FRYING PERFORMANCE AND STORAGE STABILITY OF POTATO CHIPS FRIED IN GENETICALLY MODIFIED CANOLA OILS

BY

IANA PETUKHOV

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
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A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

A study was undertaken to compare the frying performance of low linolenic (LLCO) and high oleic (HOCO) canola oils with regular (RCO) and hydrogenated (HYCO) canola oils, and the storage stability of potato chips fried in these oils. Potato chips were fried over a five day period and oil samples were collected each day for analyses.

The results from chemical and instrumental analyses performed on the oils showed that there was no single oil that had consistently lower initial amounts or rates of accumulation of the degradation products. All oils demonstrated some degree of deterioration during the 5 day frying period. The frying stability of HOCO and LLCO showed a slight improvement over RCO based on the levels of free fatty acids, conjugated dienes and polars that formed in the oil. There are several explanations as to why the differences between the genetically modified oils and RCO were not as pronounced as expected. The initial quality of HOCO was impaired by the use of a high deodorization temperature during processing; high initial levels of tocopherols were found in RCO compared to HOCO and LLCO and, therefore, may have delayed the degradation of the RCO; the practice of replenishing the used oil with fresh oil each morning prior to frying may have slowed down the decomposition particularly for the RCO by introducing the additional levels of tocopherol.

The storage stability of potato chips fried in each oil from frying day 1 and 5 was determined by storing the chips over 16 days at 60°C. The results of sensory and chemical/instrumental analyses revealed that all chips had higher sensory scores for oxidation odours and accumulated

primary and secondary oxidation products as storage increased. RCO potato chips had higher rates of accumulation of peroxides, free fatty acids, conjugated dienes and polars, and higher amounts of total volatiles compared to HYCO, LLCO and HOCO chips, indicating lower storage stability of RCO chips. HYCO potato chips had lower rates of accumulation of peroxides and conjugated dienes than LLCO and HOCO chips, and lower rates of free fatty acids accumulation than LLCO chips, demonstrating better stability. The changes in sensory quality appear to be supported by the chemical and instrumental results.

Potato chips from frying day 5 usually had a slower accumulation of degradation products during storage compared to chips from frying day 1. Thus, the prooxidative effect of frying oil degradation products on the storage stability of potato chips was not demonstrated in this study in contrast to results from other researchers. Only HYCO chips showed an increased rate of stale/musty odour accumulation for frying day 5 compared to frying day 1.

Overall, genetically modified canola oils showed slight improvements over RCO during prolonged frying of potato chips. Potato chips fried in genetically modified canola oils showed greater storage stability than chips fried in RCO.

Recommendations for future research include the use of oils that have all undergone industrial processing; improved training of the panelists to enable the use of common attributes and references thereby allowing the sensory results to be compared directly; and further investigation of the effect of oil degradation products on fried food storage stability.

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This thesis is dedicated to my father, my teacher and my friend, who always believed in me and whom I will miss forever.

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CHAPTER 1

INTRODUCTION

Canola oil is a major oilseed crop in Canada and has gained popularity in the United States (Dotson, 1991). The production of canola oil in Canada has increased from 1980 to 1994 from 346 thousand tonnes to 605 thousand tonnes, with canola oil's market share increasing from approximately 47 to 73.5% (More, 1993; Anon, 1995). In the United States the market share for canola oil for the year 2000 has been projected to reach 25% in fast foods, 27% in snack foods, and 35% in salad and cooking oils (Dotson, 1991).

The fatty acid composition of canola oil has a favourable nutritional profile (Table 1.1). It has less than 6% saturated fatty acids, high levels of monounsaturated fatty acids, and increased levels of linoleic and linolenic fatty acids both of which are considered to be essential and are present at optimum ratio of 2:1 (Ackman, 1990).

The high levels of polyunsaturated fatty acids are viewed as the reason for canola oil's low oxidative and thermal stability. Changes that occur in the oil during heating/frying can have adverse nutritional effects. These changes can also impair the sensory properties of the oil and the fried food, and can affect the behaviour of the oil as a heat transfer medium. Therefore, improvements in canola oil stability are important for both the consumer and the manufacturer.

Table 1.1. Fatty Acid Composition of Some Edible Comercial Oils (w/w% fatty acids) *.

Fatty acids	Canola var.Westar	Soybean	Corn	Sunflower	Peanut	Olive
16:0	3.9	10.8	11.4	6.2	10.0	11.0
18:0	1.6	4.0	1.9	4.7	2.3	2.2
18:1	59.1	23.8	25.3	20.4	47.1	75.8
20:1	1.4	0.2	-	-	1.4	0.3
22:1	0.5	-	-	-	-	-
18:2 n-6	18.8	53.3	60.7	68.8	33.6	8.3
18:3 n-3	8.8	7.1	0.7	-	-	0.6

* adapted from R.G.Ackman (1990).

The stability of highly unsaturated vegetable oils can be improved by changing their fatty acid composition. The most widely used method to reduce the unsaturation of the oil is hydrogenation. Studies have shown improved frying performance and storage stability of the foods fried in hydrogenated oils (Stenvenson, 1984; Mounts *et al.*, 1988; Hawrysh, 1992; Melton, 1993). However, studies have reported that *trans* fatty acids, the by-products of hydrogenation, have the same adverse effect on blood cholesterol levels as saturated fatty acids (Anon, 1990).

An alternative to hydrogenation is the reduction of linolenic acid in the oil by genetic modification. A limited number of studies have been undertaken to examine the frying performance and storage stability of genetically modified oils. Studies which evaluated the sensory attributes of canola frying oils found that the heated room odour of low linolenic canola oil had a lower intensity than regular canola oil (Prevôt *et al.*, 1990; Eskin et al., 1989; Warner and Mounts, 1993). Other studies have evaluated the levels of oil degradation and the quality of the food (Warner *et al.*, 1994; Warner and Mounts, 1993; Mounts *et al.*, 1994a and 1994b; Liu and White, 1992; Marsic, 1993). These studies suggest that there is an improvement in the frying stability of genetically modified canola oils, but the results are not consistent. Studies done to examine the storage stability of foods fried in genetically modified canola oils suggest that these oils

produced foods with better stability than regular oil (Hawrysh, 1993; Warner *et al.*, 1994).

The presence of frying oil degradation products in fried foods has been demonstrated by some workers to have a prooxidative effect on the storage stability of snack foods (Asap and Augustin, 1986; Yoon *et al.*, 1988). However, there are no reports on the effect of degradation products on storage stability of foods fried in genetically modified oils.

Therefore, there is a need for further investigation of the frying performance of genetically modified canola oils, the storage stability of foods fried in these oils, and the effects of oil degradation products on storage stability of fried foods. Thus the overall objective of this study was to evaluate the frying performance of two genetically modified canola oils, and to determine the storage stability of potato chips fried in these oils. In order to achieve this overall objective, two experiments were performed. The first experiment investigated the effect of fatty acid composition on frying stability. The specific objectives of this experiment were:

1. To determine how the genetically modified canola oils differ from hydrogenated and regular canola oils in terms of their fatty acid composition.
2. To investigate whether the differences in fatty acid composition improved the frying performance of the

genetically modified canola oils compared to regular and hydrogenated canola oils.

The second experiment examined the effect of fatty acid composition and the degree of oil degradation during frying on the storage stability of potato chips. The specific objectives of this experiment were:

1. To compare the storage stability of potato chips fried in genetically modified canola oils with chips fried in regular and hydrogenated canola oils.
2. To determine if the level of oil degradation products caused by prolonged frying influenced the storage stability of potato chips and to investigate whether this differed among the four oils.

CHAPTER 2

REVIEW OF LITERATURE

2.1 FRYING STABILITY OF VEGETABLE OILS

2.1.1 Chemical Changes in the Oil During Frying

Frying is the process of cooking food in hot oil. The temperature of the oil is usually in the range of 150-190°C. During frying a number of physical and chemical changes occur from heating, presence of oxygen in the air, release of moisture from the product due to the processes of thermal degradation, oxidation and hydrolysis (Fig 2.1). These changes can affect the performance of the oil as a heat transfer medium, as well as its nutritional value after it is absorbed by the fried food. It could also impair the shelf life of the fried product. The rate of decomposition depends on a number of factors including the composition of the oil, temperature and length of frying, continuous or intermittent frying, type of food fried, and whether or not the fresh oil is added (Melton, 1994; Boskou, 1988; Fedeli, 1988; Thompson and Aust, 1983; Blumenthal, 1991, Peers and Swoboda, 1982).

2.1.1.1 Thermal Degradation

Thermal polymerization is a term widely used in the literature to refer to the process that occurs in the oil at high temperatures in the absence of oxygen. However, polymers are not the only products that are formed. The formation of other products depends on whether saturated or unsaturated

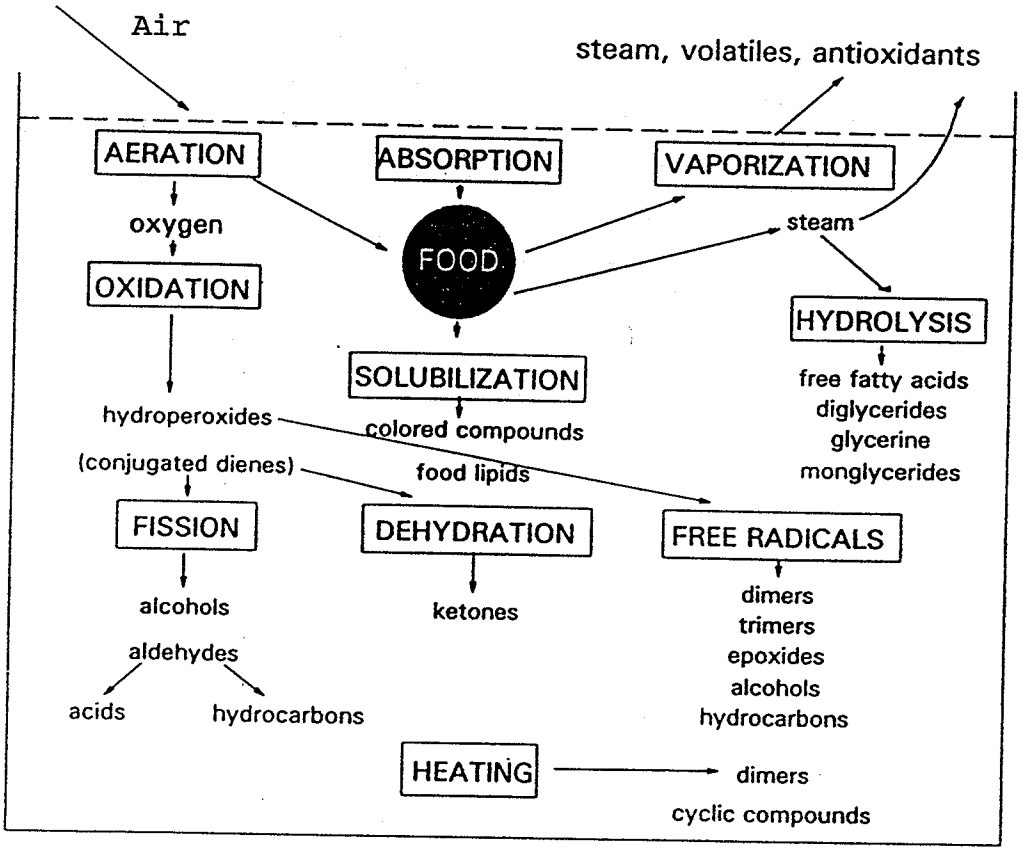


Figure 2.1. Changes Occuring During Deep Fat Frying (adapted from Fritsch, 1981).

fats are involved (Nawar, 1985a). Saturated fats produce hydrocarbons, fatty acids, ketones, acrolein, carbon monoxide, carbon dioxide and other components when heated to very high temperatures 200-700°C. However, only minute amounts of these compounds are formed at the frying temperatures.

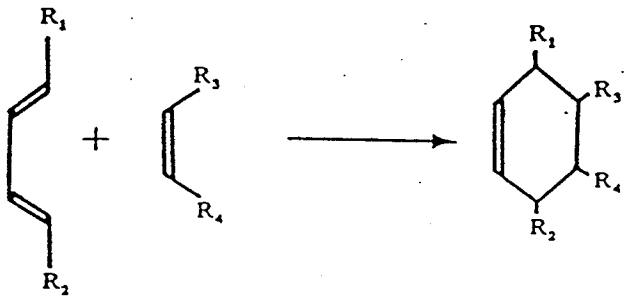
Unsaturated fats yield dimeric rather than higher polymeric compounds at temperatures below 250°C (Artman, 1969). Specifically, methyl oleate when heated produced hydrocarbons, fatty acid esters and straight chain dimers. Polyunsaturated methyl esters produced more complex components (Nawar, 1985b).

The formation of dimers in unsaturated systems occurs by a free radical mechanism. Free radicals come from the cleavage of C-C bond on either side of the double bond (Nawar, 1985b; Figge, 1971). Free radicals derived from hydroperoxides can also catalyze the formation of thermal dimers in the absence of oxygen (Nawar, 1985b). Free radicals can attack fatty acyl in the neighbouring triglyceride forming dimeric triacylglycerol, intermolecular polymer with increased molecular weight. When a free radical attacks a fatty acyl in the same triglyceride molecule an intramolecular dimer is formed (Artman, 1969). The polarity of thermal dimers is lower than that of oxidative dimers (Evans *et al.*,1965; Christopoulou and Perkins, 1989). This has implications in the measurement of oil degradation products.

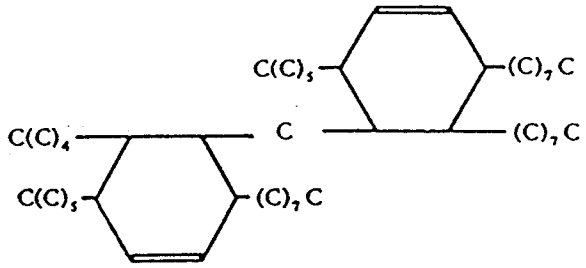
The polymers or dimers with cyclic structures could also be formed in unsaturated fats by the Diels-Alder reaction which involves a conjugated diene and a double bond (Figure 2.2). And again, the polymers formed could be inter- or intramolecular (Nawar, 1985b). Cyclic polymers have only been observed in oils that contain fatty acids with at least two double bonds (Chang *et al.*,1978).

The unsaturated fatty acids with two or more double bonds, linoleic and linolenic, can also produce cyclic fatty acid monomers as a result of a thermal degradation process (Sebedio and Grandgirard, 1989). These cyclic compounds are known to be well absorbed by the body if compared to other oxidized or polymerized compounds (Sebedio and Grandgirard, 1989; Clark and Serbia, 1991). The toxic effect of frying or heated oils has been attributed to the presence of cyclic fatty acid monomers, which has raised a lot of concern about the safety of frying fats (Clark and Serbia, 1991). However, some researchers think that the toxicity of frying fats has been greatly exaggerated because most of the clinical studies were done using heavily abused fats that were heated at temperatures much higher than those normally used for frying (Clark and Serbia, 1991).

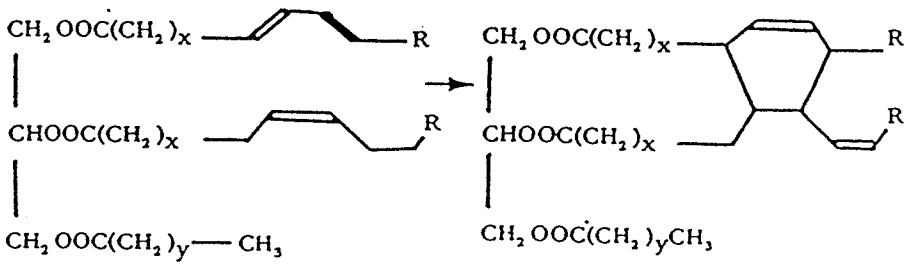
The formation of geometrical isomers of linolenic fatty acid is also attributed to the heating of the oil (Grandgirard *et al.*, 1984). The amount of these isomers that is formed are dependent on the temperature and time of heating. However, it



Diels - Alder Reaction



Intermolecular Polymer (Between Triglycerides)



Intramolecular Dimer

Figure 2.2. Cyclic Dimers and Polymers Formation (adapted from Nawar, 1985a; Figge, 1971).

has been observed that if heating is continued, the accumulation of geometric isomers of linolenic fatty acid decreased, suggesting that isomerization is followed by formation of cyclic fatty acid monomers or polymers (Grandgirard *et al.*, 1984).

2.1.1.2 Oxidation

It is well documented that initial stages of oxidation at room temperatures as well as at high temperatures follow the classical free radical mechanism of hydroperoxides formation (Nawar,1985a; Paquette *et al.*, 1985; Frankel, 1985; Porter, 1986) (Figure 2.3).

Unsaturated fatty acids are more prone to oxidation than saturated fatty acids (Frankel, 1985). In the C18 family of fatty acids, they rank as follows from the most susceptible to the least susceptible to oxidation: linolenic --→ linoleic --→ oleic --→ stearic (Nawar, 1985; Labuza, 1971; Fedeli, 1988; Miller, 1993).

The primary oxidation products are hydroperoxides and their structure and amounts are dependent on the unsaturated fatty acids present. Thus, oleic acid yields *trans* and *cis* isomers of four hydroperoxides: 9-OOH, 10-OOH, 8-OOH and 11-OOH; linoleic - a mixture of *cis,trans* and *trans, trans* isomers of 9- and 13-hydroperoxides; linolenic - a mixture of *cis, trans* and *trans, trans* isomers of 9-, 12-, 13- and 16-hydroperoxides (Frankel, 1982; Przybylski and Eskin, 1995).

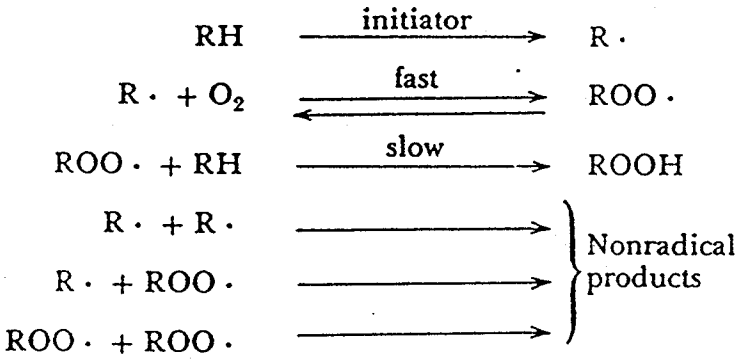


Figure 2.3. Free Radical Mechanism of Oxidation (adapted from Frankel, 1985).

The accumulation of hydroperoxides is slow initially and its rate increases as the oxidation progresses (Hahm and Min, 1995). After the initial accumulation of hydroperoxides the decomposition of hydroperoxides occurs.

At frying temperatures the rates of reactions are much higher than at room temperatures, thus the hydroperoxides are not accumulated but readily decompose to give rise to the secondary products of oxidation. At frying temperatures after the first 5-10 min of increased accumulation of peroxides, there is a fast decrease in their level within the first hour. The peroxide value (PV) decreases to zero after 30 min of heating at temperature of 250°C (Nawar, 1985b).

The hydroperoxide formation from polyunsaturated fatty acids usually results in shifting the double bonds and conjugated diene or triene groups are formed. The conjugated double bonds are more prone to further oxidation than non-conjugated (Nawar, 1985a).

The decomposition of hydroperoxides yields both volatile and nonvolatile degradation products (Chang *et al.*, 1978; White, 1991; Fritsch, 1981). The resulting low molecular weight volatiles are considered to be responsible for the off-flavours associated with oxidized lipid-containing products (Hahm and Min, 1995; Frankel, 1982; Figure 2.4). The volatile compounds derived from 18:3 are known to have lower threshold values than those derived from 18:2, and, therefore, less oxidation is needed to impair the flavour of food products

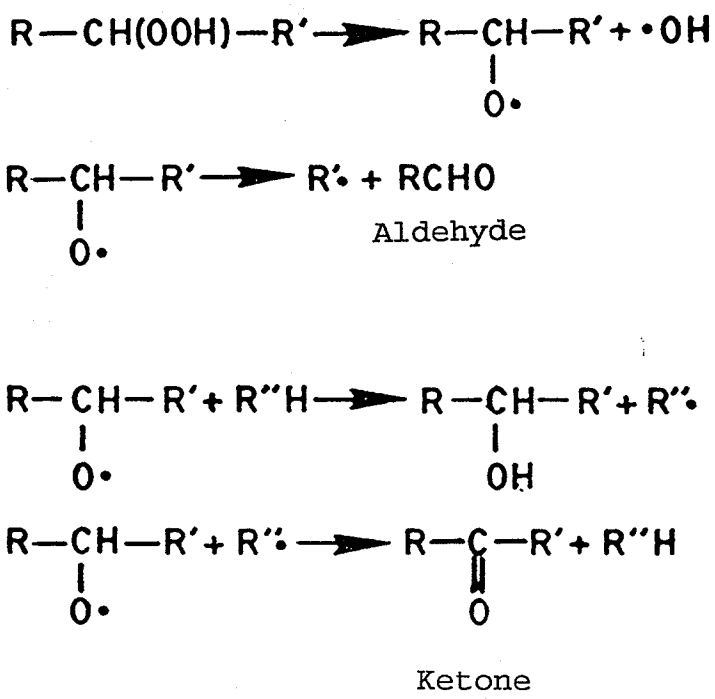


Figure 2.4. General Decomposition of Hydroperoxides into Aldehydes and Ketones (Paquette et al., 1985).

that contain considerable amounts of linolenic acid (Frankel, 1982). The volatile compounds can undergo further decomposition to yield new volatile and nonvolatile products. Due to the complex nature of the oxidation of unsaturated lipids, it is not always possible to predict, measure or describe the formed volatile products. The pathways of forming volatiles during frying and autoxidation are similar (Nawar, 1985b; Artman, 1969). However, it is also difficult to determine all of the volatile components that are formed at elevated temperatures since they immediately undergo further decomposition or interact with each other (Nawar, 1985b; Selke *et al.*, 1975, 1977, 1980, 1983).

