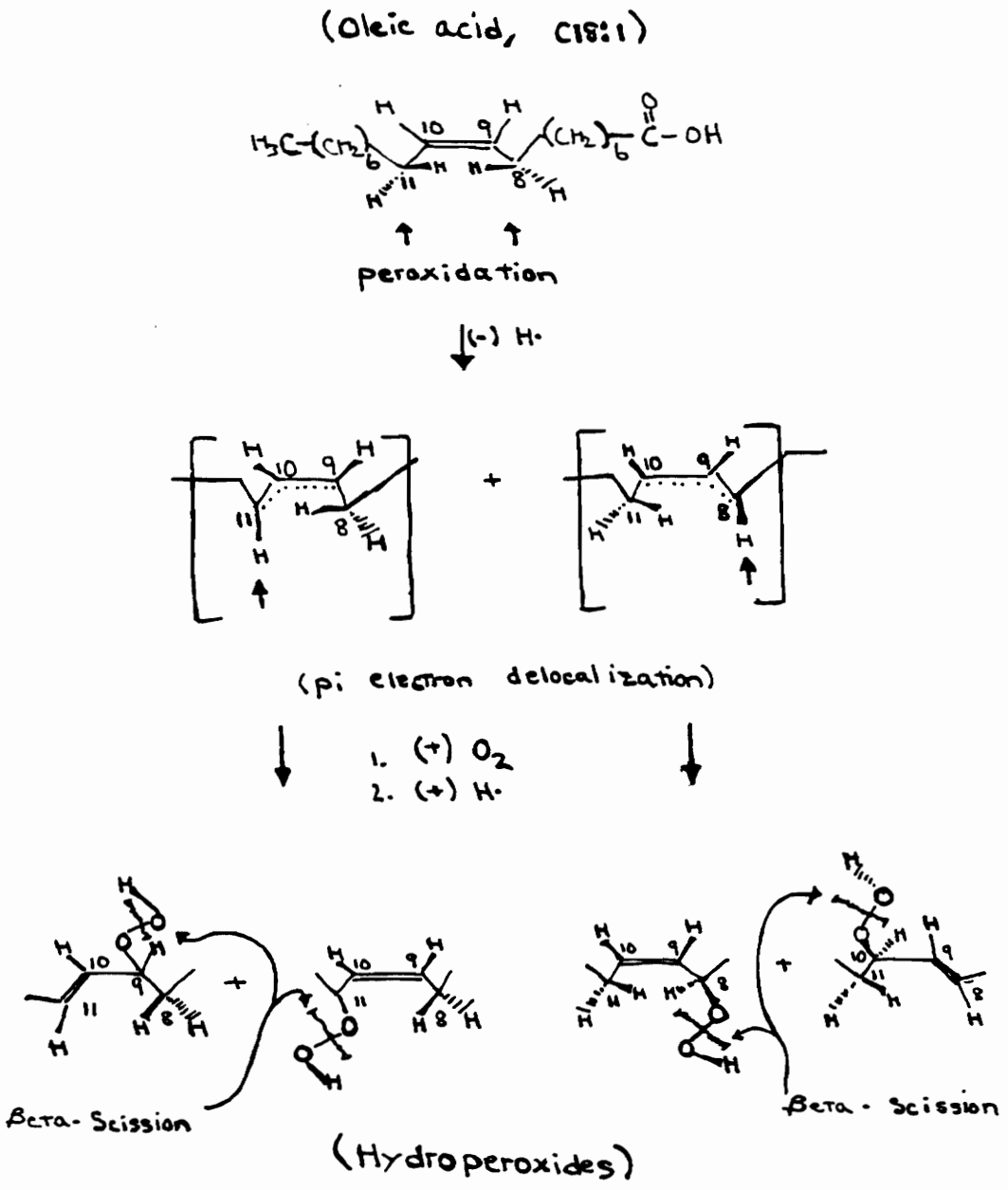


Figure K. 1. Peroxidation of oleic acid at the eighth and tenth methylene carbon, with stereochemistry shown.

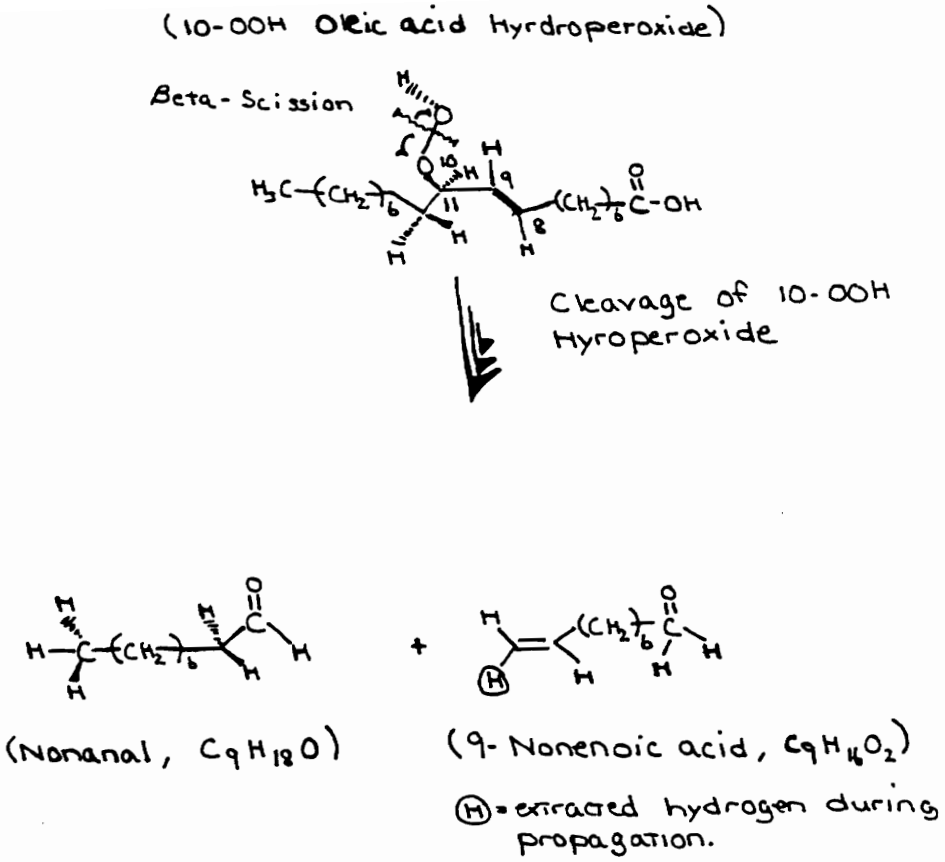


Figure K. 2. Nonanal production from oleic acid peroxidation at carbon number eight, with subsequent delocalization to carbon ten: Formation of a 10-OOH hydroperoxide intermediate (Chan and Coxon, 1987).