Compounds with higher molecular weight are also formed by the free radical mechanism. Polymers that result from oxidation have more polarity than those formed in the absence of oxygen (Figge, 1971). Dimers and trimers formed in the presence of oxygen have the ether or peroxide bridges (C-O-C or C-O-O-C) that connect monomers into dimers, trimers or higher polymers (Nawar, 1985a; Figure 2.5). Oxidation polymers also can have oxygen containing side groups in the constituting monomers (hydroxyl, carbonyl and others) (Nawar, 1985b; Paquette, 1985).

Ether Bridge

Peroxide Bridge

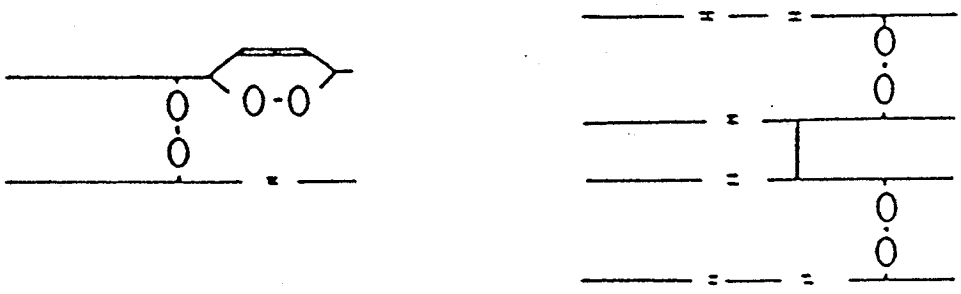


Figure 2.5. Oxydimers and Polymers (adapted from Nawar, 1985a; Figge, 1971).

2.1.1.3 Hydrolysis

Hydrolysis becomes an important chemical process of oil degradation only when the food is fried because of the moisture released from the food. The major products of hydrolysis are: free fatty acids, mono- and diglycerides, and glycerine (Fritsch, 1981).

Free fatty acids are oxidized faster than their glycerides (Nawar, 1985a; Gonzalez-Quijano and Dobarganes, 1988). There has also been speculation that free fatty acids can catalyze further oxidation of the oil. However, experiments which have been undertaken with various fatty acids added to frying oils at levels of 1% and 5% do not support this theory (Handel and Guerreri, 1990).

2.1.2 Methods to Measure Oil Deterioration

As chemical changes from thermal degradation, oxidation and hydrolysis take place, two large groups of decomposition products are formed: volatile and nonvolatile (Figure 2.6). The majority of the volatile products are continuously removed from the oil making the task of their collection and analysis very laborious and difficult (White, 1991). Nonvolatile decomposition products remain in the oil or are partially picked up by the fried food, making them more practical to study (Stevenson *et al.*, 1984).

Most of the analyses performed on frying fats are in fact based on nonvolatile products determination (White, 1991).

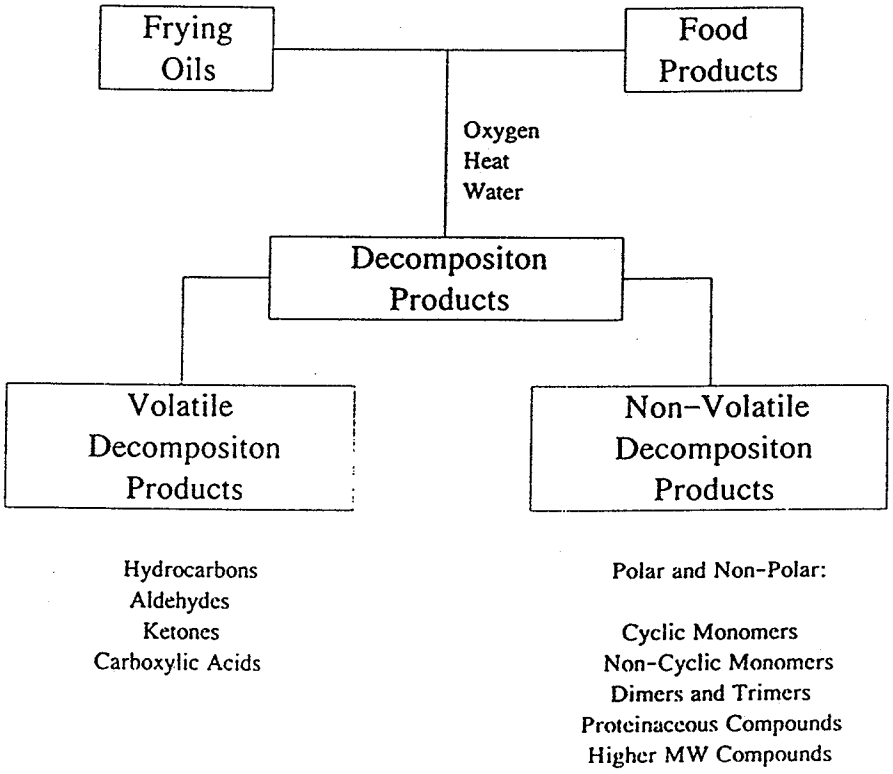


Figure 2.6. Decomposition Products Formed During Deep Fat Frying (Brooks, 1991).

One group of methods, measure the physical and specific chemical changes that have taken place in the oil, an indication that certain groups of components were formed and accumulated. These methods are widely used because they are easy, quick, inexpensive and in most cases give reproducible results. The second group of methods quantitatively evaluate the newly formed compounds (Gonzalez-Quijano and Dobarganes, 1988). These tests measure either the specific components formed in the oil (eg. polymers), overall change in the oil (eg. polars) or the overall alteration by employing complex analytical procedures (eg. combined separation techniques) (Gonzalez-Quijano and Dobarganes, 1988).

2.1.2.1 Sensory Evaluation

Sensory evaluation of the frying oil is not usually done for the same reason as volatile analysis: most odour components evaporated from the oil during frying. However, the evaluation of the heated room odour during frying has been found by some researchers to be a good measure of polyunsaturated oil deterioration (Prevôt *et al.*, 1990; Prevôt *et al.*, 1988; Durance, 1986; Mounts *et al.*, 1994; Frankel *et al.*, 1985; Warner and Mounts, 1993). Lack of consistency in the methods used to rate room odour makes it difficult to compare the results from one study to another.

2.1.2.2 Measurement of Physical Characteristics

Oil foaming during frying is attributed to the accumulation of polymers in the oil. However, it has been reported that in some cases foaming occurred after the oil was already unacceptable for use, therefore misleading the operator (Chang *et al.*,1978). Addition of antifoaming agents further complicates the use of foam height as an indicator of oil deterioration (Stevenson *et al.*,1984b). Nevertheless, some researchers have found high correlations between foam height and oil deterioration when only citric acid was added to the fresh oil (Warner and Mounts, 1993).

Viscosity measurements, which indirectly determine the accumulation of polymers, are widely used to monitor oil degradation (White,1991). Some authors point out, however, that changes in physical indices might not only be due to the presence of the altered oil components, but could be caused by some other effects. For example, an increase in viscosity might be influenced by the presence of gelatinized starch from a batter, and the instrument would not be able to distinguish between the two different sources. Dielectric constant determination which indirectly measures polar compounds, can be affected by food residues and/or electrolytes (salt, citric acid), which also increase the readings on the instrument (Blumenthal, 1993).

The colour of the oil becomes darker with continued use and can serve as an indirect measure of polymers accumulation.

At the same time, it may not be a good indicator of oil degradation since oil can change colour due to the migration of the browning compounds from food (Blumenthal,1993).

Smoke point of the oil decreases during frying due to the accumulation of free fatty acids and nontriglyceride components. The method is widely used in Europe as a general measure of oil degradation with the lower limit set at 170-180°C (Wan, 1995; Firestone *et al.*,1991). But it has been found that these measurements lack reproducibility (Wu and Nawar, 1986), and are dependent on the room temperature (Stevenson *et al.*,1984b).

Thus, evaluation of oil degradation using physical measurements only is not reliable and should be supported by chemical analyses as well.

2.1.2.3 Peroxide Value

PV is still commonly used in Scandinavian countries as a measure of frying fat deterioration (Firestone *et al.*, 1991). However, as discussed previously, peroxides do not accumulate in the heated oil since they readily undergo decomposition. Therefore, measurement of PV does not provide a meaningful indication of oil degradation (Fritsch, 1981).

2.1.2.4 Conjugated Dienoic Acids

This method is widely used in the evaluation of frying oils (White, 1991). Conjugation is the shifting of double

bonds that occurs in polyunsaturated fatty acids when they are oxidized. Conjugated dienes are primary products of oxidation, but unlike peroxides they do not decompose at frying temperatures (Frankel, 1993; White, 1995). During the early stages of oxidation, the amount of conjugated dienes increase along with increased oxygen uptake, until their amount reaches a plateau just before the end of the induction period (Kirk and Sawyer, 1991; White, 1995).

The shifting of the double bonds can also take place during deodorization if the temperature used during these procedures is above 245°C, or during hydrogenation (White, 1995; Smouse, 1995).

Determination of conjugated dienoic acids (CDA) is based on their property to absorb light in the UV range at 233 nm wavelength. The method is simple, fast and uses small amounts of the sample (100 mg) (White, 1995). Some researchers stress that CDA measurements are only useful for determining relative changes (Gonzalez-Quijano and Dobarganes, 1988; Kirk and Sawyer, 1991; White, 1995).

2.1.2.5 Free Fatty Acids

The determination of free fatty acids (FFA) is regulated or recommended for the evaluation of the frying fats quality in many European countries. The upper limit that is accepted is 2-2.5% depending on the country (Firestone, 1991).

Measurement of FFA is an easy procedure, based on titration of all free acids with an alkaline solution. The final result does not depend on the origin of the FFA (hydrolysis or oxidation) (Fritsch, 1981; Peers and Swoboda, 1982).

The controversy exists on whether FFA measurements are a good indicator of oil deterioration. Some researchers report that this value depends on the length of frying and type of food being fried (Jackobson, 1991; Stevenson et al, 1984a). Jackobson (1991) indicated that at 1.5% level of FFA the sensory quality of the oil (flavour) was rated as having very low acceptability, and other changes (polymerization) occurred more rapidly. The levels of FFA have also been found to have high correlations with the measurements of polar materials in hydrogenated solid and liquid canola and soybean fats (Stevenson *et al.*, 1984a). In contrast, Handel and Guerreri (1990) consider the FFA measurements to be unreliable. They found that when high levels (5%) of different fatty acids were added to the oils of varying unsaturation levels, the majority of FFA evaporated resulting in a decrease in the amount of FFA with increasing frying time. However, when fatty acids were added at low levels (1%), the trend was reversed with higher levels of FFA accumulating with increasing frying time (Handel and Guerreri, 1990).

Stevenson *et al.* (1984b) strongly recommended that FFA measurement alone should not be relied on as to when to

discard the oil since fats with the same amount of free fatty acids can have different degrees of deterioration (Gonzalez-Quijano and Dobarganes,1988).

2.1.2.6 Total Polar Components

Fresh oil is a nonpolar medium, and more than 96% of it is constituted by triglycerides (Table 2.1). As degradation takes place during frying the new products formed are more polar than non-altered triglycerides. This difference in polarity permits the separation of the used oil into nonpolar and polar fractions by employing solvents of different polarity. Thus, it is possible to determine the extent of oil deterioration by measuring the amount of polar fraction.

The classical method for determination of polar compounds involves the elution of nonpolar fraction from a silica column using a combination of solvents (usually petroleum ether:diethyl ether - 87:13 ratio) (Sebedio *et al.*, 1986). This fraction contains unaltered triglycerides (Melton, 1994). However, this fraction will also contain nonpolar thermal polymers (Gonzalez-Quijano and Dobarganes, 1988a; Marquez-Ruiz *et al.*, 1995). The polar fraction that is trapped on the column is usually eluted with polar solvent, methanol or diethyl ether or calculated by the difference (Sebedio, 1986; Gonzalez-Quijano and Dobarganes, 1988a; White, 1991). This fraction is comprised of triglyceride dimers and polymers,

Table 2.1. Oil Degradation During Frying *.

Oil quality	Triglycerides (%)	Polar degradation products	Polymer (%)	Free fatty acids (%)	Oxidized fatty acids (%)
New oil	>96	< 4	0.5	0.02	0.01
"Break-in"	90	10	2.0	0.50	0.08
Fresh	85	15	5.0	1.00	0.20
Optimum	80	20	12.0	3.00	0.70
Degrading	75	25	17.0	5.00	1.00
"Runaway"	65	35	25.0	8.00	2.00

* adapted from Brooks (1991).

oxidized triglycerides,diglycerides and free fatty acids (Sanchez-Muniz *et al.*,1993; Arroyo *et al.*, 1992; Marquez-Ruiz *et al.*,1995). The classical procedure can take up to 3-4 hours, therefore a less time consuming procedure has been introduced that employs the same principle but uses silica gel Sep-Pak cartridges (Sebedio *et al.*, 1986). The use of silica gel Sep-Pak cartridges permits the separation of small oil samples in 30-40 min. Good correlations have been found between the classical method and the Sep-Pak separation of polar components (Sebedio *et al.*, 1986).

The determination of the amount of total polar components is widely accepted in most European countries. The upper limits permitted vary slightly among the countries, but generally fall in the range of 25-27% (Firestone *et al.*, 1991).

There is a concern by some researchers that the amount of polar components may not be related to the level of frying oil degradation since unsaturated oils tend to yield more polymers than polar materials (Lumley, 1988). Nevertheless, most of the reports on the performance of frying fats find high correlations between total amount of polar materials and flavour quality of fried food, length of frying, and amounts of free fatty acids (Melton, 1994; Stevenson *et al.*, 1984a).

2.1.2.7 Fatty Acid Analysis by Gas Chromatography

The change in fatty acids composition is thought to be a good indication of general degradation of oil during frying (Dobarganes and Perez-Camino, 1988b; White, 1991). When the method with internal standard is used to determine the fatty acid profiles of the oils, it is possible to obtain the information about change in the amounts of individual fatty acids and the quantities of non-altered triglycerides (White, 1991; Dobarganes and Perez-Camino, 1988b). The latter determination is possible because methyl esters of polymers and oxidized materials have high molecular weights and are therefore not separated by means of gas chromatography (GC) (Waltkins *et al.*, 1975).

It has been shown that during frying, the amounts of unsaturated fatty acids decreased, and the saturated fatty acid content remain unchanged (Dobarganes and Perez-Camino, 1988b; Thompson and Aust, 1983).

2.1.2.8 Polymers

It is thought that the amount of nonpolar polymers is a direct measure of thermal alteration of fat (Dobarganes and Perez-Camino, 1988a; Marquez-Ruiz *et al.*, 1995). Although it is possible to separate polar (oxidized) and nonpolar (thermal) polymers from each other by means of gas-liquid chromatography (GLC) (Evans *et al.*, 1965), usually the total amount of polymers is determined (White, 1991). Direct

measurement of total amount of polymers has been carried out by fractionation of non-urea-adduct-forming (NUAF) esters by liquid chromatography (LC) (Chang *et al.*, 1978). Alternative to the above methods is the measurement the amounts of different classes of polymeric components are determined by separation according to their molecular weight using high-performance size-exclusion chromatography of the fatty acid methyl esters (Marquez-Ruiz *et al.*, 1995; Cuesta *et al.*, 1993; White and Wang, 1986; Arroyo *et al.*, 1992). Both methods were found to be highly correlated (Waltking *et al.*, 1975; Thompson and Aust, 1983).

In summary, no single method has been proven to be absolutely reliable for measuring frying oil deterioration, partly because there is not a full understanding of all the processes that take place during frying nor of the products that are formed. Therefore, it is necessary to use several tests which compliment one another in order to get a complete picture of oil degradation (Frankel, 1993; Wu and Nawar, 1986; Dobarganez and Perez-Camino, 1988b).

2.1.3 Effect of Altered Fatty Acid Composition on Frying Stability of Vegetable Oils

It is well established that the poor frying stability of canola oil is due to its high levels of polyunsaturated fatty acids, especially linolenic acid which is highly susceptible

to oxidation at elevated temperatures. The attempts were made to eliminate the heated odour by adding antioxidants to canola oil, but they were not successful (Vaisey-Genser and Ylimaki, 1985; Hawrysh *et al.*, 1990). Therefore, reduction in 18:3 content is needed to improve the frying stability of canola oil. The most widely used method is hydrogenation. New approaches to lower the 18:3 content include blending with a more stable oil and genetic manipulation of the fatty acid composition.

2.1.3.1 Hydrogenation

Hydrogenation is the process of transforming a liquid vegetable oil into a fat of different hardness with increased oxidative stability and improved functional properties (Haumann, 1994). The resulting fat is used in the formulations of margarines, baking shortenings, and solid and semi-solid cooking fats. Hydrogenation involves the addition of hydrogen (from hydrogen gas) to the double bonds of unsaturated fatty acids in the presence of a catalyst. The temperature used in this procedure is between 150-225°C, and this promotes the formation of *trans* fatty acids (Haumann, 1994).

Stevenson *et al.*(1984a) studied the frying performance of hydrogenated and partially hydrogenated oils during 37.5 and 75 hrs of french fries frying, respectively. Both canola and soybean oils were used. Solid fats contained less than 0.2% 18:3, whereas liquid fats contained 18:3 at levels of 1.2% -

canola and 2.8% - soybean. Frying performance was evaluated by a number of indices among which the levels of FFA and PC(polar compounds) showed the most distinct increase in solid fats during 37.5 hrs of frying. The authors mentioned that liquid fats showed FFA and PC values similar to solid fats, which was indicative of similar stability. PC values stayed below 14% for solid fats after 37.5 hrs, and below 16% for liquid fats after 75 hrs. The correlation between these two indices was equally high for all oils and fats used in the study.

Melton *et al.*(1993) evaluated the quality of potato chips fried in partially hydrogenated canola oil, cottonseed oil, and their blends (85:15 and 70:30). All oils and blends had low levels of 18:3 fatty acid (0.2-0.4%). The FFA content did not differ among the oils used during heating/frying. No differences in potato chip flavour likability and overall likability rated on 8-point hedonic scales (1=dislike extremely, 8=like extremely) were found for the oils used. Therefore, the authors concluded that partially hydrogenated canola oil performed as well as cottonseed oil during frying of potato chips.

Hydrogenation was also reported to reduce the objectionable fishy odour in heated soybean oil (Mounts *et al.*, 1988).

However, the use of hydrogenation has recently come under question due to the development of *trans* fatty acids during the process of hydrogenation. It has been reported that *trans*

fatty acids have the same adverse effect on blood cholesterol levels as saturated fatty acids (Anon, 1990). Another disadvantage of hydrogenation is the development of objectionable "hydrogenated" odour and flavour in the fried foods, even if lightly hydrogenated oil is used (Frankel *et al.*,1985; Warner and Mounts, 1993). And lastly, hydrogenation is an expensive process, costing 3-5 cents/lb (6.6-11 cents/kg) for the commercial catalytic process (Mounts *et al.*, 1988).

2.1.3.2 Blending with More Stable Oils

Blending canola oil with a more stable oil is another approach that can be taken to increase the thermal stability of the less stable oil. Durance (1986) found marked improvement in room odour of blends of canola oil with either cottonseed or sunflower oils compared to 100% canola oil. The oils and blends were heated for 10 min at 185°C and the room odour intensity and overall acceptability of the odour was evaluated by a sensory panel. The intensity was evaluated on a 15-cm line scale (0=bland, 15=strong). Canola oil had the highest intensity of heated room odour (10.8), and the lowest acceptability (10%). Sunflower oil had an intensity of 8.2 and an acceptability of 45%, and cottonseed oil had an intensity of 5.3 and an acceptability of 65%. The best results in the reducing the intensity of room odour were achieved with blends of 25% canola and 75% sunflower oil (score of 8.2), and canola

and cottonseed oil in the same proportion (score of 7.3). The best acceptability scores were found with the same canola:cottonseed oil blend (35%), and with a 50:50 blend of canola:sunflower oils (35%). These results were found to be in agreement with the content of 18:3 in the oils (canola oil - 6.4%, sunflower - 0.4%, cottonseed - 1.9%).

Similar studies have been done with blends of sesame and soybean oils. Sesame oil is considered to be one of the most stable oils known (Yen,1991). When it was added to soybean oil (18:3=7.13%) at levels of 5, 10 and 20% there was a significant ($p<0.05$) improvement in oil stability after 48 hrs of heating at 180°C for the oil containing 20% sesame oil as measured by viscosity, absorbance at 232nm, refractive index and dielectric constant (Yen, 1991).

Although, the blending of more stable oils with less stable oils can improve the stability of a latter, it is dependent on the availability and the price of the more stable oil (Warner and Mounts, 1993).