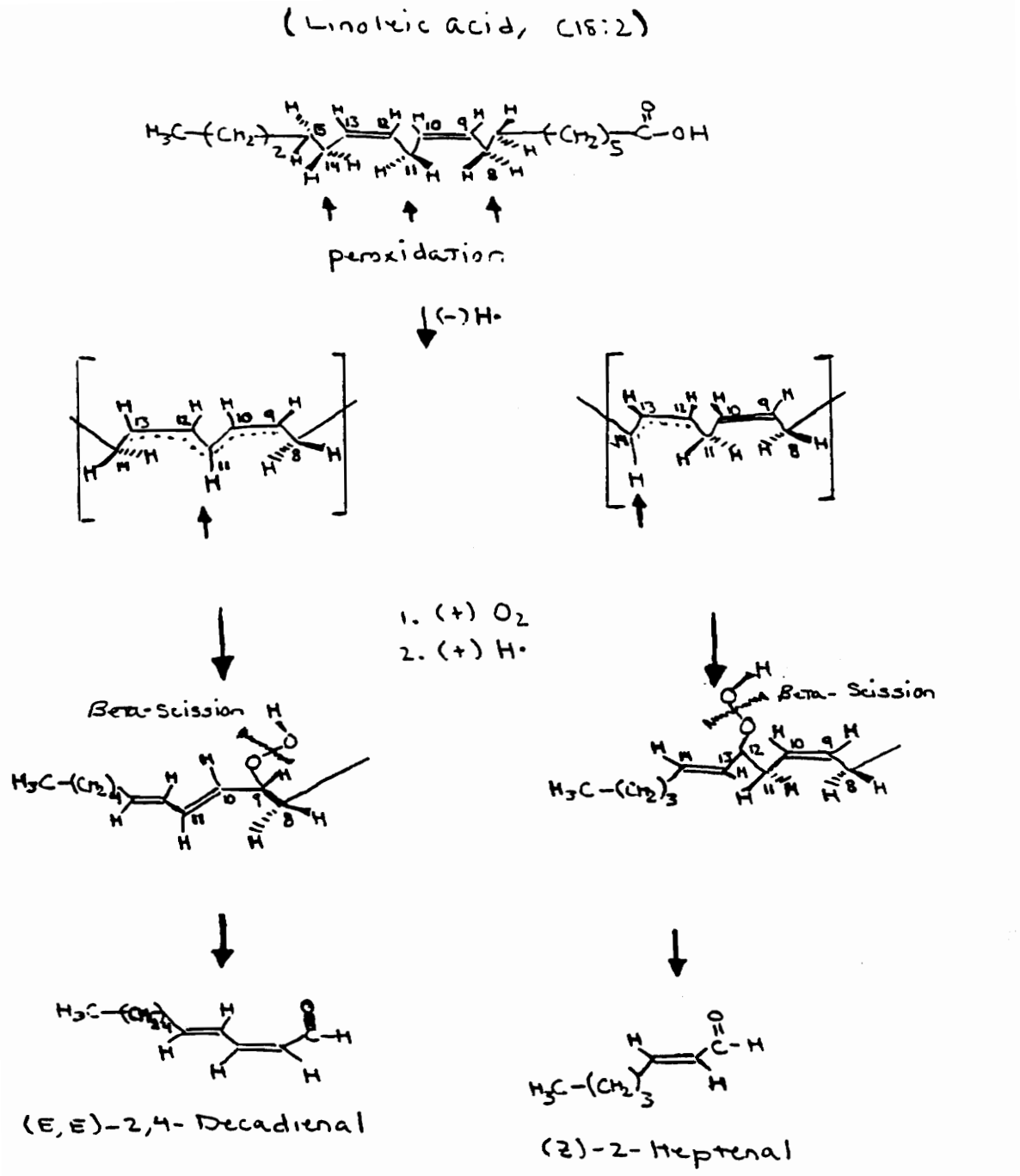


Figure K. 3. (E,E)-2,4-Decadienal and (Z)-2-heptenal production from the peroxidation of linoleic acid (Chan and Coxon, 1987).

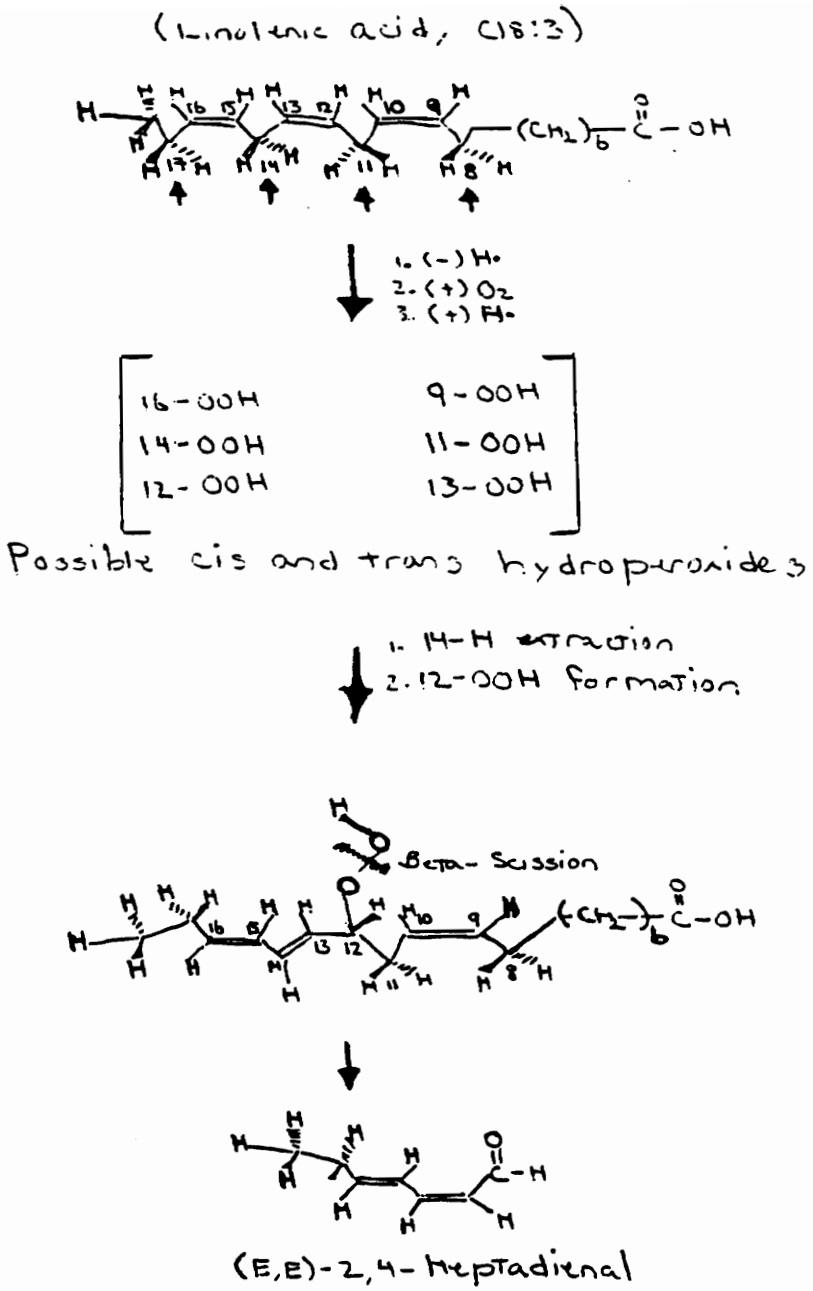


Figure K. 4. (E,E)-2,4-Heptadienal production from the peroxidation of linolenic acid (Chan and Coxon, 1987).

Appendix L

Comparative Methods for Volatile Extraction from Heated Vegetable Oils.

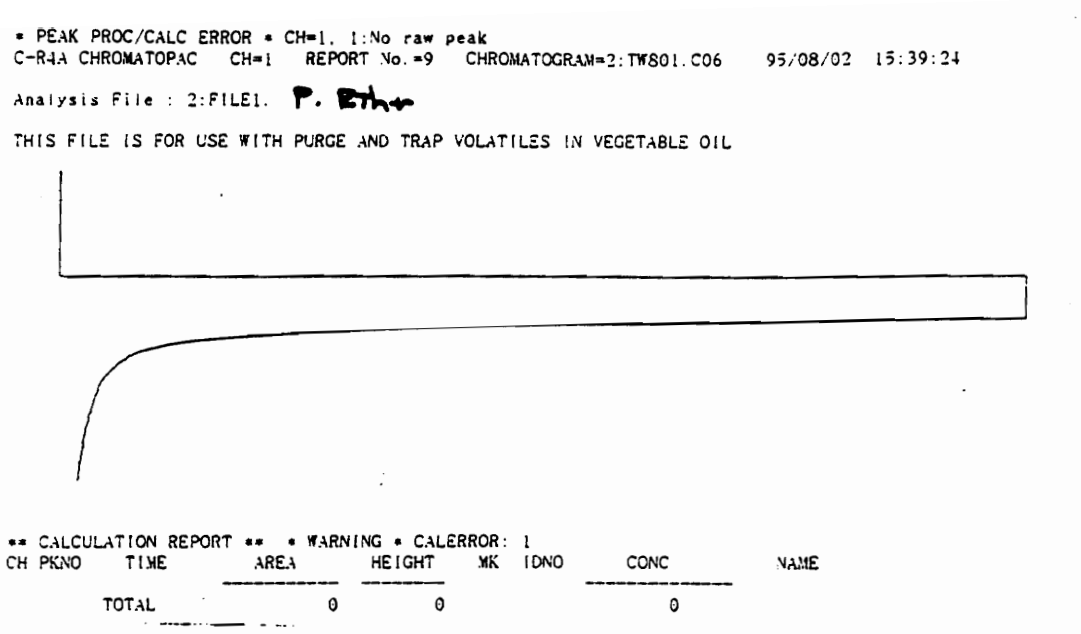


Figure L. 1. Petroleum ether solvent peak for GC/Volatile analysis.

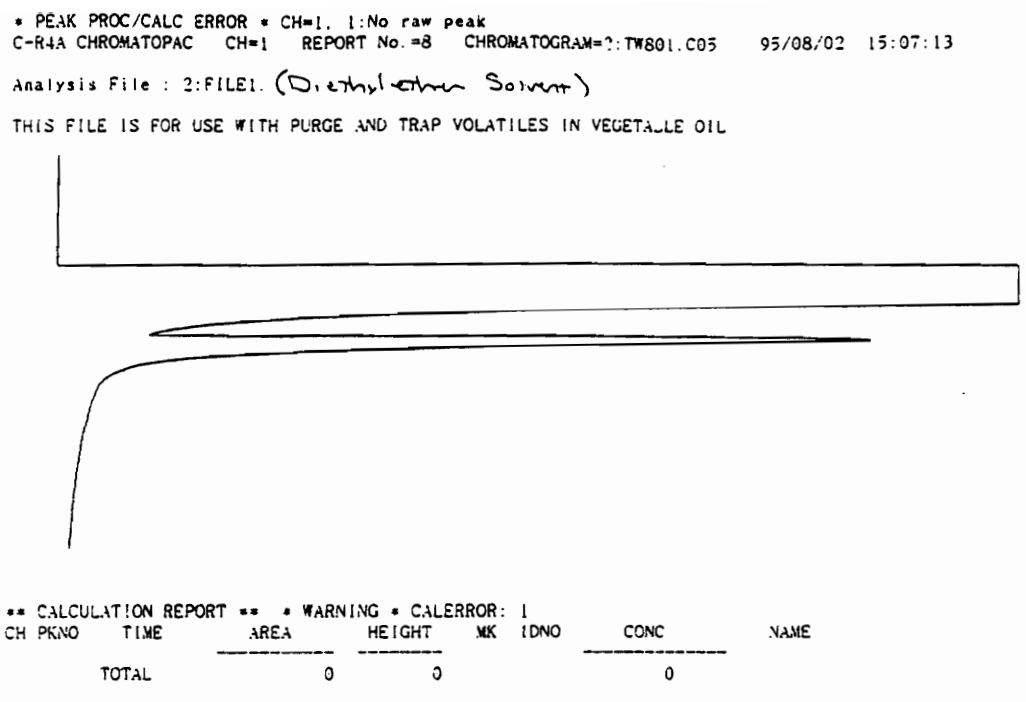


Figure L. 2. Ethyl ether solvent peak for GC/Volatile analysis.