2.1.3.3 Breeding

Crop breeding has been widely used for long time to improve yields of commodity crops, as well as their resistance to disease. Today, genetic breeding technology allows to improve the quality of the crop as well. The traditional breeding techniques combined with molecular genetic transformations allowed the development of low linolenic and

high oleic/low linolenic canola oils (Erickson and Frey, 1994). The biosynthesis of unsaturated fatty acids in canola seed involves a stepwise desaturation of stearic acid. By blocking the enzyme that catalyzes the addition of a third double bond, through mutation it is possible to achieve a canola low in linolenic acid. When desaturation is blocked earlier, between 18:1 and 18:2, there is an increase in oleic acid. By crossing the obtained mutants with canola cultivars adapted to specific growing conditions low linolenic and high oleic canola cultivars have been developed (R.Scarth, 1995). Genetic modification of the fatty acid composition of oil has reduced the amount of 18:3 in low linolenic canola oil from 10-12% to about 3% or even lower (Warner and Mounts, 1993; Eskin *et al.*, 1989; Carr, 1991). High oleic canola oil has the levels of 18:1 considerably increased from about 60% in regular canola oil to greater than 78% with a noticeable reduction in both 18:2 and 18:3 (Warner *et al.*, 1994, Miller, 1993). The fatty acid composition of other vegetable oils was also improved by genetic modification to broaden their use as frying oils, spray oils and for other applications (Erickson and Frey, 1994).

Studies on Canola Oil

Prevôt *et al.* (1990) evaluated the room odour of the low linolenic (18:3=3.1%) and regular (18:3=11.3%) rapeseed oils when they were used for frying of potatoes. Eight fryings were

done over a 2 day period. After first, fourth and eighth fryings the overall room odour intensity (10=unnoticeable frying odour, 2=very poor and repulsive odour) and the intensity of individual odours (0=none, 1=weak, 2=moderate, 3=strong) were evaluated. Low linolenic oil showed consistently higher scores for overall intensity and a better room odour profile than the regular. Low linolenic oil initially had a substantially higher intensity of fruity odour intensity than regular oil. In low linolenic oil the fruity odour had a tendency to increase throughout the whole frying period, whereas in regular oil this odour almost disappeared after eight fryings. The intensity of burnt-acrid-rancid and painty-plastic-fishy odours was dramatically reduced in low linolenic oil. The reduction in burnt-acrid-rancid odour intensity in low linolenic oil after eight fryings was about 2 times compared to regular oil, and the reduction in painty-plastic-fishy odour was approximately 4 times compared to regular. Authors attributed such a dramatic increase in the low linolenic rapeseed oil frying performance to reduction of 18:3 content.

Improved room odour and increased acceptability of heated low linolenic canola oil was reported by Eskin *et al.*(1989). The 18:3 content in low linolenic oil was 1.6% compared to 8.5 and 9.0% in two sources of regular canola oil. Room odour intensity, rated on a 15 cm line (0=bland), was significantly lower for low linolenic (7.2) compared to the two regular

(12.2 and 11.1) canola oils. The acceptability of low linolenic oil remained high - 44%, compared to regular oils - 19 and 0%. The maximum level of FFA reached in low linolenic oil was 0.15%, compared to levels of 2.16% in the regular oil. The level of carbonyls in low linolenic oil was the lowest, but was only significantly different ($p<0.05$) from that in one of the regular oils. The amount of dienals and TBA formed after 10 min of heating was also significantly lower ($p<0.05$) for the low linolenic oil. The authors concluded that the reduction in 18:3 did result in improved stability, but a further decrease in linolenic acid content would be needed to improve its heated odour.

A study by Warner and Mounts (1993) examined the frying performance of low linolenic varieties of both canola and soybean oils. Only the results for canola oil will be presented. Low linolenic canola oil was also hydrogenated (18:3=0.7%) and lightly hydrogenated (18:3=0.8%). All the oils were heated to 190°C for 5 days for a total of 45 hrs. Sixteen batches of potatoes were fried in each oil every day. Frying oil was replenished with fresh oil every day. A number of sensory and analytical methods were used to monitor the quality of the oils and french fries. The genetically modified canola oils had significantly lower values for FFA (except for low linolenic hydrogenated which had FFA the same as regular). The amount of polar compounds for all canola oils did not differ. Although, low linolenic oil (18:3=1.7%) had slightly

lower values, the limit of 25-27% set in Europe for polar compounds was reached after 25 hrs of frying. The final values for all oils were around 42%. Low linolenic canola oil had significantly lower foam heights than regular canola oil. The room odour intensity test performed using a 10-point category scale (0=none, 10=strong) showed that the low linolenic oils had a significantly lower value than regular (18:3=10.1%). The quality of french fries determined using a 10-point category scale (10=excellent, 1=bad) revealed that fries fried in the non-hydrogenated low linolenic oil were significantly better than those fried in the rest of the oils. All low linolenic oils produced fries without fishy odour/flavour which was noted in fries fried in regular canola oil. However, both hydrogenated oils had a distinct objectionable hydrogenated odour/flavour. The non-hydrogenated low linolenic oil was characterized as having a fried food, acrid, burnt and woody odour, but the french fries were characterized as only having a fried food flavour. The authors concluded that breeding improved the stability of the oil, but further reduction of the 18:3 by hydrogenation only decreased the quality of the oil and french fries due to the objectionable hydrogenated odour/flavour.

A more recent study by Warner *et al.* (1994) compared the frying performance of regular (18:3=7.7%), two low linolenic (18:3=2.8 and 2.9%), high oleic (18:3=4.2%), blend of low linolenic and high oleic (18:3=3.1%) and hydrogenated

(18:3=0.8%) canola oils. Potato chips were fried in each oil over two days nine hours a day for a total of 18 hrs. The fresh oil was added periodically to maintain the initial level of oil. Regular canola oil had the lowest level of FFA after 18 hrs of frying, whereas high oleic oil had the highest levels of FFA, these differences were significant ($p<0.05$) when compared to the rest of the oils. Low linolenic oils were all significantly different from each other. High oleic canola oil had the lowest amount of polar components that was not significantly different from one of low linolenic oils (18:3=2.9%) and the blended oil. Low linolenic oil with 2.8 % of 18:3 and hydrogenated canola oils had the highest amounts of polars. The levels of volatiles over the fryer showed that all the oils exhibited similar decreases during both frying days with an increase after overnight cooling. The measurement of volatiles in potato chips indicated that chips fried in regular canola oil showed the biggest fluctuations in the amount of volatiles with a significantly higher level ($p<0.05$) at the beginning of the second frying day. The potato chips fried in new oils had lower initial and final amounts of volatile components as compared to those fried in regular canola oil. The flavour quality scores (10=excellent, 1=bad) for potato chips fried in regular oil were the lowest at the end of day 1 frying and all day 2 than in any modified oil potato chips. Warner *et al.* (1994) determined the effect of 18:3 amounts on flavour quality and found that with an

increase in linolenic acid content the quality scores decreased. The fishy odour was rated on a 10-point individual flavour intensity scale (0=no flavour, 10=strong flavour) and it dramatically decreased in potato chips as the amount of linolenic acid decreased. The potato chip flavour was the lowest in potato chips fried in regular canola oil, intermediate in chips fried in high oleic and hydrogenated canola oils, and highest in chips fried in low linolenic canola oil. Authors also came to the conclusion that reduction in the 18:3 amounts did improve the frying stability of canola oils and potato chips. Although, high oleic did not have the best flavour scores, it could have been due to the elevated amounts of 18:1 which contributed to waxy flavour.

Studies on Other Vegetable Oils

Studies have also been undertaken to examine the stability of genetically modified soybean oils. Mounts *et al.* (1988) evaluated the heated room odour of soybean oils with modified fatty acid composition (18:3 content: regular=7.7%, hydrogenated=3.0%, low linolenic=3.3%, 4.2% and 4.8%). The tests used included the sensory evaluation of the overall odour intensity (0=none, 10=strong intensity) and the intensity of the individual odours (1=weak, 2=moderate, 3=strong). After 1 hr of heating at 190°C the hydrogenated oil had the highest room odour intensity score, and regular oil had next highest score. Two of low linolenic oils (18:3=3.3%

and 4.8%) had significantly lower ($p<0.05$) overall odour intensities compared to hydrogenated and regular. The third low linolenic oil (18:3=4.2%) was not significantly different from regular. After 5 hrs of heating, hydrogenated oil had the highest odour intensity, regular had the second highest intensity, two low linolenic oils (18:3=4.2% and 4.8%) were not significantly different from the regular, and only one modified oil (18:3=3.3%) had significantly lower ($p<0.05$) odour intensity than the regular. Therefore, only one low linolenic oil showed significantly lower overall odour intensity throughout the whole period of heating. An objectionable hydrogenated odour was only detected in hydrogenated oil. Unpleasant fishy odour was present only in regular and hydrogenated oils with the intensity decreasing after 5 hrs of heating. The low linolenic oil (18:3=4.2%) had the highest fried food odour after 1 and 5 hrs of heating. The authors concluded that reduction in the linolenic acid content significantly improved the quality of the frying oils.

Mounts *et al.* (1994b) obtained results similar to those of Mounts *et al.* (1988). In the 1994 study the modified soybean oils evaluated had much lower 18:3 content (1.7, 1.9 and 2.5%). The regular soybean oil had 6.5% linolenic acid. The oil was used for frying 5 hrs a day for 4 days (total 20 hrs), three batches of french fries were fried in the middle of each frying day. Fresh oil was added to the frying oil every day to replenish. Potatoes fried in the three modified

oils had significantly higher ($p<0.05$) flavour quality scores (scale: 10=excellent, 1=bad) than those fried in the regular oil. The range of scores for low linolenic oils was 6.6 to 7.4, compared to a score of 4.4 for regular oil. The analysis of individual odours indicated that potatoes fried in regular soybean oil had the highest intensity of fishy and stale odours during 15 hrs of using the oil. The modified oils had the highest intensities of fried food and potato odours. The analysis of free fatty acids, total volatiles and polar compounds in frying oils did not show significant differences among the oils during 20 hrs of frying.

Mounts *et al.* (1994a) also compared frying stability of regular (18:3=6.5%) and low linolenic (18:3=1.9 and 2.9%) soybean oils. The heating and frying continued for three days, 7 hrs a day (total about 20 hrs) with two batches of french fries fried in the middle of each frying day. Fresh oil was added every day as a makeup oil. The heated room odour intensity (0=none, 10=strong) of the modified oils was significantly lower ($p<0.05$) than that of regular soybean oil throughout the entire frying period. The flavour quality (10=excellent, 1=bad) of the french fries was significantly higher ($p<0.05$) for low linolenic than for regular after 10 hrs of oil use. After 20 hrs of use, the flavour quality scores were higher for french fries fried in the modified oils but only the oil with an 18:3 content of 1.9% was significantly different ($p<0.05$) from regular oil. The results

of polar components, total volatiles and FFA in oils did not show any significant differences during 20 hrs of heating and frying.

Liu and White (1992) compared the stability of the three low linolenic soybean oils (18:3=1.5, 1.8 and 1.9%) with two regular (18:3=5.9 and 6.8%) oils during 40 hrs of heating and frying of bread cubes. The amount of conjugated dienoic acids (CDA) in the oil and the flavour of the bread cubes was evaluated. The levels of CDA in both regular soybean oil were higher but were not significantly different ($p<0.05$) from two low linolenic oils (18:3=1.8 and 1.9%) throughout the entire frying period. Sensory evaluation performed on the quality (10=excellent, 1=extremely poor) and intensity of oxidized flavour (10=bland, 1=extremely intense) of bread cubes revealed that there was no significant difference ($p<0.05$) between the oils either at the beginning or at the end of frying. Using a similar frying protocol, Miller and White (1988) found that bread cubes fried in low linolenic (18:3=3.5%) soybean oil had significantly lower ($p<0.05$) amounts of CDA than those fried in regular oil (18:3=6.9 and 8.1) throughout the whole period of frying. The oxidized flavour intensity scores (10=bland, 1=extremely intense) were also significantly higher ($p<0.05$) in low linolenic bread cubes as compared to regular after 40 hrs of heating and frying. The improved stability of the new oil was attributed to the reduced amounts of 18:3.

There has also been studies done to evaluate the frying performance of modified high oleic oils. Marsic (1993) compared high oleic sunflower oil to hydrogenated soybean oil containing an antioxidant during thirteen days of frying (8 hours a day). A range of different foods were fried in the oils in rotation. The oils were filtered and replenished with fresh oil every day. FFA, dielectric constant, polar components were assessed on the used oil and overall likability of the fried foods (1=dislike extremely, 9=like extremely) was evaluated. No significant differences were found in FFA and dielectric constant between the oils, however, high oleic sunflower oil had significantly lower amount of polars than hydrogenated soybean oil, and the preference scores were consistently higher for chicken nuggets fried in high oleic oil.

Dobarganes *et al.* (1993) used conventional and three high oleic sunflower oils for frying of 15 batches of french fries over a 5 hour period. The oils were not replenished. The amounts of total polars and individual polar components were determined to monitor oil stability during frying. All three modified sunflower oils showed lower accumulation of total polars at the end of frying than the conventional oil, in particular, the greatest changes were in the amounts of triglyceride dimers and oxidized triglycerides. The amounts of free fatty acids and diglycerides were similar for all oils.

The authors concluded that the high oleic oils showed better frying stability than conventional sunflower oil.

In summary, most of the studies show that genetically modified oils have better frying stability than their regular counterpart and hydrogenated oils, as reflected in improved odour/flavour scores of both heated oil and fried products. Genetic modification also decreased the levels of degradation compounds present in the used oil.

2.2 STORAGE STABILITY OF FRIED PRODUCTS

The best test to monitor the frying performance of an oil is to evaluate the sensory properties of the fried product during storage (Brooks, 1991). Generally, snack foods (e.g. potato chips) should withstand several months of storage at ambient temperatures since they are low in moisture (potato chips - 1-2%) (Mottur, 1989). The biggest factor in the storage stability, therefore, is the high fat content (potato chips 30-40%) (Mottur, 1989). The major defect associated with stored snack foods, is the development of rancid flavour. The degree of unsaturation of the oil is the major factor that affects the formation of rancidity (Robards *et al.*,1988).

2.2.1 Accelerated Storage

Although some researchers evaluate shelf-life of potato chips under practical storage conditions (room temperature) it takes weeks before any results can be obtained (Hawrysh, 1992;

Asap and Augustin, 1986). Therefore, more rapid tests are employed using elevated temperatures since it is known that the rate of oxidation increases exponentially with temperature increase (Ragnarsson and Labuza, 1977).

Schaal oven test which using temperatures of 55-60°C is widely accepted for evaluating the stability of fats, oils and lipid containing foods because at these temperatures the mechanism of oxidation is similar to what occurs at ambient temperatures (Ragnarsson and Labuza, 1977; Frankel, 1993). The only weak feature of the Schaal Oven test is the lack of a standard storage protocol (Malcolmson *et al.*, 1994).

Other accelerated storage methods include oxygen absorption (OAM), active oxygen (AOM), Rancimat methods. These methods use high temperatures of 80-140°C and the end of shelf-life is determined when PV reaches an agreed upon value. However, according to Frankel (1993) these tests may not be reliable since at the high temperatures, used in these tests, side reactions of polymerization and cyclization take lead, altering the mechanism of oxidation observed at room temperature. Also, the end point of PV=100-120 is too high since rancid flavour develops at PV as low as 10. It should also be stressed, that storage stability tests should be conducted without light, since the presence of light involves a different mechanism of oxidation (Frankel,1993).

2.2.2 Methods to Measure Storage Stability

Frankel (1993) evaluated some of the methods used to assess lipid oxidation and ranked them in descending order according to their ability to predict stability and consumer acceptability as follows: sensory → volatile analysis → oxygen absorption → PV.

PV and CDA methods measure the primary products, sensory evaluation and volatile analysis measure the secondary products of oxidation.

2.2.2.1 Sensory Evaluation

Sensory evaluation is considered to be the ultimate method for measuring the flavour quality and stability of lipid foods (Warner, 1995). The humans' ability to integrate different compounds and their concentrations into the perceived flavour quality and intensity has not been surpassed by any chemical or instrumental test (Warner, 1995).

There are two different types of sensory panels - consumer and trained. For assessing the shelf-life of lipid containing foods a trained panel is more useful. Although, a consumer panel can be used to determine if there is a decrease in quality during storage, most often the cause for that decrease is unknown and the results are greatly affected by individual preferences (Warner, 1995; Labuza and Schmidt, 1988).

There are two types of tests that a trained panel can employ: difference tests and descriptive analysis tests (Warner, 1995; Labuza and Schmidt, 1988). Although descriptive tests give more information about flavour quality and intensity, extensive training with reference samples is needed.

Different scales for measuring the flavour quality and intensity in fried foods have been used by researchers. The scales most often used are similar to the AOCS recommended practice for testing of oils (Warner, 1995). Overall flavour quality and intensity are measured on a 10-point category scales (Figures 2.7 and 2.8) (Warner, 1995; Frankel, 1985; Mounts *et al.*, 1994a; Mounts *et al.*, 1994b; Warner and Mounts, 1993). Individual flavour attributes are also rated on a 10-point category scale (1=no odour, 10=strong intensity) (Warner and Mounts, 1993; Warner *et al.*, 1994).

In order to achieve the reliable sensory data with reduced variability the level of training is critical (Frankel, 1993). Differences in descriptive terms makes it difficult to compare results from different laboratories (Frankel, 1982; Przybylski and Eskin, 1995). There are also differences in testing procedures, training and reference samples used. Other drawbacks of sensory testing include: high cost to conduct and maintain a sensory panel; the presence of other ingredients (spices, flavourings) may mask the rancid

AOCS Flavor Quality Evaluation

Directions: Take 5–10 mL of warm oil into mouth; pull air through the oil and exhale through the nose. Rate samples for overall flavor quality on a 10-point scale; identify flavors and rate as weak (W), moderate (M), or strong (S).

Quality		Overall Quality Scores			
		492	716	258	931
10	Excellent				
9	Good				
8					
7	Fair				
6					
5	Poor				
4					
3	Very Poor				
2					
1	Bad				

Descriptions	Description Intensity			
Nutty				
Buttery				
Corny				
Beany				
Hydrogenated				
Burnt				
Weedy				
Grassy				
Rubbery				
Melon				
Rancid				
Painty				
Fishy				
Other _____				
Other _____				

Figure 2.7. Quality Scales for Oil Evaluation (Warner, 1995).

AOCS Flavor Intensity Evaluation

Directions: Take 5–10 mL of warm oil into the mouth; pull air through the oil and exhale through the nose.
Overall Intensity Scores: Rate samples on a 10–1 scale.
Descriptions: Identify flavors and rate as weak (W), moderate (M), or strong (S).

Intensity		Overall Intensity Scores			
		492	716	258	931
10	Bland				
9	Trace				
8	Faint				
7	Slight				
6	Mild				
5	Moderate				
4	Definite				
3	Strong				
2	Very strong				
1	Extreme				

Descriptions	Description Intensity			
Nutty				
Buttery				
Corny				
Beany				
Hydrogenated				
Burnt				
Weedy				
Grassy				
Rubbery				
Melon				
Rancid				
Painty				
Fishy				
Other _____				
Other _____				

Figure 2.8. Intensity Scales for Oil Evaluation (Warner, 1995).

flavour at the initial stages of oxidation (Robards *et al.*, 1988; Frankel, 1993).

2.2.2.2 Volatile Component Analysis

The major change in potato chips volatiles during storage is attributed to oxidation (Min and Schweizer, 1983). There has been a great deal of effort made over the years to identify the classes and specific components responsible for the oxidized flavours and odours. Most chromatographic studies were done with the purpose of relating the results to sensory evaluation (Robards *et al.*, 1988). It has been found, that increases in carbonyl compounds, both saturated and unsaturated are associated with deterioration of flavour in aged potato chips. At the same time a decrease in the amounts of dienals, specifically 2,4-decadienals, is related to a decrease or disappearance of fresh potato chip flavour (Mookherjee *et al.*, 1965; Warner *et al.*, 1994). Ullrich and Grosch (1988a and 1988b) were able to identify specific compounds that were important in oxidized soybean oil by equipping the gas chromatograph with a sniffing port. Linoleic and linolenic acids were identified as important precursors of oxidized odour in soybean oil (Ullrich and Grosch, 1988a and 1988b). Although GC results are not always well correlated with sensory scores, much can be learned about the mechanisms of flavour formation from the GC results (Frankel, 1993).

The analysis of volatiles in potato chips is challenging since nonlipid components can produce volatiles not related to oxidation in the lipid fraction (Przybylski and Eskin, 1995). The choice of method for determining potato chip volatiles is dependent on how much the sample will be manipulated before the analysis. Direct gas chromatography as one of the purge-and-trap methods is preferred because no oil extraction from potato chips is needed thus bringing the determination as close to sensory testing as possible (Przybylski, 1991; Robards *et al.*, 1988). This method is also characterized by good recovery of volatile components (Przybylski and Eskin, 1995). More detailed discussion of different methods for volatile analyses can be found elsewhere (Przybylski and Eskin, 1995).

2.2.2.3 Peroxide Value

Hydroperoxides or peroxides are primary products of oxidation. Peroxides are flavourless compounds that are unstable, especially at elevated temperatures, and readily decompose to yield low molecular weight volatiles (Fritsch, 1994; Robards *et al.*, 1988; Gray, 1978; Hahm and Min, 1995). There are several methods available to determine PV's. The AOCS official method employs an iodometric titration, which has been found to produce somewhat inaccurate values due to binding of the iodine to unsaturated bonds in the oil and the release of iodine from potassium iodide by air oxygen (Gray,

1978; Robards *et al.*, 1988; Hahm and Min, 1995). Due to the arbitrary determination of the end point of titration the iodometric method is not accurate for measuring peroxides at low levels (Gray, 1978). Several colorimetric and spectrophotometric methods have also been developed over the years (Gray, 1978; Robards, 1988), but the AOCS method remain the most widely used.