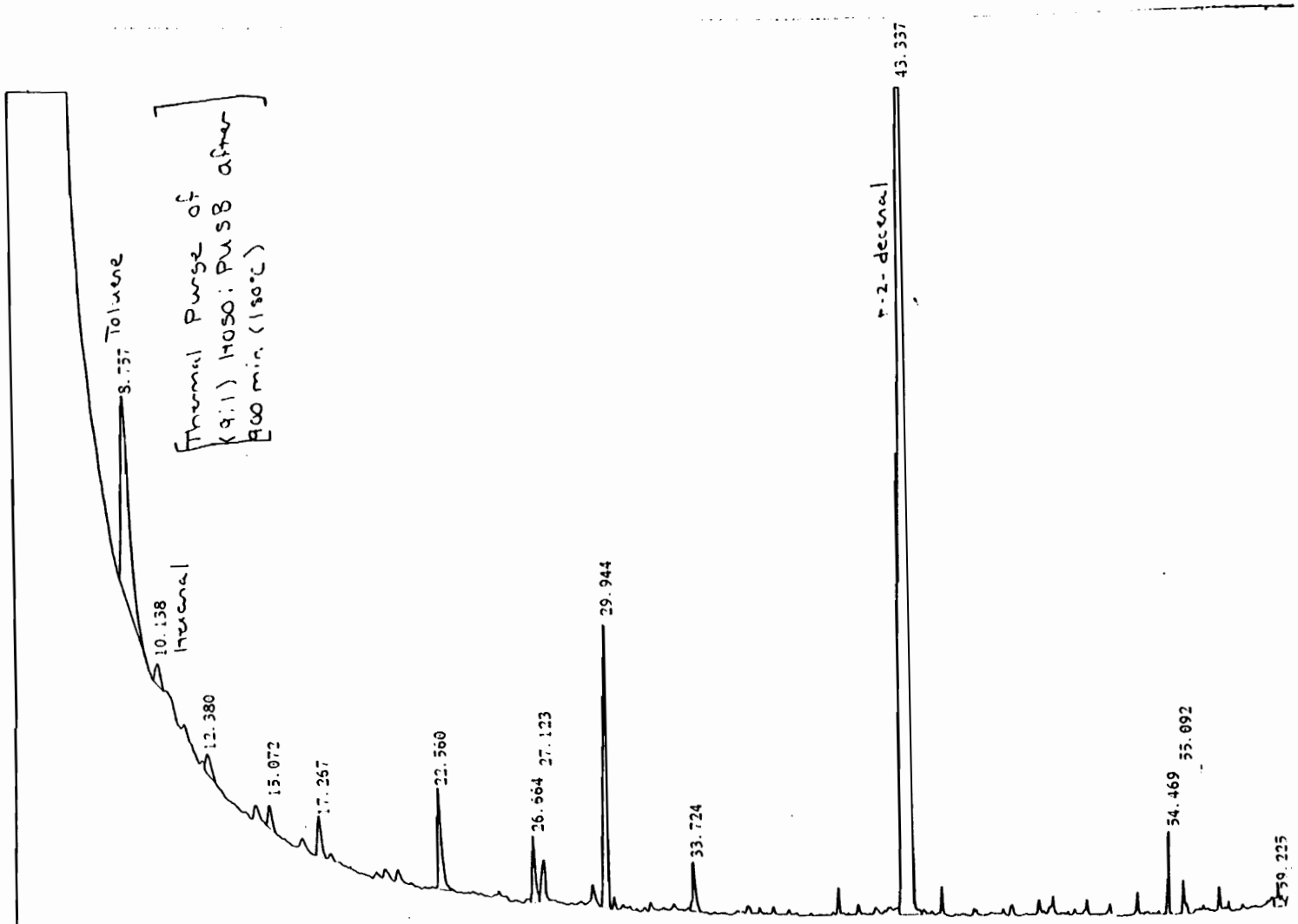


Figure L. 3. GC/Volatile analysis of volatiles thermally purged from 500 ml of (9:1) high oleic acid sunflower to soybean oil after heating at 180°C for 900 minutes.