Min and Schweitzer (1983) found PV to be highly correlated with volatiles formation in potato chips. Liu and White (1992) reported high correlations between PV and flavour quality and intensity of stored bread cubes fried in soybean oil. Hawrysh (1992) found high correlations between PV and the intensity of painty and rancid odour/flavour of stored potato chips. Similar findings were reported for stored tortilla chips by Hawrysh (1993).

2.2.2.4 Conjugated Dienoic Acids

Determination of CDA has been found to be useful in evaluating the storage stability of fried products (White, 1995). Hawrysh (1992, 1993) reported high correlations between CDA and painty and rancid odours/flavours in stored potato and tortilla chips, respectively. The CDA method is simple, faster than PV measurements, does not require any colour development or chemical reaction, and does not depend on arbitrary judgements (Gray, 1978). However, since CDA is measured on the slope of the absorbance curve, it is only useful for

determining relative changes (Gonzalez-Quijano and Dobarganes, 1988; Kirk and Sawyer, 1991; White, 1995).

In summary, the evaluation of oxidation in stored lipid containing food should include variety of tests that measure both primary and secondary products (Frankel, 1993). Several tests are needed since no single test have been shown to be absolutely reliable in measuring the oxidation products.

2.2.3 Effect of Altered Fatty Acid Composition on Storage Stability of Fried Products

The stability of potato chips fried in regular and partially hydrogenated canola oils during 12 days of accelerated storage at 60°C was evaluated by Hawrysh (1992). The characteristic potato chip odour was higher initially for potato chips fried in regular canola oil which then dropped dramatically by the end of the storage period. In contrast, the intensity of characteristic potato chip odour for potato chips fried in hydrogenated oil decreased very slightly during the 12 days. The off-odour intensity in potato chips fried in hydrogenated canola oil was significantly higher ($p<0.05$) initially than in chips fried in regular oil, which then decreased during storage. The off-odour intensity in chips fried in regular canola oil increased during storage but was not significantly different from that of chips fried in hydrogenated oil. Rancid and painty odour intensities in chips fried in regular and hydrogenated canola oils increased during

storage and was higher in chips fried in regular oil, but was significantly higher ($p<0.05$) only for painty odour. Potato chips fried in hydrogenated canola oil had significantly lower ($p<0.05$) accumulation of PV, CDA and total volatiles than chips fried in regular canola oil after accelerated storage. There was no significant difference in the accumulation of FFA between chips fried in regular and hydrogenated canola oils.

Melton *et al.* (1993) have found stored potato chips fried in hydrogenated canola oil and in blends of hydrogenated canola oil with cottonseed oil (85:15 and 70:30) to have similar stability as potato chips fried in 100% cottonseed oil, measured by PV. The authors concluded that reduction of 18:3 in canola oil by hydrogenation have improved the stability of potato chips fried in hydrogenated canola oil and blends with cottonseed oil.

Hawrysh *et al.* (1993 and 1995) evaluated tortilla chips fried in regular, partially hydrogenated and low linolenic canola oils subjected to accelerated storage at 60°C for 16 days. The intensity of characteristic odour in chips fried in regular canola oil dropped dramatically during storage and was significantly lower ($p<0.05$) than in chips fried in hydrogenated and low linolenic canola oils. The intensity of characteristic odour did not change dramatically in chips fried in hydrogenated and low linolenic oils. The intensities of off, rancid and painty odours increased rapidly for tortilla chips fried in regular canola oil and were

significantly higher ($p<0.05$) than intensities of the same odours in chips fried in hydrogenated and low linolenic oils after 16 days of accelerated storage. The results of chemical and instrumental tests revealed that although the initial values for PV, CDA, FFA and total volatiles were similar for chips fried in the three oils, the final measurements of the same indices after storage were significantly higher ($p<0.05$) for tortilla chips fried in regular canola oil compared to chips fried in hydrogenated and low linolenic canola oils.

Warner *et al.* (1994) evaluated the storage stability of potato chips fried in regular (18:3=7.7%), hydrogenated (18:3=0.8%), low linolenic (18:3=2.8 and 2.9%) and high oleic (18:3=4.2%) canola oils. Chips were sampled every three hours during 18 hrs of frying and were stored for four months at 25°C. Flavour quality measurements (10=excellent quality, 1=bad quality) indicated that potato chips fried in regular canola oil had the lowest scores compared to the chips fried in the other three oils. Chips fried in hydrogenated oil had the lowest scores. The amount of total volatiles in chips fried in regular canola oil was significantly higher ($p<0.05$) than in potato chips fried in other three oils at all sampling times.

2.2.4 Effect of Oil Degradation on Storage Stability of Fried Products

The effect of frying oil quality on potato chip shelf-life was studied by Asap and Augustin (1986). They fried 10 batches of potato chips a day (5 hours of frying) for 8 consecutive days in palm olein. At the end of each day one batch was subjected to storage at room temperature or at 60°C. The end of shelf-life was considered when 4 out of 6 judges could detect a rancid flavour in the chips. The chips stored at 60°C showed a rapid decrease in shelf life after the first day of frying and did not change after the fourth day of frying (Table 2.2). A similar pattern but less dramatic was observed at room temperature. The authors attributed this to the accumulation of polar compounds in the oil and an increase in the acid value. The limits set in this study were 27-30% for polars and acid value of 1-2 mg KOH per g of oil. As the polars and acid value increased, the shelf life of potato chips decreased.

Yoon *et al.* (1988) examined the prooxidative effect of frying degradation products. Thermally oxidized oil (heated at 180°C for 96 hrs, polar compounds - 31.3%) was added to fresh oil (soybean) at different levels (0.5, 1.0, 1.5, 2.0%). The oil was subjected to accelerated storage at 55°C for 10 days. There was a direct relationship between the amount of thermally oxidized oil added and volatiles formation. The accumulation of volatiles was increasing significantly

Table 2.2. Effect of Frying Oil Deterioration on Potato Chips Shelf-Life*

Day of Frying	Storage Days Required to Reach Rancidity			
	Fry Number	Stored at 60°C	Fry Number	Stored at 27-30°C
1	1	41	2	75
1	10	27	9	50
2	20	9	19	37
3	30	7	29	27
4	40	6	39	20
5	50	6	49	16
6	60	6	59	12
7	70	6	69	12
8	80	6	79	10

* Adapted from Asap and Augustin (1986)

($p<0.05$) as the levels of oxidized oil added to the fresh soybean oil increased. The oxygen disappearance in the headspace was also significantly increasing ($p<0.05$) as the amount of thermally oxidized oil added to the fresh oil increased. These findings suggest that oil degradation products have a prooxidative effect.

In summary, most of the studies show that foods fried in genetically modified oils have better storage stability compared to traditional oils as reflected by odour/flavour stability and reduced accumulation of primary and secondary oxidation products. There is an indication that degradation products of frying can impair the storage stability of fried foods. However, there is no information available on how these products affect the storage stability of snack foods fried in genetically modified oils.

CHAPTER 3

FRYING PERFORMANCE OF REGULAR, HYDROGENATED, LOW LINOLENIC AND HIGH OLEIC CANOLA OILS

3.1 INTRODUCTION

During frying, a number of changes takes place in the oil as a result of thermal degradation, oxidation and hydrolysis. The rate of decomposition depends on a number of factors including the composition of the oil, temperature and length of frying, continuous or intermittent frying, type of food fried, and whether or not fresh oil is added throughout frying (Melton, 1994; Boskou, 1988; Fedeli, 1988; Thompson and Aust, 1983; Blumenthal, 1991, Peers and Swoboda, 1982). Among the factors listed above the degree of unsaturation of canola oil is considered to be the major factor influencing an oil's poor frying stability. Canola oil has high levels of linolenic acid and therefore limited in its ability to withstand frying temperatures. By reducing the levels of linolenic acid present in canola oil by genetic modification it is possible that a stable frying oil can be achieved.

A limited number of studies have been undertaken to examine the sensory attributes of canola frying oils and found that the heated room odour of low linolenic canola oil had a lower intensity than regular canola oil (Prevôt *et al.*, 1990; Eskin et al, 1989; Warner and Mounts, 1993). The odour/flavour quality of french fries fried in low linolenic canola oil was shown by Warner and Mounts (1993) to be higher compared to fries fried in regular and hydrogenated canola oils. Potato

chips fried in low linolenic and high oleic canola oils had improved flavour quality over those fried in regular canola oil (Warner *et al.*, 1994). The reduction in 18:3 content resulted in significantly lower levels of free fatty acids, carbonyls and dienals in low linolenic canola oil as compared to regular canola oil during 10-minute heating (Eskin *et al.*, 1989). Low linolenic canola oil has also shown significantly lower accumulation of free fatty acids and lower foam height compared to regular canola oil during 45-hour frying of french fries. The amount of polars was not significantly different among the two oils (Warner and Mounts, 1993). In contrast, Warner *et al.* (1994) found the amount of polars in low linolenic and high oleic canola oils was significantly lower than those in regular canola oil, but the free fatty acid levels were significantly lower in regular canola oil compared to low linolenic and high oleic canola oils.

Although these studies suggest that there is an improvement in the frying stability of genetically modified canola oils, the results are not consistent. Therefore, there is a need for further investigation of the frying performance of genetically modified canola oils. Thus, this study was undertaken to meet the following objectives:

1. To determine how the genetically modified canola oils differ from hydrogenated and regular canola oils in terms of their fatty acid composition.

2. To investigate whether the differences in fatty acid composition improves the frying performance of the genetically modified canola oils compared to regular and hydrogenated canola oils.

3.2 MATERIALS AND METHODS

3.2.1 Oils and Potatoes

Four types of canola oil were selected for the study. Regular and low linolenic canola oil were refined, bleached and deodorized at CanAmera (Altona, MB). Only citric acid was added to these oils during processing. Hydrogenated canola frying fat, obtained from CanAmera (Nipawin, SK), was a refined, bleached, deodorized regular canola oil which was then hydrogenated and bleached. High oleic canola oil was refined, bleached and deodorized on a laboratory scale at Anderson Clayton/Humko (Memphis, TN).

The potatoes were Norchip variety which is used in commercial frying of potato chips, obtained from Southern Potato Company (Winkler, MB).

3.2.2 Frying Protocol

The antifoaming agent (dimethylpolysiloxane) was added in the amount of 2 ppm (Hawrysh, 1992). To achieve the required concentration, a mix of silicone in oil was prepared as follows: 0.17 g of silicone was added to 20 g of oil and 1 g of this mixture was added to 4.25 kg of the oil.

Unpeeled potatoes were washed with a brush under water. One medium potato or two small were sliced on a Hobart slicer to a thickness of 1.2-1.3 mm (Mottur, 1989). The slices were washed twice under running cold water to remove surface starch which is released from the cells during slicing, then left in

a pan covered in cold water. Failure to do so causes the chips to stick together (Mottur,1989). The required amount of potato slices (approx.60-70 g) were taken from the pan, blotted with paper towels and spread in a single layer on a wire rack. The potato slices were then covered with a second wire rack and placed onto the tray attached to the fryer. A Model 611 mini fryer (Belshaw Bros., Inc., Seattle, WA) with 5 kg capacity was used for frying of potato chips. The initial amount of oil used was 4.25 kg. On the first day of frying, the oil was seasoned by being heated to 185±5°C and kept at this temperature for 30 min. The chips were fried until the bubbling of the oil from the released moisture ceased (2 min to 2'15"). After frying, the chips were allowed to drain for 5 min on the tray attached to the fryer. The potato chips were then transferred onto the table covered with paper towels and blotted with other paper towels. The chips were left on the table for 15 min and then collected in a container where all chips from one day of frying were kept. Thirty two batches of potato chips were fried each day, 15 min apart for a total of 8 hrs of frying. Chips were fried in each oil for 5 days (40 hours of frying).

On the second and consecutive days the oil was weighed in the morning to determine the amount of fresh oil that needed to be added in order to start every day with 4.25 kg of oil in the fryer. The antifoaming agent:oil mixture was added to achieve 2ppm of silicone in the make-up oil. Next, the oil was

heated to reach the temperature 185±5°C before frying the first batch of chips.

The order in which the oils were used for frying of potato chips was as follows: hydrogenated, low linolenic, regular, high oleic.

3.2.3 Sampling of Oil for Analyses

The samples of oils for chemical and instrumental analyses from each frying day were taken each morning after the oil had cooled overnight and before it was measured, replenished with fresh oil and heated. The 0 h time oil (fresh oil heated for 30 min at 185±5°C) was collected hot.

The oil (approx. 20 g) was collected into 20 mL screw top vials. For each oil, six samples of oil were gathered (0 h, day 1, day 2, day 3, day 4 and day 5).

3.2.4 Chemical and Instrumental Analyses

The initial quality of the oils (fresh, non-heated) was determined by measuring peroxide value (PV), free fatty acids (FFA) and fatty acid composition (FA composition). PV was determined by titration using the AOCS method Cd 8-53. FFA were determined by titration using the AOCS method Ca 5a-40. Samples for both PV and FFA were done in duplicate.

FA composition (relative %) - was carried out by gas chromatography following the AOCS method Ce 1-62 with methylation of the samples modified according to Przybylski

(1994, personal communication). A Hewlett Packard 5890A gas chromatograph (GC) with fused silica capillary column 30m x i.d. 0.25mm coated with polar phase Supelcowax 10 (Supelco Inc., Bellefonte, PA) was used. The GC was equipped with autosampler, 3392A Integrator and a flame ionization detector. The temperature of the detector and the injection port was held at 250°C. The carrier gas was hydrogen. The column temperature was programmed from 195°C to 235°C at a rate 2°C/min and held at lower and upper temperature for 3 and 4 min, respectively. The injected amount was 3 μL.

The methylation of the oil samples was performed as follows: 100 mg of the oil (or melted hydrogenated fat) was measured with 0.01 g accuracy into the 20 mL threaded top tube. One mL of petroleum ether was added and the content was mixed well to obtain a monophase system, 6 mL of 0.5N HCl in CH_3OH was added. After vortexing, the tube was heated in an oven at 70-80°C for 1 hour. The sample was cooled down to room temperature and 2.5 mL of iso-octane (2,2,4-trimethyl pentane) and 3 mL of glass distilled water were added. The content was mixed by turning upside down only. After the upper layer became clear, half of it was pipetted into a 2 mL capacity GC vial and analyzed for FA composition.

Frying performance of the oils was determined by measuring FFA (by both titration and thin layer chromatography with flame ionization detector), conjugated dienoic acids

(CDA) by the AOCS method Ti 1a-64 (duplicate readings were taken), FA composition by quantification with internal standard, polymers and oxidation products by quantification of non-eluted materials according to AOAC method 977.17 and polar components (by both gravimetric method and thin layer chromatography with flame ionization detector).

FA composition (quantification with internal standard) - the same instrument settings and injected amounts as described previously were used. The amounts of individual fatty acids were quantitated using an internal standard. The internal standard was prepared by dissolving 100 mg of trinonadecanoin (Nu-Chek-Prep. Inc., Elysian, MN) in 50 mL of 1:1 mixture of benzene and chloroform, thereby giving a 2 mg of internal standard in every mL of solution. A 50 mL volumetric flask was used to prepare the solution. One mL of internal standard solution was added to the sample before the methylation procedure.

Polymers and Oxidation Products

It is possible to estimate the polymers from the fatty acid composition results if the analysis with internal standard is run (Waltking *et al.*, 1975). The polymers and oxidation products formed are the components that have high molecular weight and not represented in the chromatogram (they are not eluted). The formula used is provided in AOAC Method 977.17 and is as follows:

$$\% \text{ Non-eluted material} = \frac{[PA' \cdot ((W' + W)/W') - PA] \cdot 100}{PA' \cdot [((W' + W)/W') - 1]}$$

where PA′= peak area of internal standard; W′ and W = weights of internal standard and sample, respectively; PA = total area of chromatogram.

Polar components analysis

Polar components were determined by using two methods: gravimetrically with Silica gel Sep-Pak cartidges and thin-layer chromatography with flame ionization detector (TLC-FID).

Gravimetric Method

The method for separation of polar and nonpolar fractions in frying fats using Sep-Pak Cartridges was published by Sebedio *et al.* (1986) and according to the authors was found to be as good as the separation on the silica gel column (the IUPAC method).

The following method for separation of nonpolar and polar fractions was adapted from the method described by Sebedio *et al.* (1986). A Sep-Pak Vac 6 cartridge (Waters Co. Division of Millipore Corp., Milford, Massachusetts) containing 1 g of silica was used for separation. The cartridge was secured at

the stand with a clamp, and N_2 pressure was used to achieve a solvent flow of 2 mL/min.

50 mg of the oil (or melted hydrogenated fat) was weighed into a 5 mL screw top vial with 0.0001 g accuracy. Petroleum ether (4.5 mL) was added to dissolve the sample. Empty 20 mL vials, two for each fraction collected, were weighed and numbered.

The Sep-Pak cartridge was activated with 5 mL of hexane. The dissolved sample was transferred from the vial onto the cartridge and the flow kept at 2 mL/min. As soon as the sample was placed into the column the collection of the nonpolar fraction began. The vial after the sample was washed with 10 mL of petroleum ether which was then transferred onto the column to ensure that the sample was transferred quantitatively. The separation of nonpolar fraction was done using a 20 mL mixture of petroleum ether : diethyl ether (90:10 v/v). The polar fraction was eluted with 30 mL of methanol (CH_3OH) and collected in a clean vial. The Sep-Pak cartridge was never allowed to dry because this would impair the recovery.

Solvent from collected fractions was evaporated under nitrogen (N_2). Then the vials with the nonpolar fraction were weighed and the amount of polar fraction was determined by the difference. The formula provided in AOAC Method 982.27 was used to calculate the %(w/w) of polar components in the sample:

Polar components (%) = [(E - A)/E] x 100

where A = g nonpolar fraction; E = g sample.

Thin layer chromatography with flame ionization detector

The following method was adapted from the technique described by Sebedio *et al.* (1987) with developing solvents prepared according to Przybylski and Eskin (1994).

Thin layer chromatography in combination with a flame ionization detector allows the researcher to separate in one step polar (monoglycerides, diglycerides, free fatty acids and highly polar components) and nonpolar (triglycerides) components and determine their respective amounts.

The separation of the lipid classes in this method is very much like the method used in classical thin layer chromatography except different solvent systems are used to achieve the desired effect. The flame ionization detector allows the GC principle for detection of the separate components to be employed, i.e. burning releases the ions which cause the change in the current and this change is registered (Tvrzicka and Votruba, 1994).

An Iatroscan TH-10 analyzer (Iatron, Tokyo, Japan) was used in conjunction with the ChromPerfect Direct software (Justice Innovations Inc., Mountain View, CA). The hydrogen and air flow rates were constant at 190 ml/min and 2.15 l/min respectively. Thin layer consisted of quartz rods covered with a layer of silica - Chromarods S III. A stainless steel frame

with 10 rods was used to hold the rods during spotting, developing and burning. The scan speed was set at 35 seconds per rod (mode 3 at the instrument).

The oil was dissolved in a chloroform : methanol mixture (2:1 v/v) to give a 4% solution (w/v). The rods were burned twice before applying the sample. A volume of 0.4 μL was spotted on the rods. The solvent was allowed to evaporate under N_2 giving the amount of pure sample on the rod equal to 16 μg, high amounts improve the detector response (Tvrzicka and Votruba, 1994). The rods were developed in dichlorethane: chloroform : acetone : acetic acid (59:10:1.4:0.4 v/v) for 55 min (Przybylski and Eskin, 1994). After developing, the frame with rods was placed into an oven at 120°C for 5 min. The frame was then placed into the instrument and the rods were scanned. The determination of polars in oil samples was run in duplicate.

The quantification of the individual polar compounds present was based on the individual calibration curves for triglycerides, free fatty acids, diglycerides and highly polar compounds. The regression equations obtained represent the relationship between the known concentration of the component of interest and its peak area on the chromatogram. The free fatty acids and diglycerides were purchased from Doosan Serdary Research Labs (Englewood Cliffs, NJ) and represented a mixture of isolates from pig liver and eggs. To obtain a pure fraction of highly polar compounds canola oil which was

used for frying potato chips for 9 days (8 hrs a day) was separated on Sep-Pak cartridges to isolate the fraction.

A separate calibration curve is needed for each component because detector response depends on the differences in chemical structure (Tvrzicka and Votruba, 1994). Each point on the calibration curve represented the mean of duplicate analyses. After fitting the regression curves the equations with the greatest coefficient of determination were chosen (Table 3.1). For all five components cubic equations showed the highest coefficients of determination (Appendices 1.1 to 1.5).

The total amount of polar components was determined as a sum of free fatty acids, 1,2- and 1,3-diglycerides, and highly polar compounds. The % polars (w/w) was calculated based on the amount of the sample spotted on the rods:

$$\% \text{ Polars} = (B / E) \times 100$$

where B = total amount of polars (μg); E = sample amount (μg).