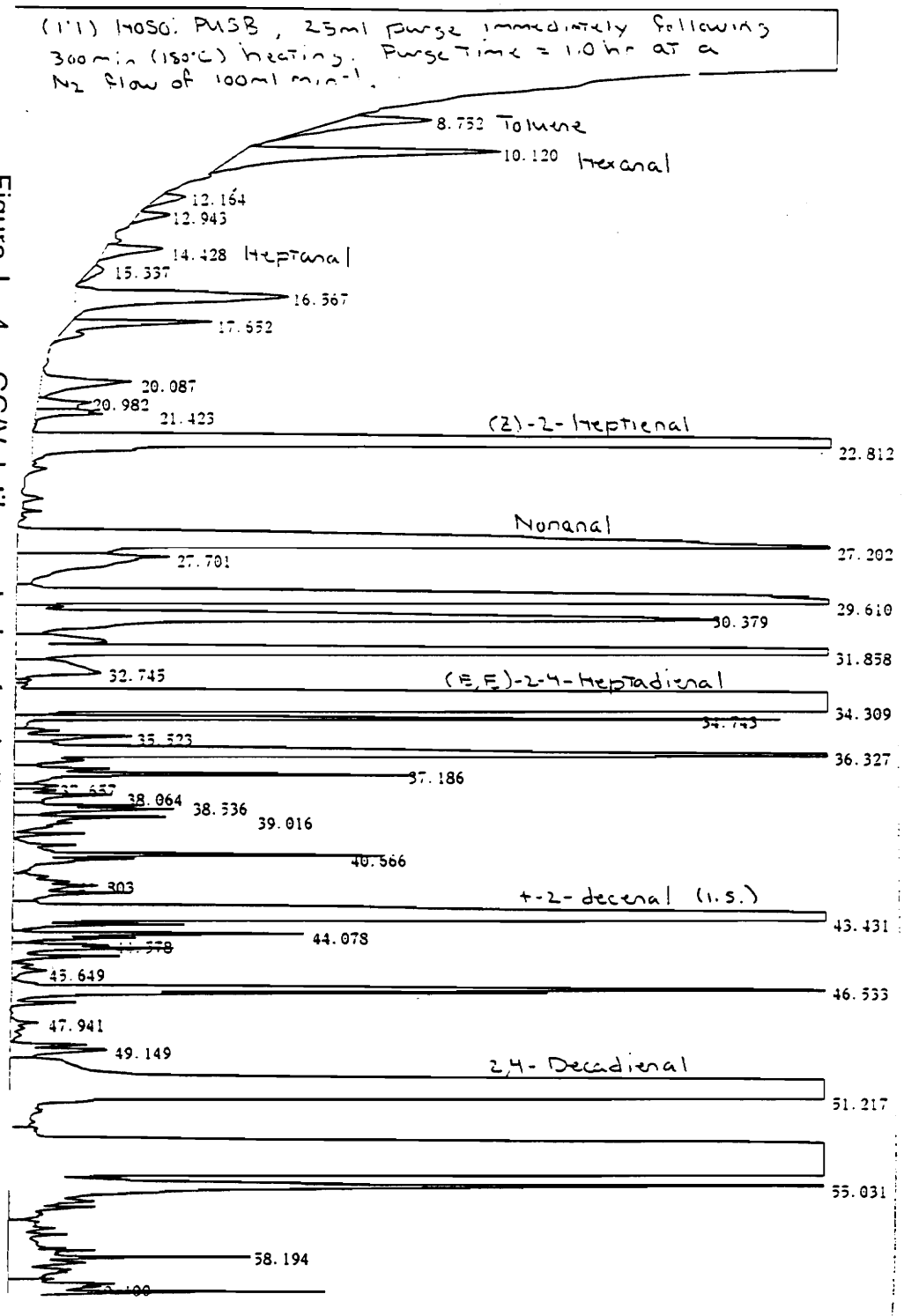
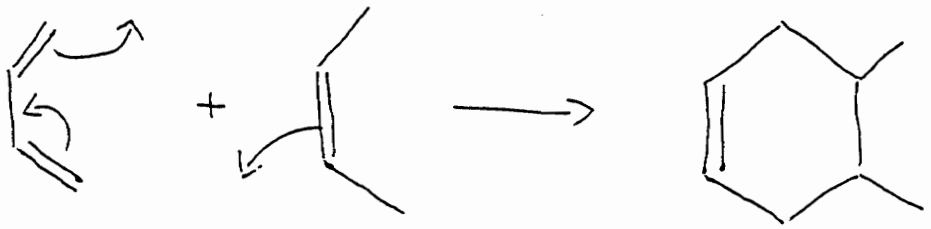


Figure L. 4. GC/Volatile analysis of volatile purged for 1 hour at a nitrogen flow rate of 100 ml/minute from 25 ml of (1:1) high oleic acid sunflower to soybean oil, immediately after heating for 300 minutes at 180°C.