3.2.5 Statistical Analysis

Analysis of Covariance (ANCOVA) was used to analyze the results obtained from all chemical and instrumental tests performed on the frying oils. The advantage of this analysis is that it combines regression analysis which treats all

Table 3.1. Regression Equations for Polar Components Determination by TLC-FID

Component	Equation	r^2
TG	$y=5205.4x$	.9552
	$y=3152.7x + 165.51x^2$	.9859
	$y=665.86x + 696.58x^2 - 24.746x^3$	**.9968**
	$y=1183.38098x^{1.6266}$	.9649
FFA	$y=367.99x$	.9248
	$y=165.16x + 169.43x^2$	.9867
	$y=204.806x + 80.767x^2 + 41.699x^3$	**.9873**
	$y=362.24271x^{1.57851}$	.9718
1,3 DG	$y=648.92x$	.8969
	$y=209.05x + 609.96x^2$	.9912
	$y=102.27x + 1032.7x^2 - 340.47x^3$	**.9926**
	$y=778.90594x^{1.55447}$	.9907
1,2 DG	$y=771.33x$	.8968
	$y=239.22x + 737.86x^2$	.9948
	$y=239.61x + 735.66x^2 + 1.6507x^3$	**.9948**
	$y=925.90790x^{1.55093}$	.9923
Polars	$y=2130.2x$	.8806
	$y=381.8x + 243.57x^2$	.9983
	$y=598.07x + 161.653x^2 + 6.409x^3$	**.9987**
	$y=4552.10504x^{1.82519}$	.9837

variables as continuous and analysis of variance (ANOVA) which treats all variables as categorical. This allows the reduction in the variance of the model (Neter *et al.*, 1990). ANCOVA allows the researcher to specify which variables they want to treat as continuous variables. In our analyses, frying days were treated as a continuous variable since we were interested in the changes that took place during the frying period and how this differed among the oils. The general linear model procedure - PROC GLM (SAS, 1988) - was used to run the ANCOVA. The model included type of oil, frying day and their interaction term. A t-test with α=0.05 was used to estimate the difference in slopes and intercepts, above and beyond error variability. When the distribution of the residuals lacked normality a natural logarithm (ln) or square root data transformation was used in the model.

Due to the greater amount of the samples from frying oils compared to the extracted from potato chips oils it was possible to determine FFA in frying oils by both titration and TLC-FID, and polars by both Sep-Pak cartridges method and TLC-FID. The TLC-FID method for FFA and polars measurements in extracted oils is preferred due to the smaller amount needed for the analysis. In order to determine the relationship between the two methods used to measure the FFA and the two methods used to measure the polar components - Pearson's correlation coefficients were obtained using PROC CORR (SAS, 1988). High correlation coefficients between the two methods

for either FFA or polars measurements would indicate that these respective methods could be used interchangeably.

3.3 RESULTS

3.3.1 Initial Quality of the Oils

The assessment of the fresh oils revealed that they were of good quality with PV's ranging from 0.2 (regular canola oil) to 1.0 (low linolenic canola oil) meq/kg (Table 3.2). Hawrysh (1990) reported that PV less than 2 meq/kg is an indication of a high quality canola oil. The levels of FFA in the four oils were no higher than 0.3 % oleic (Table 3.2), which is also an indication of good quality oils.

The fatty acid composition of the four oils is provided in Table 3.3. Regular canola oil (RCO) had expected levels of oleic (56.5%), linoleic (22.27%) and linolenic (10.79%) fatty acids. Hydrogenated canola oil (HYCO) had no linolenic acid present, but it had some linoleic (7.98%) and considerable amounts of oleic (73.72%) present. Low linolenic canola oil (LLCO) had smaller amounts of linolenic acid than RCO (3.7% versus 10.07%). A slight increase in the levels of oleic (58.20%) and linoleic (27.88%) fatty acids were also observed compared to RCO. High oleic canola oil (HOCO) had higher levels of oleic acid compared to RCO (75.17% vs 56.50%). This level was similar to the amounts found in HYCO. HOCO also showed a reduction in the content of linolenic acid (5.52%) compared to RCO, which was closer to the amounts of this acid in LLCO. However, increased levels of linolenic acid *trans* isomers were found in the HOCO. This fact could indicate that elevated temperatures were used during deodorization step in

Table 3.2. Chemical Analyses of Fresh Canola Oil *

Canola Oil	Peroxide Value (meq/kg)	Free Fatty Acids (% Oleic)
Regular	0.2	0.03
Hydrogenated	0.4	0.03
Low Linolenic	1.0	0.03
High Oleic	0.4	0.02

* Mean of duplicate analyses

Table 3.3. Fatty Acid Composition of Fresh Canola Oils.

Oil	18:1	18:2	18:3[1]	SFA[2]	MUFA[3]	PUFA[4]
RCO	56.50	22.27	10.79	7.25	58.43	33.06
HYCO	73.72	7.98	-	16.02	75.82	7.98
LLCO	58.20	27.88	3.70	6.43	60.00	31.58
HOCO	75.17	8.01	5.52	6.55	76.92	13.53

[1] - Linolenic Acid - combined *cis* and *trans* 18:3 isomers,

[2] - Saturated Fatty Acids,

[3] - Monounsaturated Fatty Acids,

[4] - Polyunsaturated Fatty Acids.

processing of the oil (Grandgirard *et al.*, 1984; Smouse, 1995). The content of linoleic acid (8.01%) in HOCO was similar to that of HYCO.

3.3.2 Stability of the Oils During Potato Chips Frying

FFA were determined by both titration and TLC-FID. The titration values were ln transformed for statistical analysis. There is an increased accumulation of FFA over frying days for all four oils (Figure 3.1). The analysis of covariance indicated that RCO had significantly higher initial amount of FFA compared to HOCO (t_{16}=-2.35, p=0.0319). During five days of frying the pairwise comparisons of the rates of accumulation of FFA for all four oils showed that HYCO had a significantly higher rate of FFA accumulation than LLCO (t_{16}=-2.65, p=0.0175).

Similar results for the accumulation of FFA in frying oils using the TLC-FID (Figure 3.2). The data was statistically analyzed using a square root transformation. Here, HYCO had a significantly more rapid production of FFA than LLCO (t=-2.11, p=0.0509).

The relationship between the two methods used to measure FFA was determined to find out whether the TLC-FID method could replace the traditional titration method for evaluating the lipids extracted from potato chips (Chapter 4). The

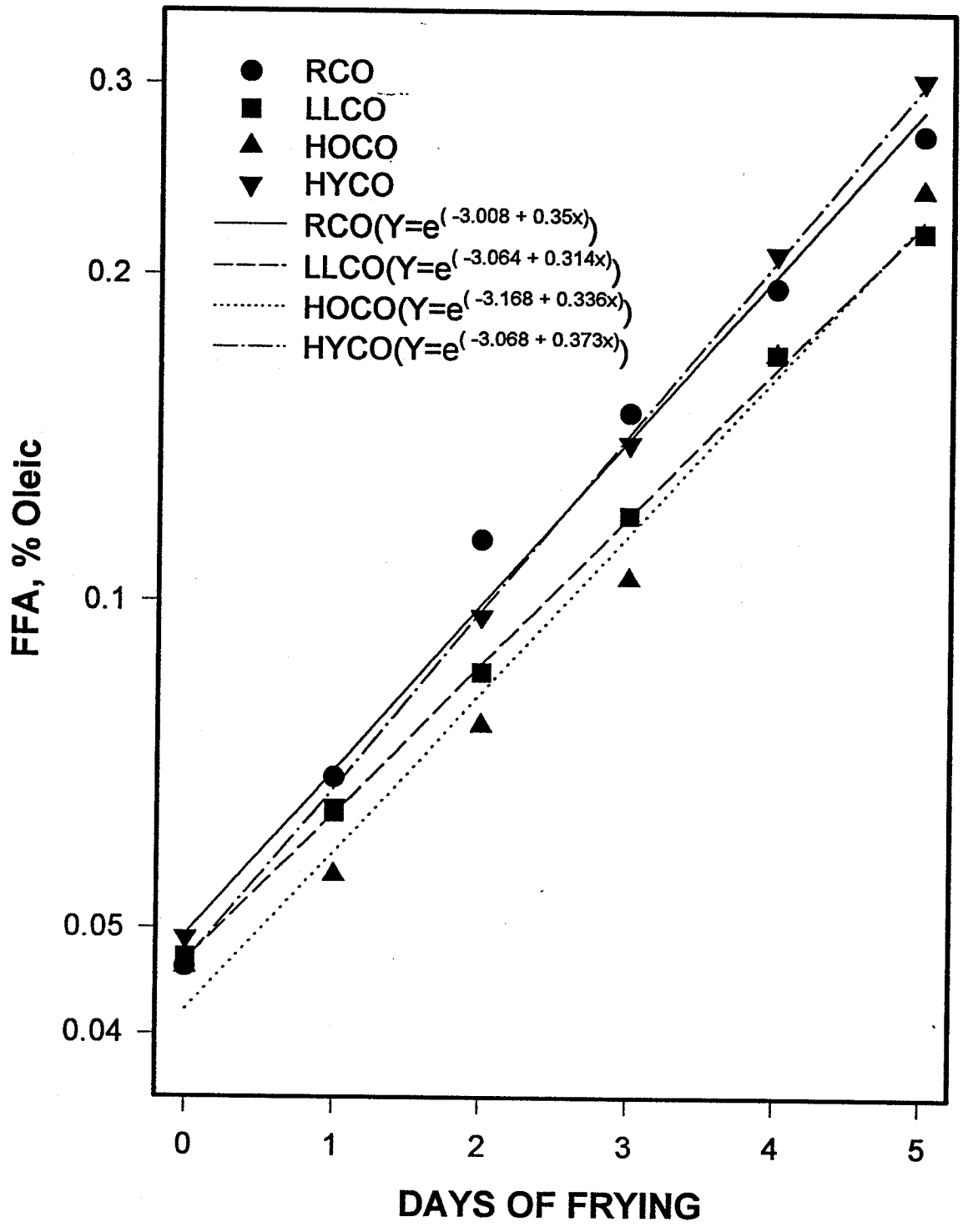


Figure 3.1. Free Fatty Acids (by Titration) in Canola Frying Oils.

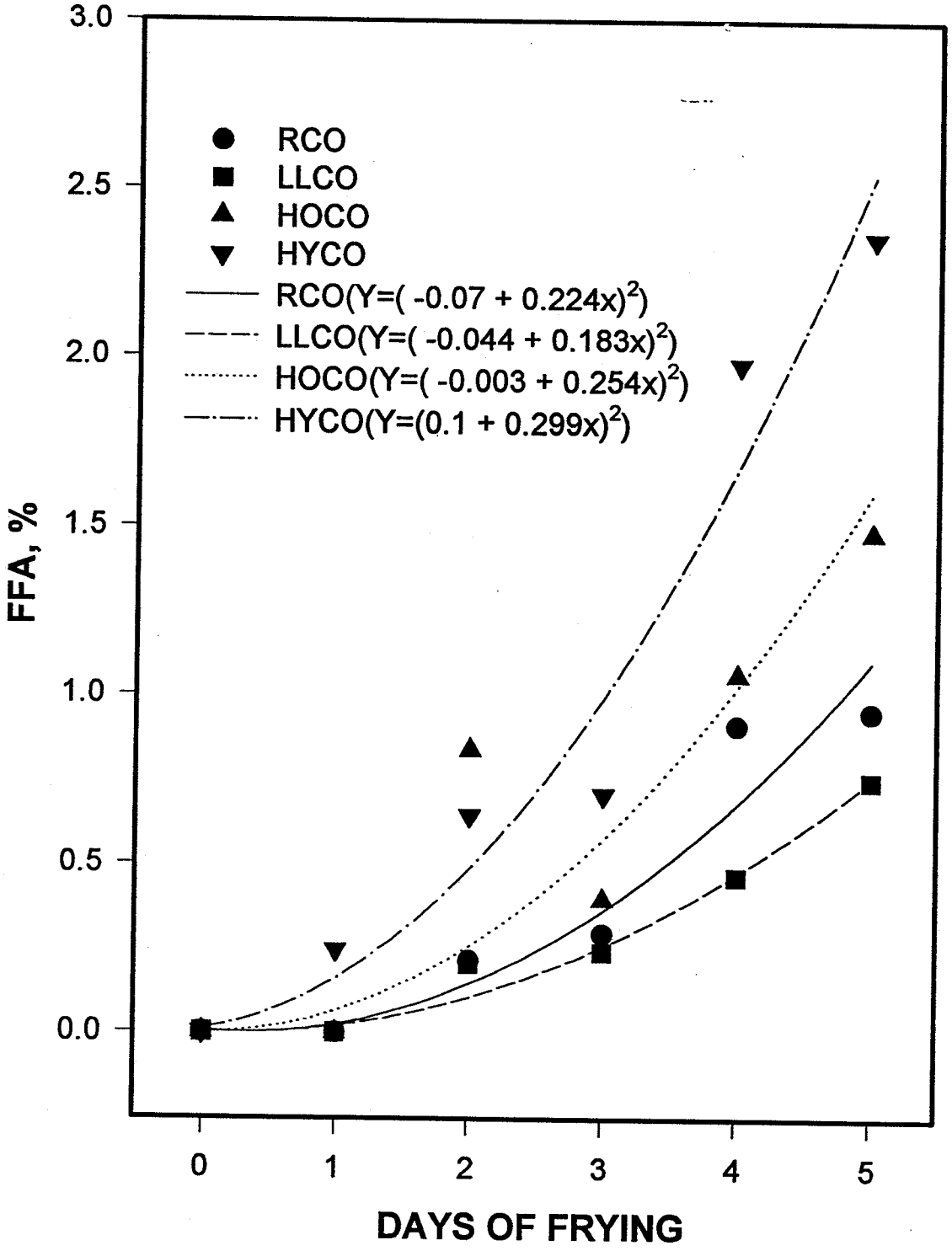


Figure 3.2. Free Fatty Acids (by TLC-FID) in Canola Frying Oils.

advantage of the TLC-FID method is that considerably less sample is required. When Pearson's correlation coefficients were calculated on the two sets of FFA results (titration vs TLC-FID) the value obtained was r=0.85 (Figure 3.3). This indicates that the TLC-FID method can be used with reasonable confidence to determine the levels of FFA in frying oils.

The statistical analysis of CDA values was performed using the ln transformation. The results of the analysis are presented in Figure 3.4. HYCO had the lowest rate of CDA accumulation which was significantly lower than all other oils (LLCO t_{16}=6.29, p=0.0001; RCO t_{16}=4.88, p=0.0002; HOCO t_{16}=2.73, p=0.0149). LLCO and RCO had the highest rates of CDA accumulation which were significantly more rapid than that in HOCO (t_{16}=3.55, p=0.0027; t_{16}=2.15, p=0.0474 respectively). The initial amounts of CDA were the lowest in LLCO and this was significantly lower than in other three oils (HYCO t_{16}=5.59, p=0.0001; RCO t_{16}=3.91, p=0.0012; HOCO t_{16}=-5.06, p=0.0001). The initial amounts of CDA in the other three oils were not significantly different from each other. A study by Liu and White (1992) found that canola oil, which had the highest amount of 18:3 compared to various soybean oils exhibited the lowest accumulation of CDA during 40 hrs of frying of bread cubes. They also found that these results were directly related to the total amount of polyunsaturated fatty acids (PUFA) in the oil (18:2 and 18:3 combined), i.e. canola oil had the lowest content of PUFA compared to the other oils

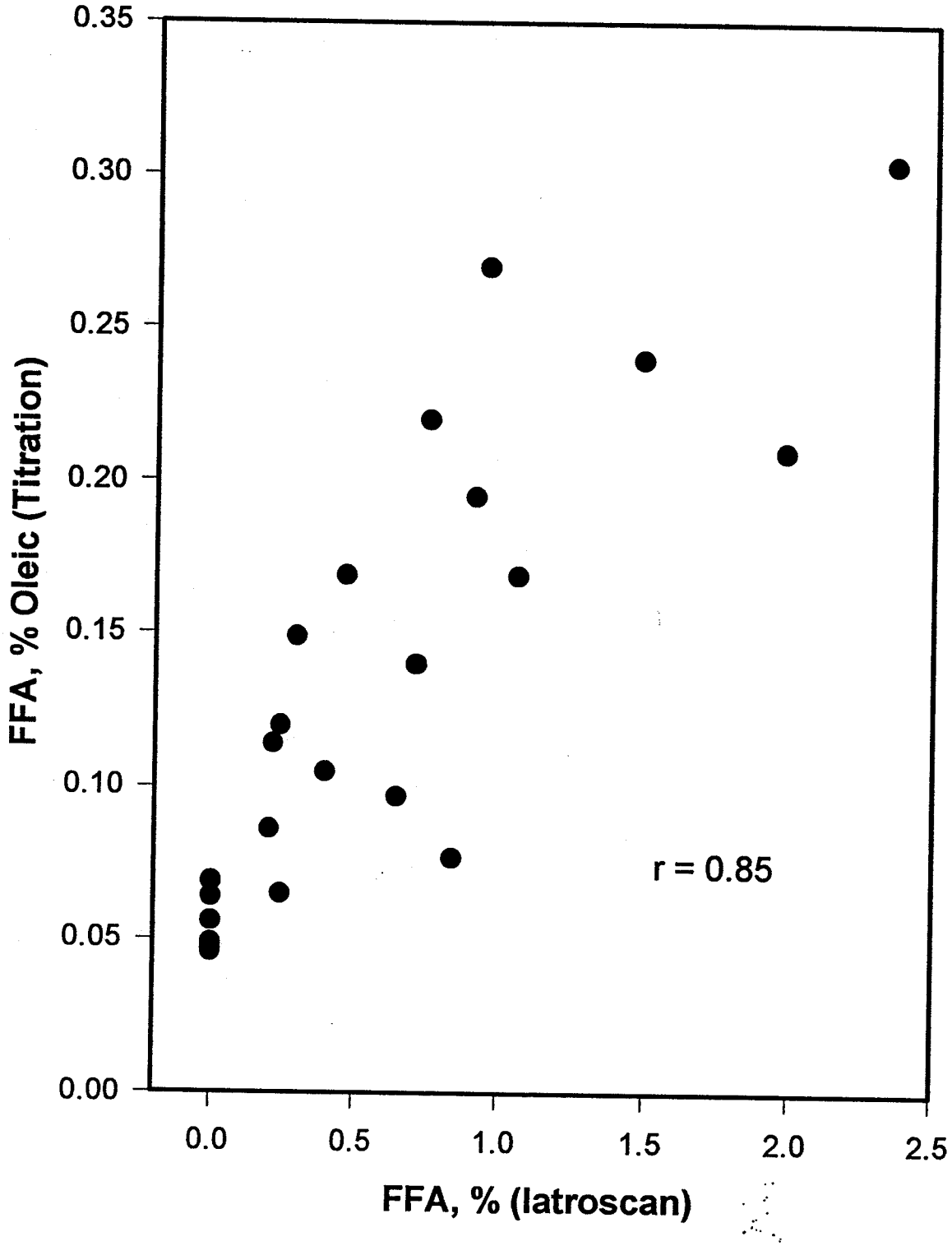


Figure 3.3. Relationship between Free Fatty Acids Determined by Titration and TLC-FID in Canola Frying Oils.

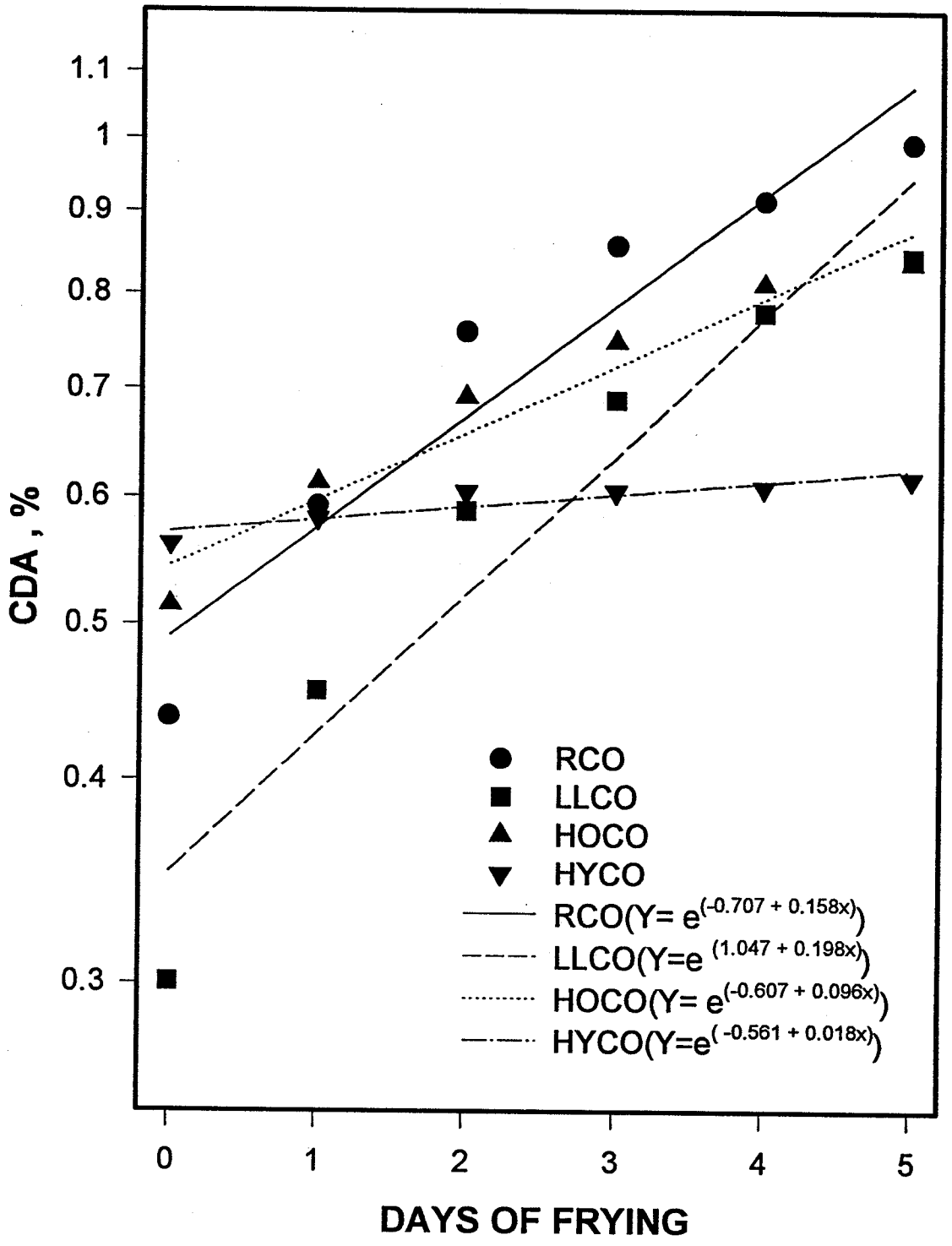


Figure 3.4. Conjugated Dienoic Acids in Canola Frying Oils.

studied (Table 3.4). These findings are in agreement with what was found in the present study where RCO, that had the highest content of 18:3 and the highest amount of PUFA (Table 3.3), exhibited the highest accumulation of CDA.