Appendix M

Chemistry, Reactions, and Structures for Vegetable Oils and Related Compounds.

Diels Alder Reaction

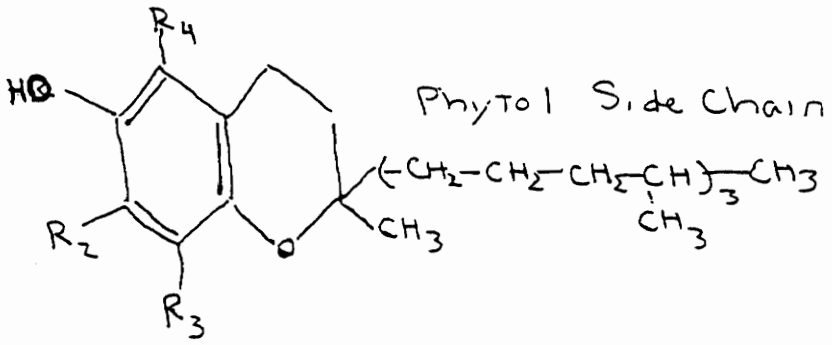


Conjugated Diene

Monoene

Cyclic Dimer

Core Structure of Tocopherol Isomers (Vitamin E)



Hydroxylated dihydrochroman ring

Appendix N

Fatty Acid Nomenclature of Saturated and Unsaturated Fatty Acids.

Table N. 1. Fatty acid nomenclature for saturated fatty acids.

Chemical name	Formula	Abbr.	Trivial name
Methanoic	$CHOOH$	1:0	Formic
Ethanoic	$CH_3—COOH$	2:0	Acetic
Propanoic	$CH_3(CH_2)—COOH$	3:0	Propionic
Butanoic	$CH_3(CH_2)_2—COOH$	4:0	Butyric
Pentanoic	$CH_3(CH_2)_3—COOH$	5:0	Valeric
Hexanoic	$CH_3(CH_2)_4—COOH$	6:0	Caproic
Heptanoic	$CH_3(CH_2)_5—COOH$	7:0	Enanthic
Octanoic	$CH_3(CH_2)_6—COOH$	8:0	Caprylic
Nonanoic	$CH_3(CH_2)_7—COOH$	9:0	Pelargonic
Decanoic	$CH_3(CH_2)_8—COOH$	10:0	Capric
Undecanoic	$CH_3(CH_2)_9—COOH$	11:0	
Dodecanoic	$CH_3(CH_2)_{10}—COOH$	12:0	Lauric
Tridecanoic	$CH_3(CH_2)_{11}—COOH$	13:0	
Tetradecanoic	$CH_3(CH_2)_{12}—COOH$	14:0	Myristic
Pentadecanoic	$CH_3(CH_2)_{13}—COOH$	15:0	
Hexadecanoic	$CH_3(CH_2)_{14}—COOH$	16:0	Palmitic
Heptadecanoic	$CH_3(CH_2)_{15}—COOH$	17:0	Margaric
Octadecanoic	$CH_3(CH_2)_{16}—COOH$	18:0	Stearic
Nonadecanoic	$CH_3(CH_2)_{17}—COOH$	19:0	
Eicosanoic	$CH_3(CH_2)_{18}—COOH$	20:0	Arachidic
Docosanoic	$CH_3(CH_2)_{20}—COOH$	22:0	Behenic
Tetracosanoic	$CH_3(CH_2)_{22}—COOH$	24:0	Linoceric
Hexacosanoic	$CH_3(CH_2)_{24}—COOH$	26:0	Cerotic
Octacosanoic	$CH_3(CH_2)_{26}—COOH$	28:0	Montanic
Triacontanoic	$CH_3(CH_2)_{28}—COOH$	30:0	Melissic
Dotriacontanoic	$CH_3(CH_2)_{30}—COOH$	32:0	Lacceroic

Table N. 2. Fatty acid nomenclature for monoenic fatty acids.

Systematic name	Abbr.	Trivial name
9-Decenoic	10:1Δ9	Caproleic
cis-4-Decenoic	10:1Δ4c	Obtusilic
cis-4-Dodecenoic	12:1Δ4c	Linderic
cis-4-Tetradecenoic	14:1Δ4c	Tsuzuic
cis-5-Tetradecenoic	14:1Δ5c	Physeteric
cis-9-Tetradecenoic	14:1Δ9c	Myristoleic
cis-9-Hexadecenoic	16:1Δ9c	Palmitoleic
cis-6-Octadecenoic	18:1Δ6c	Petroselinic
cis-9-Octadecenoic	18:1Δ9c	Oleic
cis-11-Octadecenoic	18:1Δ11c	Asclepic or *cis*-vaccenic
trans-11-Octadecenoic	18:1Δ11t	Vaccenic
cis-9-Eicosenoic	20:1Δ9c	Gadoleic
cis-11-Docosenoic	22:1Δ11c	Cetoleic
cis-13-Docosenoic	22:1Δ13c	Erucic
cis-15-Tetracosenoic	24:1Δ15c	Selacholeic or nervonic
cis-17-Hexacosenoic	26:1Δ17c	Ximenic
cis-19-Nonacosenoic	26:1Δ19c	Lumequic

Table N. 3. Fatty acid nomenclature for methylene interupted polyenic fatty acids.