The quantitative analysis of the fatty acid composition of the frying oils over the 5-day frying period showed that the levels of unsaturated fatty acids decreased in all oils (results not shown). The rates of disappearance were expressed as the ratios of the amounts of specific fatty acids to the amounts of 18:0. 18:0 was used since saturated fatty acids experience the least amount of change during high temperature treatments (Dobarganes, 1988). During five days of frying, changes in the rate of disappearance of 18:1 (Figure 3.5) were not very pronounced for all oils, although LLCO showed a drop at frying day 4. A decrease in the 18:2 amounts was observed in LLCO and RCO across frying days as compared to HYCO and HOCO (Figure 3.6). The disappearance of 18:3 in RCO was more pronounced than it was for HOCO and LLCO (Figure 3.7). HYCO is not included in the Figure 3.7 since it contained no 18:3.

The results of the calculated amounts of polymer and oxidation products were statistically analyzed using ln transformation. Overall there was an increase in the amount of polymers in all oils, but the rates of polymers accumulation were not significantly different among the oils (Figure 3.8). The pairwise comparisons of the initial amounts of polymers

Table 3.4. Fatty Acid Composition (relative area %) of Oils Used for Frying Bread Cubes.

Oil Type	Relative FA Composition by GLC, %			
	18:1	18:2	18:3	PUFA[1]
Soybean:				
Hardin	25.2	54.8	5.9	60.7
BSR 101	22.6	56.7	6.8	63.5
A17	29.3	49.4	1.5	50.9
A16	31.8	50.7	1.9	52.6
A87	29.1	54.7	1.8	56.5
Canola	63.0	21.3	10.3	31.6

(adapted from Liu and White, 1992)

[1] - Polyunsaturated Fatty Acids.

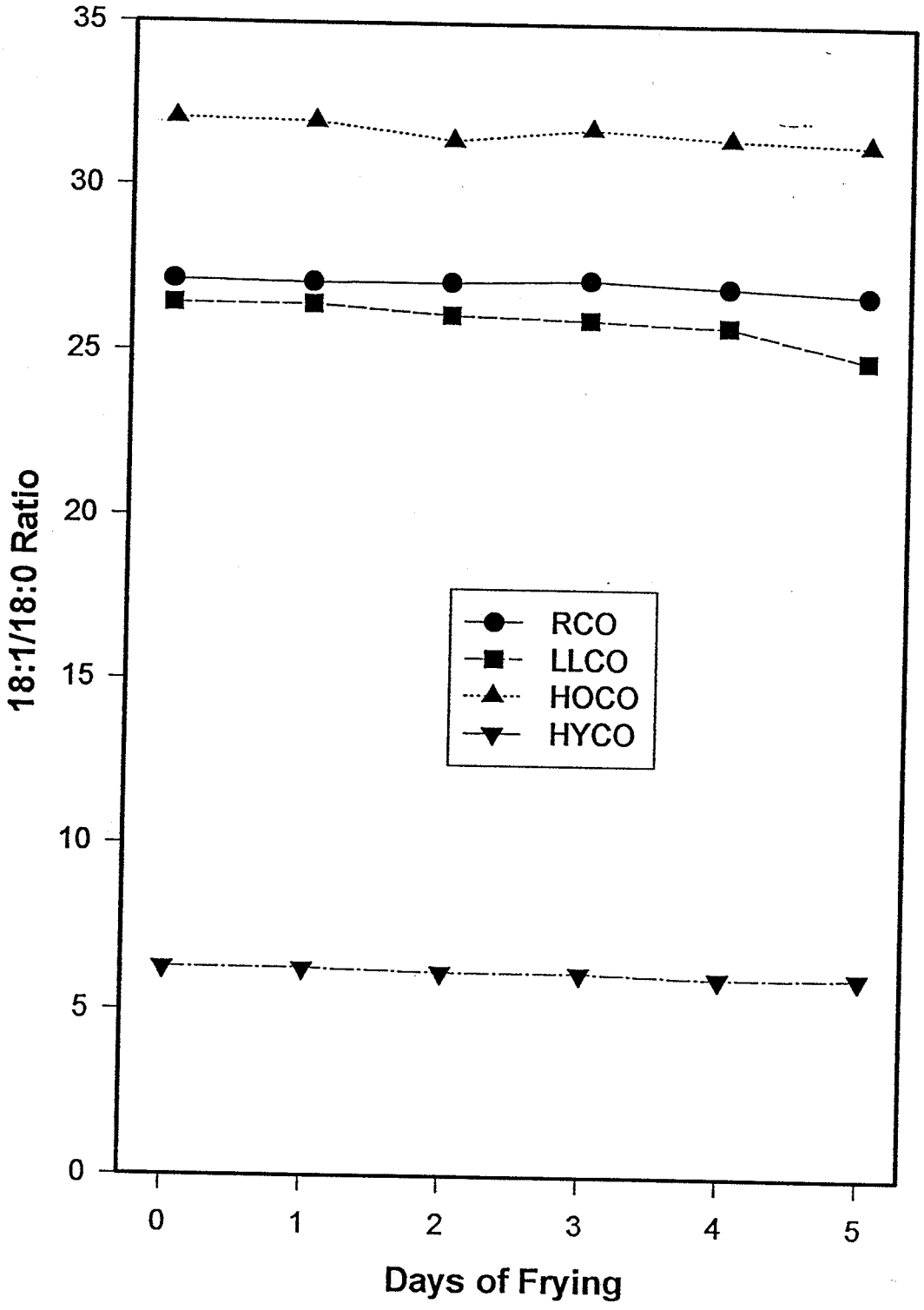


Figure 3.5. Changes in Oleic Acid Content in Canola Frying Oils.

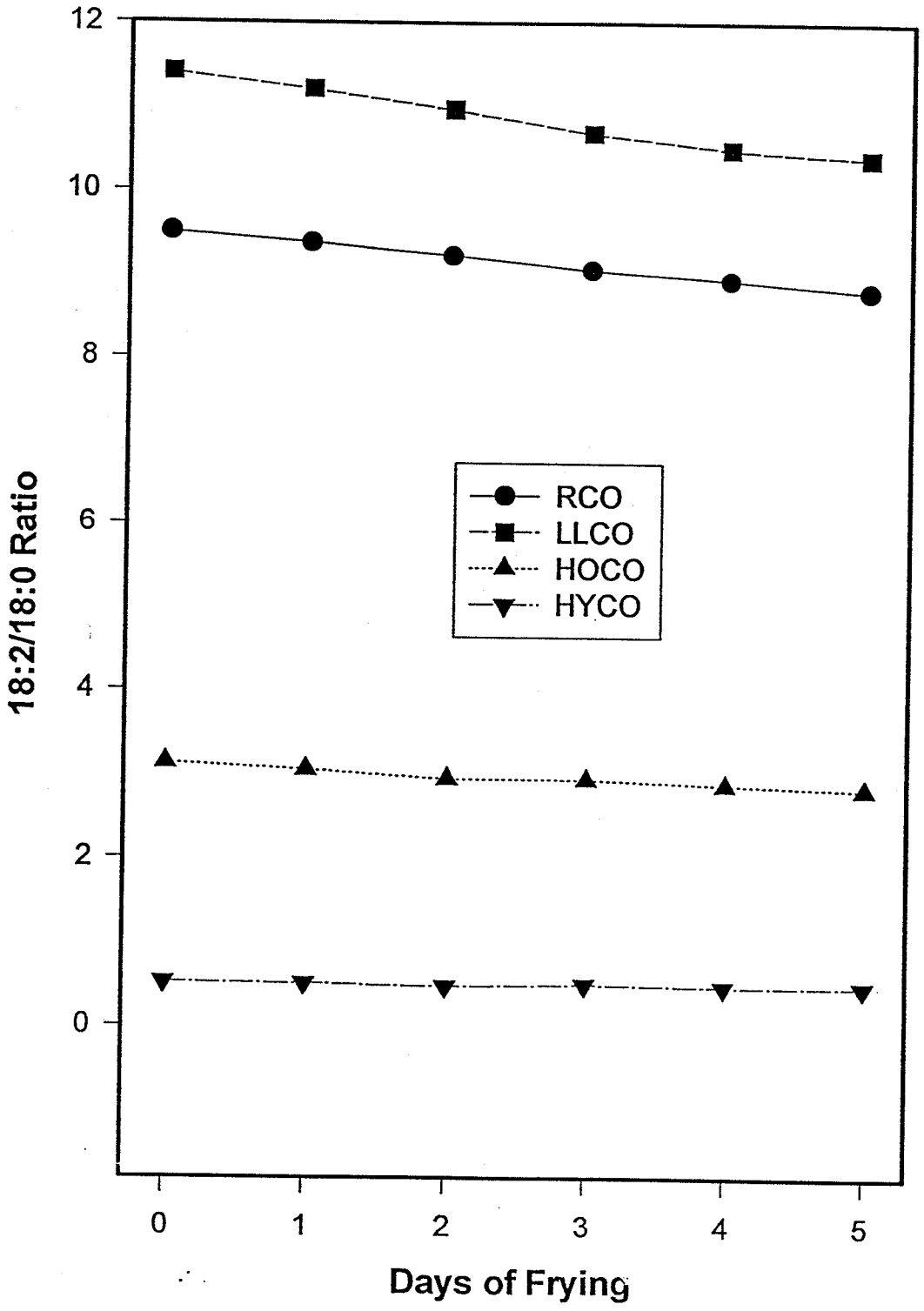


Figure 3.6. Changes in Linoleic Acid Content in Canola Frying Oils.

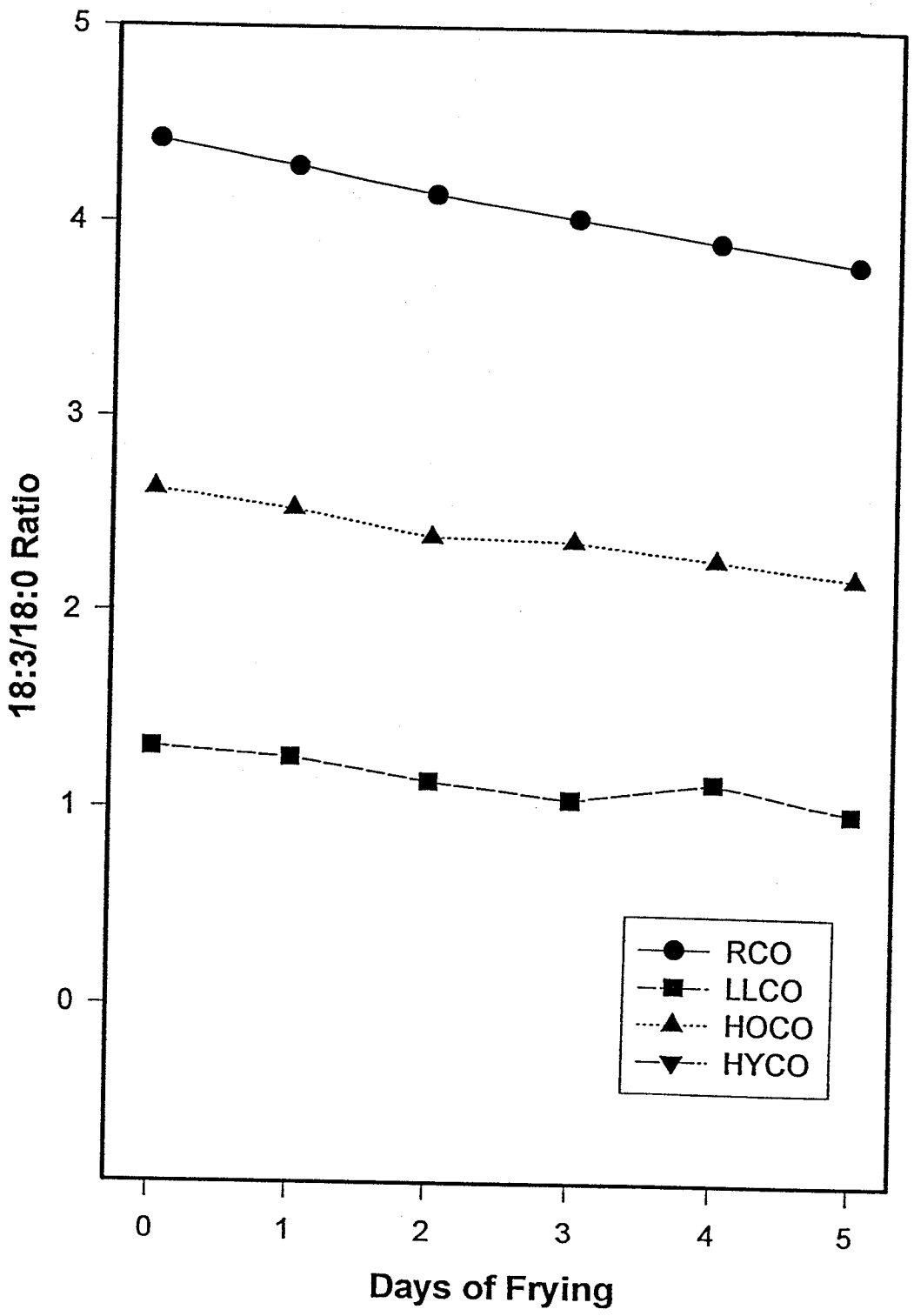


Figure 3.7. Changes in Linolenic Acid in Canola Frying Oils.

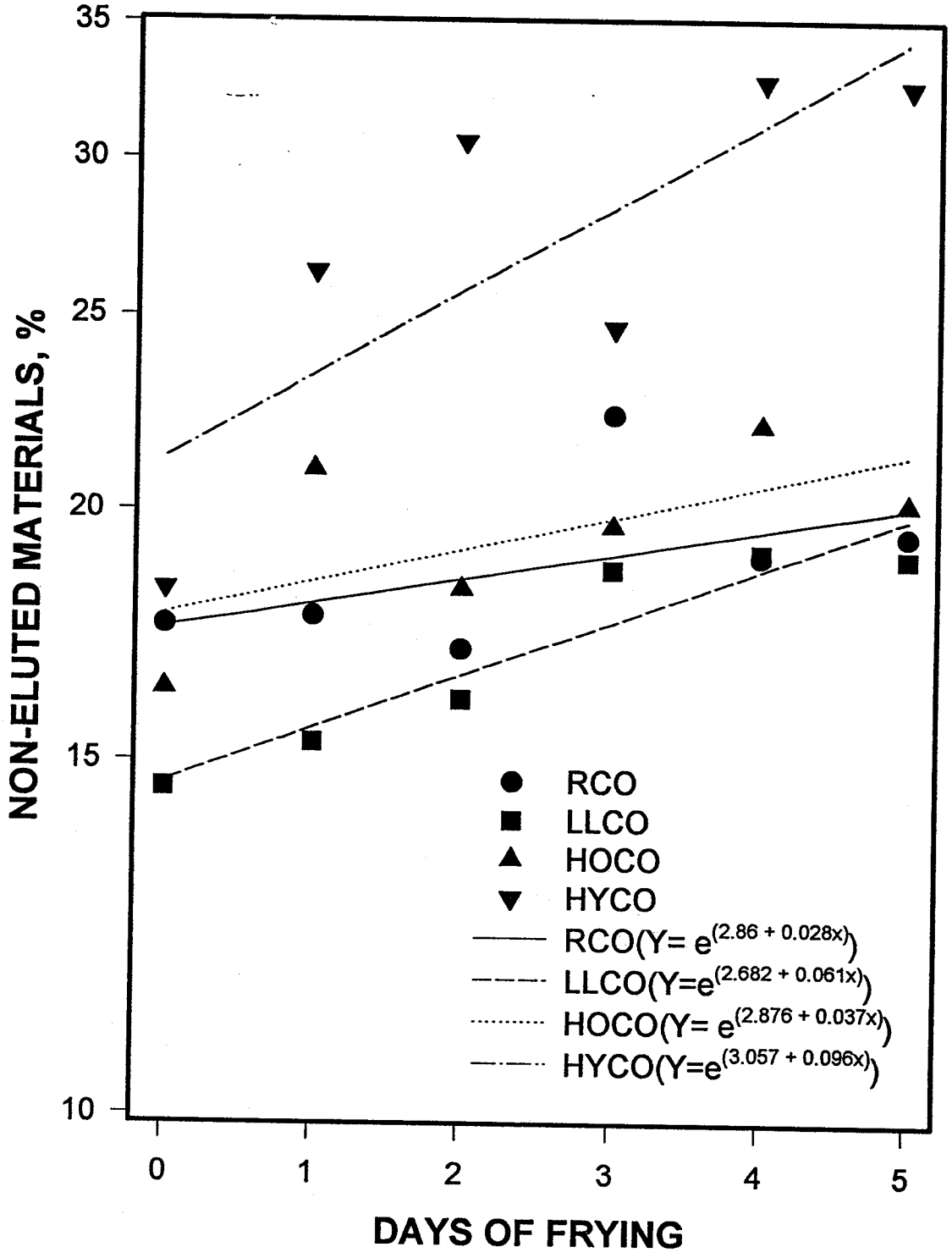


Figure 3.8. Polymers and Oxidized Materials (Noneluted Materials) in Canola Frying Oils.

revealed that HYCO and LLCO were significantly different from each other (t_{16}=-3.58, p=0.0025).

The results of gravimetric measurements of the amount of polars were statistically analyzed using ln transformation. The results of the analysis indicated that all oils showed increased accumulation of polars as frying days increased (Figure 3.9). There were no significant differences observed in the rates of accumulation of polar materials. The only differences observed were in the initial amounts of polars with a significant difference between RCO and the other three oils: HOCO (t_{16}=-3.22, p=0.0054), LLCO (t_{16}=-2.79, p=0.0132) and HYCO (t_{16}=-1.98, p=0.0653). As indicated earlier, HOCO had high levels of 18:3 positional isomers suggesting that deodorization took place at temperatures higher than 245°C (Smouse, 1995). At such high temperature some degradation of the oil could have taken place which may explain the high initial amount of polars present in the HOCO.

The data from polars measurement by TLC-FID was statistically analyzed using ln transformation. Overall, the amount of polars increased as the frying days increased for all oils (Figure 3.10). No significant differences were found in the rates of accumulation of polar components among the four oils. The initial amounts of polars for HOCO and HYCO were found to be significantly different from each other (t_{16}=-2.34, p=0.0323).