Family	Systematic name	Abbr.	Trivial name
n-3	9,12,15-Octadecatrienoic	18:3(n-3)	α-Linolenic
	6,9,12,15-Octadecatetraenoic	18:4(n-3)	Stearidonic
	5,8,11,14,17-Eicosapentaenoic	20:5(n-3)	
	4,7,10,13,16,19-Docosahexaenoic	22:6(n-3)	
n-6	9,12-Octadecadienoic	18:2(n-6)	Linoleic
	6,9,12-Octadecatroenoic	18:3(n-6)	γ-Linolenic
	5,8,11,14-Eicosatetraenoic	20:4(n-6)	Arachidonic
	7,10,13,16-Docosatetraenoic	22:4(n-6)	Adrenic
n-7	9-Hexadecenoic	16:1(n-7)	Palmitoleic
	11-Octadecenoic	18:1(n-7)	Vaccenic
n-9	9-Octadecenoic	18:1(n-9)	Oleic
	15-Tetracosenoic	24:1(n-9)	Nervonic

Table N. 4. Fatty acid nomenclature for nonmethylene interupted polyenic fatty acids.

Systematic name	Abbr.
5,9-Octadecadienoic	18:2Δ5,9
5,11-Octadecadienoic	18:2Δ5,11
2t,9,12-Octadecatrienoic	18:3Δ2t,9,12
3t,9,12-Octadecatrienoic	18:3Δ3t,9,12
5t,9,12-Octadecatrienoic	18:3Δ5t,9,12
5,9,12-Octadecatrienoic	18:3Δ5,9,12
5,11,14-Octadecatrienoic	18:3Δ5,11,14
3t,9,12,15-Octadecatetraenoic	18:3Δ3t,9,12,15
5,9,12,15-Octadecatetraenoic	18:3Δ5,9,12,15
5,11-Eicosadienoic	20:2Δ5,11
5,13-Eicosadienoic	20:2Δ5,13
7,11-Eicosadienoic	20:2Δ7,11
7,13-Eicosadienoic	20:2Δ7,13
5,11,14-Eicosatrienoic	20:3Δ5,11,14
7,11,14-Eicosatrienoic	20:3Δ7,11,14
5,11,14,17-Eicosatetraenoic	20:4Δ5,11,14,17
5,11-Docosadienoic	22:2Δ5,11
5,13-Docosadienoic	22:2Δ5,13
7,13-Docosadienoic	22:2Δ7,13
7,15-Docosadienoic	22:2Δ7,15
7,17-Docosadienoic	22:2Δ7,17
9,13-Docosadienoic	22:2Δ9,13
9,15-Docosadienoic	22:2Δ9,15

VITAE

Troy A. Wilkerson is the son of James G. Wilkerson and Carolyn H. Wilkerson, with one brother Bryant W. Wilkerson. He is a native of Richmond, Virginia and grew up in Stafford County, Virginia. He served four years in the United States Navy in a tactical electronic warfare squadron (VAQ-137) reaching the rank of E-5. While in the Navy he visited ports around the world, including Italy, Spain, France, Yugoslavia, Singapore, Greece, Egypt, The Bahamas, Las Vegas, Florida, California, Washington State, and Canada. Also while in the Navy he became fully licensed in Scuba diving and placed third in the Navy's West coast physical fitness testing.

After returning from the service he began his studies at Northern Virginia Community College and Mary Washington College of Fredericksburg, VA, completing an associates of science and a BS in Biology. While attending both colleges he worked as a fitness instructor for youths and adults, Nautilus instructor, and a dietary analyst, where he was featured on video and on local television. Upon completing his undergraduate studies he pursued studies in food science and analysis and nutrition at Virginia Polytechnic Institute and State University. His awareness of health and fitness began at the age of twelve, when his grandmother Catherine Harding was diagnosed with bone marrow cancer.

Today he has a growing concern for the increasing levels of

artificial food products and altered food products that are appearing on the supermarket shelves and their implication on health and longevity. His belief is that you must understand the chemistry and composition of a food product in order to assess how it will react once consumed, especially now more than ever. The Science of Food Program in the Human Nutrition and Foods Department gave him the opportunity to begin exploring and experiencing the developments in food science and chemistry while completing a Masters of Science degree in December of 1996.