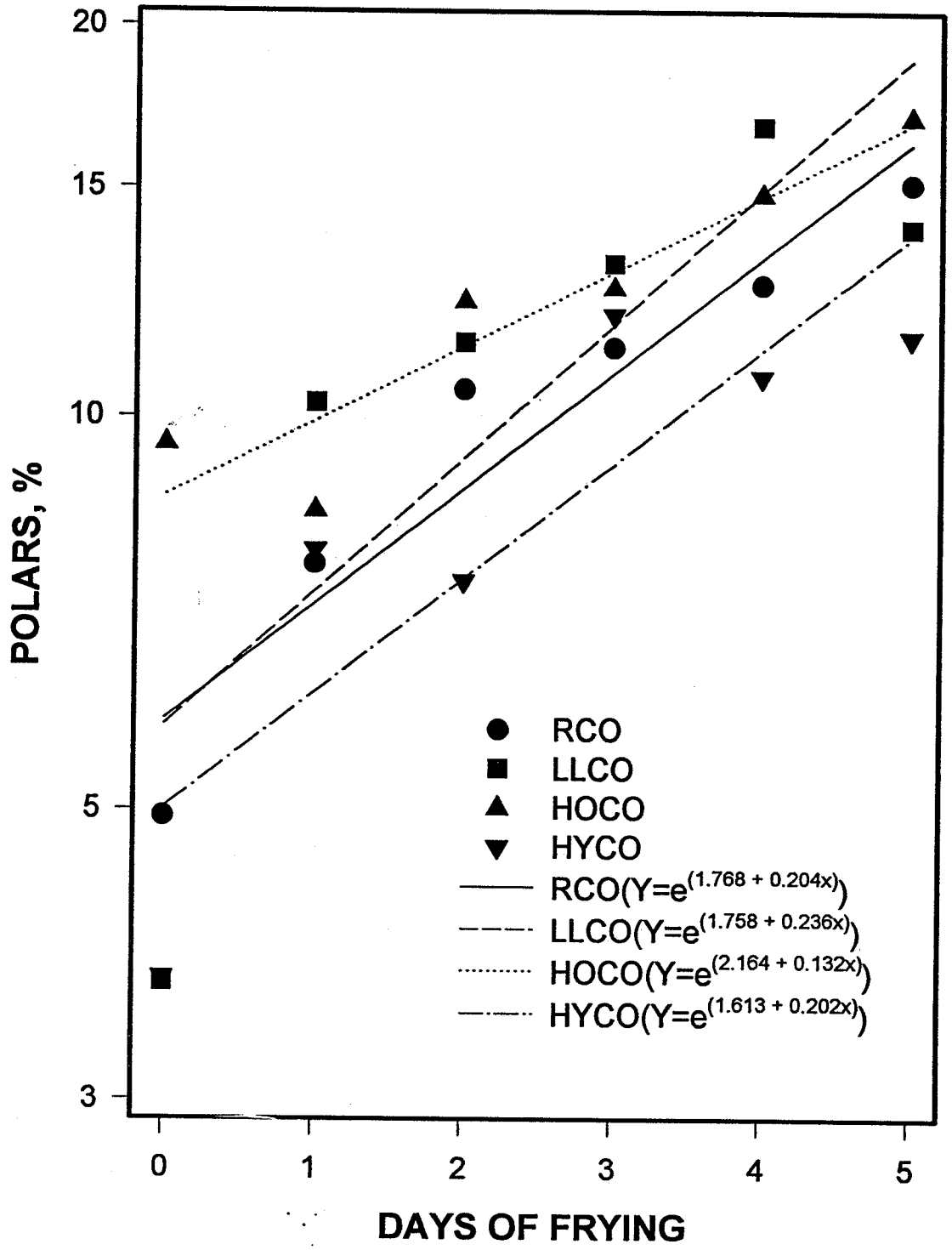


Figure 3.9. Polar Components (by gravimetric method) in Canola Frying Oils

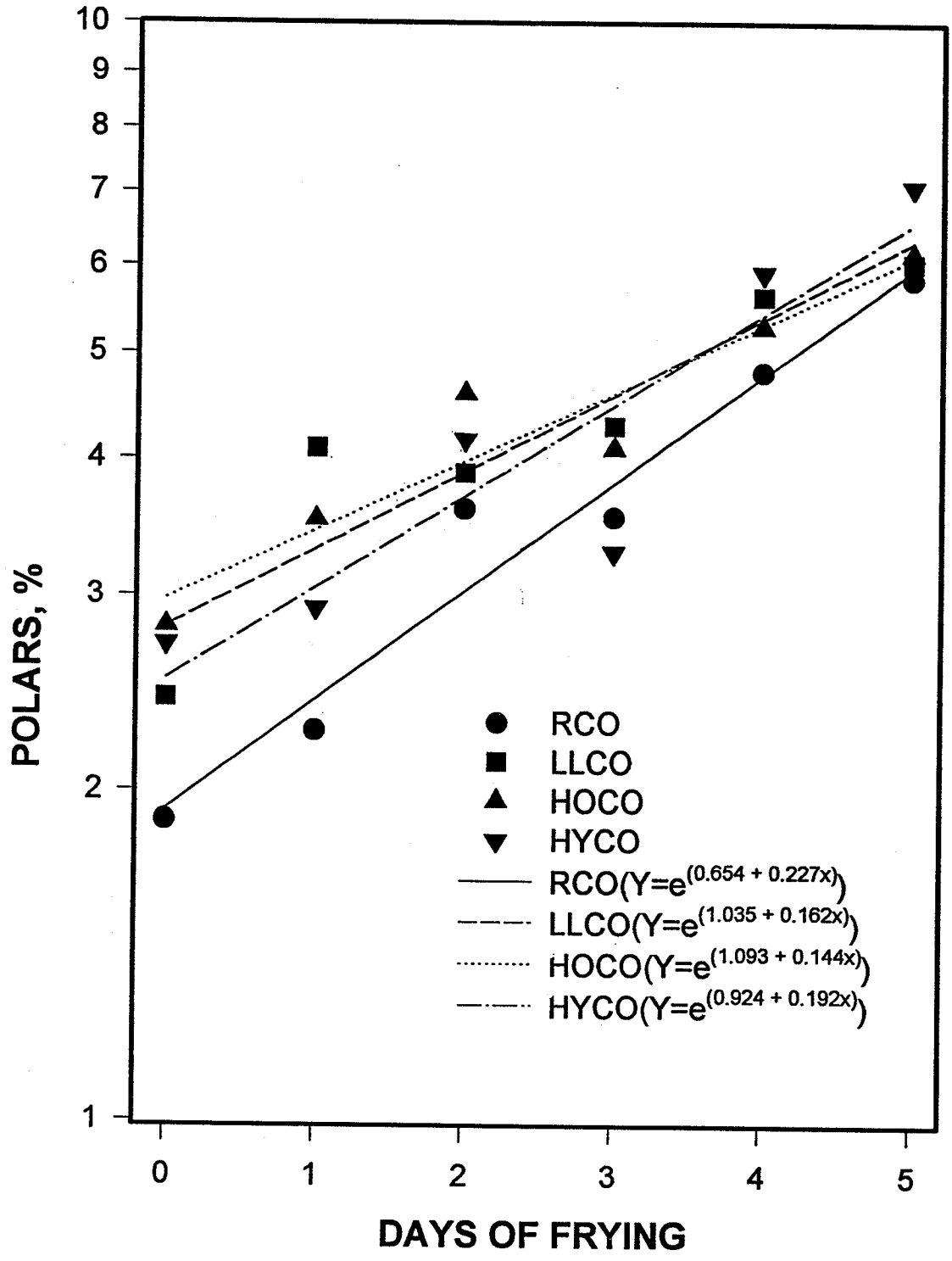


Figure 3.10. Polar Components (by TLC-FID) in Canola Frying Oils.

A correlation coefficient of 0.77 was found between TLC-FID and the gravimetric method, indicating that TLC-FID could be used with some confidence to determine the amount of polar components present in frying oils (Figure 3.11). This coefficient was smaller than the correlation reported for the same two methods by Sebedio *et al.* (1987), 0.77 versus 0.95. However, there was a difference in how the amount of polars were assessed in Sebedio's study and this study. Sebedio *et al.* (1987) calculated the amount of polars for both methods based on the amount of nonpolar fractions. In this study, the levels of polars in the TLC-FID method were calculated as a sum of FFA, diglycerides and highly polar compounds, thus taking into account differences in chemical structure between newly formed degradation products.

3.3.3 Summary of Results

In summary, when the results from the various chemical and instrumental tests were grouped for each oil the following was observed:

RCO

- intermediate rate of accumulation of FFA and high initial amounts of FFA which were significantly different from HOCO,
- high rate of CDA accumulation which was significantly different from both HYCO and HOCO; intermediate initial amounts of CDA which were significantly different from LLCO,

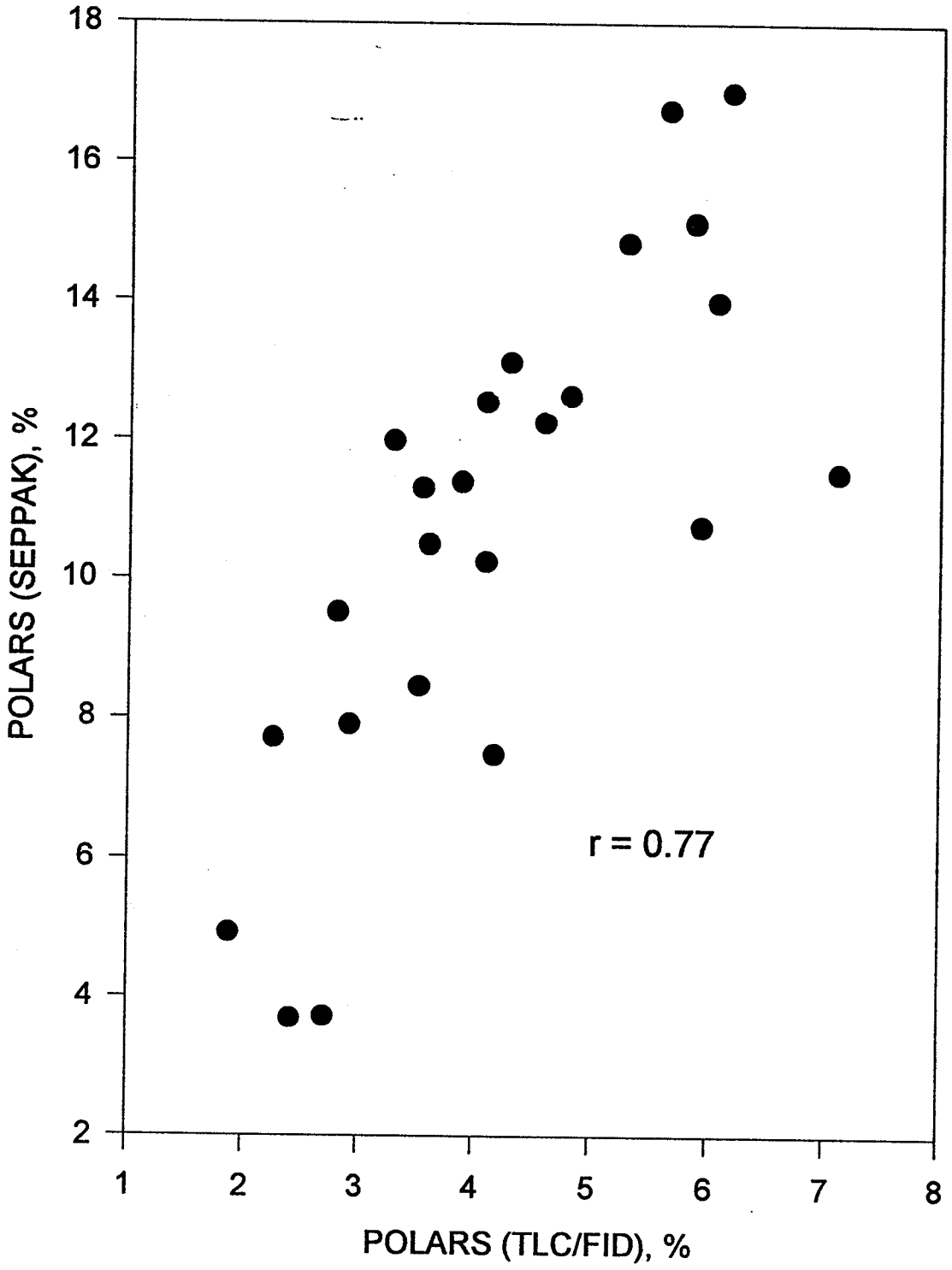


Figure 3.11. Relationship between Polar Components Determined Gravimetrically and by TLC-FID in Canola Frying Oils

- low rate of polymers accumulation and intermediate initial levels of polymers,
- intermediate to high rate of polars production and intermediate to low initial levels of polar components which were significantly different from HOCO.

HYCO

- high rate of FFA accumulation which was significantly different from LLCO; intermediate initial amounts of FFA,
- low rate of CDA production which was significantly different from all other oils; high initial amounts of CDA which were significantly different from LLCO,
- high rate of polymers accumulation and high initial amounts of polymers which were significantly different from LLCO,
- intermediate rates of polars accumulation and low to intermediate initial amounts of polar components.

LLCO

- significantly slower production of FFA than HYCO; intermediate initial amounts of FFA,
- significantly more rapid accumulation of CDA than HYCO and HOCO; significantly lower initial amounts of CDA compared to the other three oils,
- intermediate rate of accumulation of polymers and significantly lower initial levels of polymers than HYCO,
- intermediate to high rate of polar components production and intermediate initial amounts of polars.

HOCO

- intermediate rate of FFA accumulation and low initial amounts of FFA which were significantly different from RCO,
- intermediate rate of CDA accumulation which was significantly different from the other oils; intermediate initial levels of CDA which were significantly different from LLCO,

- intermediate rate of polymers production and intermediate initial amounts of polymers,

- low rate of polars production and high initial levels of polars which were significantly different from HYCO and RCO.

3.4. DISCUSSION AND CONCLUSIONS

The results from chemical and instrumental tests showed that there was no single oil that had consistently lower initial amounts or rates of accumulation of the degradation products. All oils demonstrated some degree of deterioration after 5 days of frying (total of 40 hours). The frying stability of both genetically modified canola oils showed a slight improvement over RCO based on the amounts of FFA, CDA and polars. HOCO and LLCO had lower rates of accumulation and initial amounts of FFA than RCO; HOCO had a significantly lower rate of CDA accumulation and LLCO had a significantly lower initial amount of CDA than RCO; HOCO had a significantly lower rate of polar components accumulation, although the initial amounts of polars was higher than in RCO. The rate of polymers accumulation in RCO in the present study was lower than in both HOCO and LLCO, but the initial amount was lower in HOCO. This observation contradicts the statement by Lumley (1988) that fats high in unsaturated fatty acids yield more polymers than fats low in unsaturated fatty acids.

Similar to the present study research by other workers has revealed no consistent trends in the frying performance of canola oils. Warner *et al.* (1994) found that RCO used for frying of potato chips over two 9 h periods, had significantly lower accumulation of FFA and intermediate amounts of polars compared to genetically modified and hydrogenated canola oils. HOCO had significantly higher accumulation of FFA and lower

amount of polars; HYCO had intermediate amounts of FFA and significantly higher amounts of polars; LLCO had amounts of FFA similar to that of HYCO, and the second highest accumulation of polars after HYCO. This study differs from the present study in that citric acid was added to all the oils prior to frying. Citric acid is a known metal chelating agent which could improve the oxidative stability of the oils (List and Erickson, 1980). Similar to the present study, fresh oil was added to replenish the frying oil.

Warner and Mounts (1993) found that RCO had higher amounts of FFA than LLCO after 45 hours of frying french fries. Accumulation of polars did not differ among the oils, although measurement of foam height (an indirect measure of polymers) singled out RCO as having the highest foaming after 20 hrs of frying and until the end of frying. Citric acid was also added to these oils prior to frying (Warner and Mounts, 1993).

Similarly, Mounts *et al.*(1994a) found that regular and low linolenic soybean oils did not differ significantly in the accumulation of FFA and polars during heating and frying of french fries (20 hrs). All oils had added citric acid and fresh oil was used to replenish the frying oil. Liu and White (1992) also compared two regular and three low linolenic soybean oils during heating and frying of bread cubes for 40 hrs. Although an increase in CDA was demonstrated by all oils, there was no significant differences in the accumulation of

CDA throughout the frying period. In this study, the oils were not topped up with fresh oil.

In contrast, Eskin *et al.* (1989) found that LLCO had significantly lower TBA and dienals than RCO after heating for 10 min without frying at 185°C. The amount of FFA and carbonyls were significantly lower in LLCO than in one of two RCO included in the study. Dobarganes *et al.* (1993) showed that high oleic sunflower oils had lower initial amounts and lower accumulation of polars than regular sunflower oil during frying of 15 batches of french fries over a period of 5 hours. No additives were added to the oils and the oils were not replenished with fresh oil during frying.

In the present study, there are several explanations as to why the observed differences were not as pronounced as was expected. The analysis of tocopherols, which are considered to have an antioxidant effect in the oils, revealed that RCO and HYCO had twice as much total tocopherols than LLCO and HOCO at the beginning of the frying period. After 5 days of frying, the total amount of tocopherols was reduced in both LLCO and RCO to two times their initial level, whereas the reduction in HOCO was 2.5 times the initial level, and for HYCO it was 16.5 times the initial level (Li, 1996).

Another factor which may have slowed down the degradation of the oils could be the use of an antifoaming agent. Antifoaming agents have been reported to exhibit a substantial antioxidant effect when added to soybean frying oils (Brekke,

1980). However, during preliminary frying the addition of an antifoaming agent was found to be necessary to prevent excessive foaming of the RCO.

Finally, the addition of fresh oil every morning to maintain the level of oil for frying likely slowed down the accumulation of degradation products (Aust and Thompson, 1983; Blumenthal, 1991). In the present study, 10-15% of fresh oil was added to the used oil every morning prior to frying.

CHAPTER 4

STORAGE STABILITY OF POTATO CHIPS FRIED IN REGULAR, HYDROGENATED, LOW LINOLENIC AND HIGH OLEIC CANOLA OILS

4.1 INTRODUCTION

The the most important factor in the storage stability of potato chips is their high fat content (30-40%), with the major defect being the development of oxidative rancidity (Mottur, 1989). Unsaturation of the oil is the major factor affecting the formation of rancidity (Robards *et al.*,1988). Canola oil contains high levels of linolenic acid (10-12%) which is responsible for the limited storage stability of snack foods fried in canola oil. Decreasing the 18:3 content by hydrogenation has been shown to reduce the development of rancid and painty odours/flavours in potato chips during 12 days of accelerated storage (Hawrysh, 1992). There was also a reduction in the accumulation of PV, CDA and total volatiles in the potato chips fried in HYCO compared to RCO. Melton *et al.* (1993) found that potato chips fried in hydrogenated canola oil and in blends of hydrogenated canola oil with cottonseed oil (85:15 and 70:30) and stored at 23°C for 4 weeks had similar stability to potato chips fried in 100% cottonseed oil as measured by PV.

More recently genetic modification has been used to reduce the 18:3 levels in soybean and canola oils. Studies have shown an improvement in the storage stability of bread cubes fried in modified soybean oil (Miller and White, 1988; Liu and White, 1992). Tortilla chips fried in low linolenic

canola oil had higher intensity of characteristic odour and lower intensities of off, rancid and painty odours after 16 days of storage at 60°C compared to chips fried in RCO (Hawrysh, 1993; Hawrysh *et al.*, 1995). The initial values for PV, CDA, FFA and total volatiles were similar for chips fried in LLCO and RCO, but after 16 days of storage the chips fried in RCO had significantly higher values compared to LLCO chips. Warner *et al.* (1994) evaluated the stability of potato chips fried in RCO, HYCO, LLCO and HOCO after four months of storage at 25°C. Chips fried in RCO had the lowest flavour quality scores (10=excellent quality, 1=bad quality) after storage compared to all other chips. Chips fried in HOCO and LLCO had higher flavour quality scores compared to HYCO chips. Similarly, RCO chips had significantly higher amounts of total volatiles than chips fried in other three oils, and HOCO and LLCO chips had lower amounts of total volatiles than HYCO chips.

The presence of frying oil degradation products in fried foods has been demonstrated by some workers to have a prooxidative effect on the storage stability of snack foods (Asap and Augustin, 1986; Yoon *et al.*, 1988). However, there are no reports on the effect of frying oil degradation products on storage stability of foods fried in genetically modified oils.

Thus, there is limited information on the storage stability of foods fried in genetically modified canola oils,

and there is no information on the effects of frying oil degradation products on the storage stability of fried foods. Therefore, the objectives of this study were:

1. To compare the storage stability of potato chips fried in genetically modified canola oils with chips fried in regular and hydrogenated canola oils.
2. To determine if the level of oil degradation products caused by prolonged frying influences the storage stability of potato chips and to investigate whether this differed among the four oils.

4.2. MATERIALS AND METHODS

4.2.1 Packaging of Potato Chips

Potato chips from one day of frying representing 32 batches were packaged in commercial potato chip packaging which was comprised of a plastic laminate with a foil lining. Strips of the packaging film, 10cm x 25cm were formed into a bag and sealed using a heat sealer. 4.5±0.2 g of potato chips were placed into each bag and the top sealed. 130 bags were prepared from each day of frying for a total of 650 bags (130 x 5 frying days). Each bag from one day of frying was given a number from 1 to 130.

4.2.2 Storage Protocol

The packaged chips were stored under accelerated conditions to evaluate their storage stability. All bags from one day of frying were put in a box and placed into a storage cabinet with a controlled temperature of 60±2°C. The numbered bags for a specific frying day were removed from the storage cabinet in the order determined by randomization of numbers from 1 to 130. Twenty six bags were removed at each storage interval (days 1, 2, 4, 8 and 16), and kept at -25°C until required for training or analysis (approx. 2 months). Twenty six bags, without accelerated storage representing day 0 from day one of frying were also kept at -25°C until required.

4.2.3 Sensory Evaluation

4.2.3.1 Selection and Training of Odour Profile Panel

The use of sensory panel to evaluate the odour of potato chips was approved by the Faculty of Human Ecology Ethics Review Committee (Appendix 2). Panelists were recruited by a letter of invitation (Appendix 3) sent to staff and graduate students in the Human Ecology Faculty. One hundred and fifteen letters were sent out and eighteen people agreed to participate. Two screening sessions were then scheduled on two separate days. Samples for screening were: chips fried in regular canola oil and stored for 1, 2, 4 and 8 days. Panelists were asked to rank the samples according to the intensity of painty odour. A painty reference sample (storage day 16) was provided to assist panelists in their evaluation. The criteria used for selecting the panelists for further training was based on their ability to rank the samples in order of intensity and to duplicate their judgements, and their availability for future sessions. Ten panelists (6 females and 4 males) were selected after screening to continue with training.

For the training program, one orientation session was held to familiarize panelists with the use of 15 cm unstructured line scales (Appendix 4) and the technique for handling and evaluating the potato chips. This technique involved cutting the bag just below the seal with the scissors provided; pushing the sides of the bag together at the bottom,

so that the bag opened at the top. Panelists were then asked to take three short sniffs, and close the bag by rolling down the top of the bag and securing with a paper clip. They then recorded their odour judgements on the line scales.

In total, twenty six, half-hour training sessions took place over a period of seven weeks as follows: nine sessions for hydrogenated potato chips, 4 sessions each for low linolenic and regular potato chips, and 9 sessions for high oleic potato chips. During the first one or two sessions for each oil, panelists were asked to describe the odour attributes present in the samples provided. Once they agreed on the terminology to characterize the samples, subsequent sessions were held to rate the samples for their intensities using unstructured line scales. During training, it was determined that panelists were not able to evaluate the odour of potato chips fried in the four oils using common attributes nor reference samples. As a result, different odour parameters were used as follows: painty for RCO chips; stale/musty for HYCO chips; fresh potato chip/frying oil and painty/rancid for LLCO chips; fresh frying oil/potato chip and painty for HOCO chips. Line scales for all attributes were labelled from "none" to "intense odour". Reference samples were provided for each attribute and panelists agreed on where to anchor them on the line scales. The reference used for painty odour in regular potato chips was a sample of chips fried in RCO from frying day 3 stored for 16 days (R2). For stale/musty odour in

hydrogenated potato chips was a sample of chips fried in HYCO from frying day 1 stored for 20 days (R2) was used. References for fresh potato chip/frying oil (R1) and painty/rancid (R2) odours in low linolenic potato chips were samples of chips fried in LLCO from frying day 1 stored for 0 and 20 days respectively. Fresh frying oil/potato chip (R1) and painty (R2) references for high oleic potato chips were samples of chips fried in HOCO from frying day 1 and stored for 0 and 20 days respectively. The references were placed on the line scales as follows: hydrogenated potato chips R2 = 13, regular potato chips R2 = 15, low linolenic potato chips R1 = 15 and R2 = 13, high oleic potato chips R1 = 15 and R2 = 13. An example of the final ballot used for odour evaluation of high oleic potato chips is provided in Figure 4.1. Throughout the training period the panelists were encouraged to discuss any difficulties they encountered with the samples, references or evaluation techniques. The training sessions were also aimed at improving the consistency of the panelists' scores and building their confidence in evaluating the samples.

4.2.3.2 Test Sessions

Only potato chips from frying day 1 and day 5 were evaluated by the sensory panel. Test sessions were held in the computerized sensory facility of the George Weston Ltd. Sensory and Food Research Centre located in the Department of Foods and Nutrition, University of Manitoba. Evaluations were

BALLOT FOR ODOUR EVALUATION

POTATO CHIPS

Name _____
Date _____

You are presented with potato chips in bags coded with 3 - digit numbers and reference samples marked as R 1 and R 2.

To evaluate the odour cut the bag just below the seal with the scissors provided. Push the sides of the bag together at the bottom so that the bag will open at the top. Take three short sniffs. Roll the opening down and secure it with a paper clip.

First smell the reference **R 1** (Fresh frying oil/potato chip odour) then **R 2** (Painty odour) noting the presence and intensity of those odour attributes.

Evaluate both odour attributes in the samples in the order indicated in the ballot. Rate the intensity of the odour attribute by placing a slash across the line at the point which best describes that sample. Go to the next sample.

Do **not** go back to the reference samples until **all** coded samples are evaluated.

Sample code _____

FRESH FRYING OIL/POTATO CHIP ODOUR

Ref *R 1*

↓ _____ ↓

none — intense FFO/ PC odour

PAINTY ODOUR

Ref *R 2*

↓ _____ ↓

none — intense painty odour

Sample code _____

FRESH FRYING OIL/POTATO CHIP ODOUR

Ref *R 1*

↓ _____ ↓

none — intense FFO/ PC odour

PAINTY ODOUR

Ref *R 2*

↓ _____ ↓

none — intense painty

Figure 4.1. Ballot for Odour Evaluation.

made in individual booths under red lights to mask any possible colour differences among the samples. Each panelist was provided with a set of reference samples, scissors, paper clips, a blank sheet for comments, and a pencil. The CSA software (Compusense Inc., Guelph, ON) was used to record the panelists' ratings of the samples directly on the computer.

Samples of potato chips fried on day 1 and day 5 of frying were evaluated for one oil on each testing day. One set of six samples representing storage days 0, 1, 2, 4, 8 and 16 from frying day one was evaluated first, followed by a five minute break after which a second set of five samples representing storage days 1, 2, 4, 8 and 16 from frying day five was tested. Two replications were completed on two consecutive days. The sample sets were presented in the reverse order (frying day 5 followed by frying day 1) in the second replication. All judges evaluated the same set of samples at a given session, but the sample order within the set was randomized for each panelist. Eight sessions were required in total (4 frying oils x 2 replications).

4.2.4 Odour Component Analysis

The purge-and-trap method used to assess odour volatiles was based on the procedure described by Przybylski (1991). A Perkin-Elmer 8500 gas chromatograph (GC) (Norwalk, CT) with a built-in integrator was used. The column consisted of two parts: a packed pre-column to trap the volatiles, which was

connected to the capillary column to separate the volatile components. The pre-column was packed with bonded packing CSP-20M (Chromatographic Specialties Ltd., Brockville, Ont.) and shaped into a coil to facilitate cooling with liquid nitrogen. Fused silica capillary column (60m x 0.32mm i.d.) with 1 μm of DB-5 was purchased from J & W Scientific (Folsom,CA). The injector and flame ionization detector temperature was 125°C and 250°C respectively. The column temperature was programmed from 45°C to 85°C at a rate 2°C/min, then 85°C to 125°C at 3°C/min and finally from 125°C to 235°C at 4°C/min. Initial and final temperatures were held for 2 and 10 min, respectively. Total length of one run was 73.03 min. An internal standard tridodecane in fresh canola oil was run along with the sample to enable the quantification of individual volatiles.

The glass insert tube with an opening on the side close to one end was used to hold the sample in the injector. A glass wool plug was inserted into the tube 15 mm opposite to the side opening. The chips were crushed in a mortar, were placed into the tube and protected with a second glass wool plug. The weight of the sample was in the range of 0.2-0.3g measured with an accuracy of 0.01g.

Before adding the internal standard, the pre-column was prepared for purging. The container was placed under the coil of the pre-column, liquid nitrogen was poured into the container, the valve with purging gas was opened. After this

preparation was completed a 5 μL of internal standard, 187.175 ng of dodecane per sample, was applied onto the glass wool plug at the end opposite the side opening. After the tube with the sample was placed in the injector it was closed immediately. Purging continued for 15 min, and liquid nitrogen was topped up as required. After 15 min had elapsed, the tube with the sample was replaced with a blank one, the pressure in the system was allowed to normalize, liquid nitrogen removed, and the run started.

A reference sample with standard components of interest was run along with the internal standard to enable the identification of the individual components in the experimental samples. This sample was run every day to determine if there was a shift in retention time.

4.2.5 Lipid Extraction from Potato Chips

The lipid extraction from potato chips was based on the method of Folch *et al.* (1957). Crushed chips (about 8-9 g) were measured with 0.01 g accuracy into 100 mL beaker and 50 mL of chloroform/methanol mixture (2:1 v/v) was added. The mixture of chips and solvent was homogenized in a POLYTRON homogeniser (Kinematica, GMBH, Lucerne - SCHWEIZ) for 30 sec at speed 4 and the upper layer carefully transferred into a separatory funnel. Another 50 mL of chloroform/methanol mixture was added to the residue and homogenized again. The content of the beaker was transferred into the same separatory

funnel. The crude extract was mixed with 0.2 of its volume of glass distilled water (20 mL). The mixture was gently shaken and was let stand overnight to separate the mixture into two phases. The lower layer from the separatory funnel was transferred into the a pre-weighted round bottomed flask and the extract was evaporated to dryness in a rotary vacuum evaporator. One to two mL of isopropanol or benzene was added at the end of evaporation and the solvent was again evaporated to ensure that there was no water in the sample. The extracted oil was then transferred with a pipette into a 5 mL vial, flushed with nitrogen and stored at -25°C until required for analysis (approx. 2 months).

4.2.6 Chemical and Instrumental Analyses of Extracted Lipids

The extracted oil was analyzed for PV using colorimetric method. The levels of FFA, CDA, polar components by TLC-FID, were measured using the methods described in Chapter 3.

Peroxide values were determined using a colorimetric method based on the conversion of Fe^{2+} into Fe^{3+} by hydroperoxides present in the solution. The resulting ferric ion forms a purple coloured complex with xylenol orange which has an absorbance maximum at 560 nm.

$$Fe^{2+} + ROOH \longrightarrow Fe^{3+} + RO\cdot + OH^{-}$$

$$Fe^{3+} + \text{Xylenol Orange} \longrightarrow \text{Purple Complex}$$

The FOX 2 reagent was prepared according to Nourooz-Zadeh *et al.*(1995), but the amounts of xylenol orange and ammonium ferrous sulfate were increased five times. The procedure for FOX 2 reagent preparation and PV analysis used is provided in Appendix 5. The amount of Fe^{3+} in the oil samples was determined using the absorbance reading and the calibration curve provided in Figure 4.2. The procedure for obtaining the calibration curve is provided in Appendix 6.

The Official AOCS method for peroxide value determinations measures the peroxides by oxidizing the potassium iodide (KI) to free iodine, while the colorimetric method measures by oxidizing the ferrous ion into the ferric ion. Thus, some differences between these methods in absolute values could be expected. Therefore, PV's were obtained for a set of samples using both tests to determine if the two tests could be used interchangeably. Oxidized low linolenic canola oil with a titrated PV $\approx$ 100 was mixed with fresh low linolenic canola oil with a titrated PV $\approx$ 1.0 in different proportions, to cover a range of PV's from 0 to 100 with close attention paid to PV's in the low end of the range, i.e. between 0 and 10 (Table 4.1). The mean results of duplicate analyses for both tests were plotted against each other and

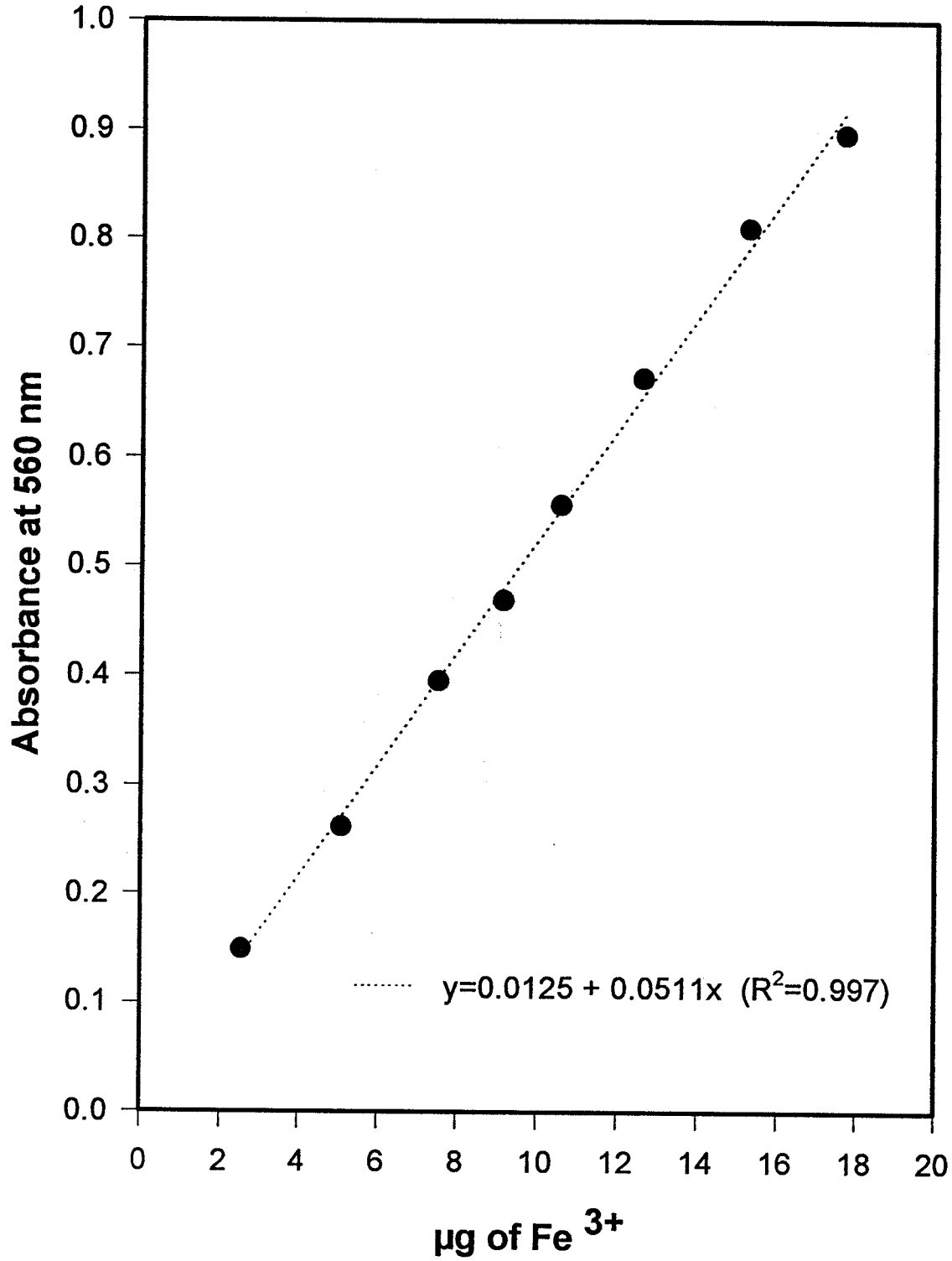


Figure 4.2. Calibration Curve for Fe^{3+} Determination for Colorimetric Peroxide Value Measurements.

Table 4.1. Samples for comparison between titrated and colorimetric PV

Sample	Mixtures	Expected PV
# 1	100% "O"[1]	100
# 2	90% "O" + 10% "F"[2]	90
# 3	80% "O" + 20% "F"	80
# 4	70% "O" + 30% "F"	70
# 5	60% "O" + 40% "F"	60
# 6	50% "O" + 50% "F"	50
# 7	40% "O" + 60% "F"	40
# 8	30% "O" + 70% "F"	30
# 9	20% "O" + 30% "F"	20
# 10	10% "O" + 90% "F"	10
# 11	7.5% "O" + 92.5% "F"	7.5
# 12	5% "O" + 95% "F"	5.0
# 13	2.5% "O" + 97.5% "F"	2.5
# 14	100% "F"	1.0

[1] "O" - Oxidized low linolenic oil,
[2] "F" - Fresh low linolenic oil.

the correlation between the two tests determined using PROC CORR (SAS, 1988).

4.2.7 Statistical analyses

4.2.7.1 Sensory evaluation

The analysis of the sensory data was carried out using a mixed model which permits specification of both random and fixed effects. Although analysis of variance (ANOVA) has a means of designating random effects, the procedure PROC GLM treats the effects as fixed (SAS, 1992; L.Armstrong, personal communication). The procedure PROC MIXED (SAS, 1992), a recent introduction, allows the random effects to be treated as such during the analysis. The estimates of variance used in the analysis of the mixed model, REML (restricted maximum likelihood) and ML (maximum likelihood), are preferred over ANOVA's least square estimates since the mixed linear model estimates have 'built-in optimality properties' (Searle, 1988).

The analyses were done for each oils separately because the sensory parameters used to evaluate the oils were different for each oil. Thus, only the effect of storage day and frying day within an oil could be determined.

The steps for analysis were as follows:

1) a full model was specified followed by a stepwise reduction of the model. The reduced model was tested for any significant loss of information using a likelihood ratio test statistic -

G^2 - which has a χ^2 distribution. A difference between full and reduced model was considered significant at $p \leq 0.05$.

2) the reduced model was also tested to determine if it was a significant improvement over the null model, which included only the error term.

3) after the reduced model was fitted, the fixed effects were tested for significance using the F-statistic with $\alpha=0.05$.

4.2.7.2 Chemical and Instrumental Tests

Chemical and instrumental data was analyzed by the analysis of covariance with days of storage treated as the covariate or continuous variable using the PROC GLM procedure (SAS, 1988). See Chapter 3 for a more detailed discussion of this analysis. The model used included the following terms: oil, storageday, storageday*oil, storageday*oil*fryday, fryday, oil*fryday, storageday*fryday.

4.3. RESULTS

4.3.1 Sensory Evaluation of Potato Chip Odour.

The first step in analysis of the sensory data involved the fitting of a reduced model. The results of these analyses are provided in the Table 4.2.

The reduced model for analysis of painty odour in potato chips fried in RCO included the panelist term. The mixed model was reduced without any significant loss of information according to the test statistic LRT G^2 which was equal to $-2REML_{(Reduced)} - (-2REML_{(Full)})$, which in turn was equal to: 714.1524-714.1467= 0.0057, with 11 df. This had a p-value > 0.9999, and was therefore not considered significant. Thus, our conclusion was that the reduced model does not result in a loss of information.

The second step in the analysis involved testing the reduced model to see if it was a significant improvement over the null model which contains only the error term. This was determined using the test statistic LRT $G^2 = -2REML_{(Null)} - (-2REML_{(Reduced)})$. A value of 10.814 with 1 df was obtained with p-value = 0.0010, which was significant. Thus, the reduced model was an improvement over the null model.

The final step in the analysis involved determining whether or not the fixed effects were significant. The results of these analyses are presented in Table 4.3. Only storage day had a significant effect on painty odour intensity of chips

Table 4.2. Fitting of Mixed Model for Sensory Evaluation Results of Canola Potato Chips Odour

Oil	Sensory Attribute	Model	df of LRT [1]	-2 REML [2]	LRT G^2	p-value
RCO	Painty	FULL [3] REDUCED (**Panelist**) NULL [4]	 11 1	714.1467 714.1524	 0.0057 10.814	 >0.9999 0.0010
HYCO	Stale/Musty	FULL [3] REDUCED (**Panelist, Storeday*Panelist**) NULL [4]	 8 2	1248.809 1249.789	 0.98 5.5802	 0.9984 0.0614
LLCO	Fresh Potato Chip/Frying Oil	FULL [3] REDUCED (**Panelist**) NULL [4]	 11 1	1210.514 1212.319	 1.805 13.3626	 0.9991 0.0003
	Painty/Rancid	FULL [3] REDUCED (**Panelist, Storeday*Panelist**) NULL [4]	 3 2	1096.715 1097.423	 0.708 26.009	 0.8713 <0.0001
HOCO	Fresh Frying Oil/ Potato chip	FULL [3] REDUCED (**Panelist**) NULL [4]	 10 1	1110.231 1110.233	 0.002 6.304	 >0.9999 0.0121
	Painty	FULL [3] REDUCED (**Panelist, Storeday*Panelist*Rep**) NULL [4]	 10 2	990.3695 1007.800	 17.4305 14.2377	 0.6536 0.0008

Table 4.3 (cont.)

[1] - LRT = Likelihood Ratio Test,

[2] - REML = Restricted Maximized Likelihood,

[3] - FULL Model includes the following terms: Panelist, Rep, Panelist*Rep, Storageday*Panelist, Storageday*Rep, Storageday*Panelist*Rep, Panelist*Fryday, Rep*Fryday, Panelist*Rep*Fryday, Storageday*Panelist*Fryday, Storageday*Rep*Fryday, Storageday*Panelist*Rep*Fryday and Error,

[4] - NULL Model includes only Error term.

Table 4.3. Results of Testing Fixed Effects for Sensory Data of Potato Chips.

Oil	Sensory Attribute	Effect	Numerator df	Denominator df	F	p-value
RCO	Painty	Storeday	1	207	576.12	<0.00001
		Fryday	1	207	0.51	0.4766
		Storeday*Fryday	1	207	0.08	0.7826
HYCO	Stale/Musty	Storeday	1	9	51.53	0.0001
		Fryday	1	198	4.40	0.0372
		Storeday*Fryday	1	198	5.88	0.0162
LLCO	Fresh Potato chip/ Frying Oil	Storeday	1	207	209.06	<0.00001
		Fryday	1	207	0.65	0.4199
		Storeday*Fryday	1	207	0.71	0.3995
	Painty/Rancid	Storeday	1	9	161.43	<0.00001
		Fryday	1	198	0.00	0.9590
		Storeday*Fryday	1	198	1.10	0.2952
HOCO	Fresh Frying Oil/ Potato Chip	Storeday	1	186	104.53	<0.00001
		Fryday	1	186	0.01	0.9341
		Storeday*Fryday	1	186	0.07	0.7987
	Painty	Storeday	1	17	118.87	<0.00001
		Fryday	1	169	0.31	0.5814
		Storeday*Fryday	1	169	0.01	0.9143

fried in RCO, the intensity increased as storage time increased. No significant difference was found between frying days nor was there a significant interaction between storage day and frying day. The mean sensory scores for painty odour for frying day 1 and 5 are presented in Figure 4.3 along with the fitted lines for this data. These results verify the statistical analysis, i.e. painty scores increase with storage interval and no difference could be perceived between frying day 1 and 5.

For potato chips fried in HYCO the reduced model for stale/musty odour contained panelist and storageday*panelist terms (Table 4.2). The LRT G^2 = 0.98 (df 8) with p=0.9984 indicated that there was no significant loss of information for the reduced model. The reduced model was also a significant improvement over the null model (LRT G^2 = 5.5802 (df=2), p=0.0614) (Table 4.2). The test of the fixed effects showed that storage day, frying day and storageday*fryday were significant (Table 4.3). For both frying days, the intensity of stale/musty odour increased with increased storage and the rate of accumulation of the odour was greater for frying day 5 than for frying day 1 (Figure 4.4).

The reduced model for analysis of fresh potato chip/frying oil odour in chips fried in LLCO included panelist term (Table 4.2). There was no significant loss of information (LRT G^2 = 1.805, df=11, p=0.9991) and the reduced model was a

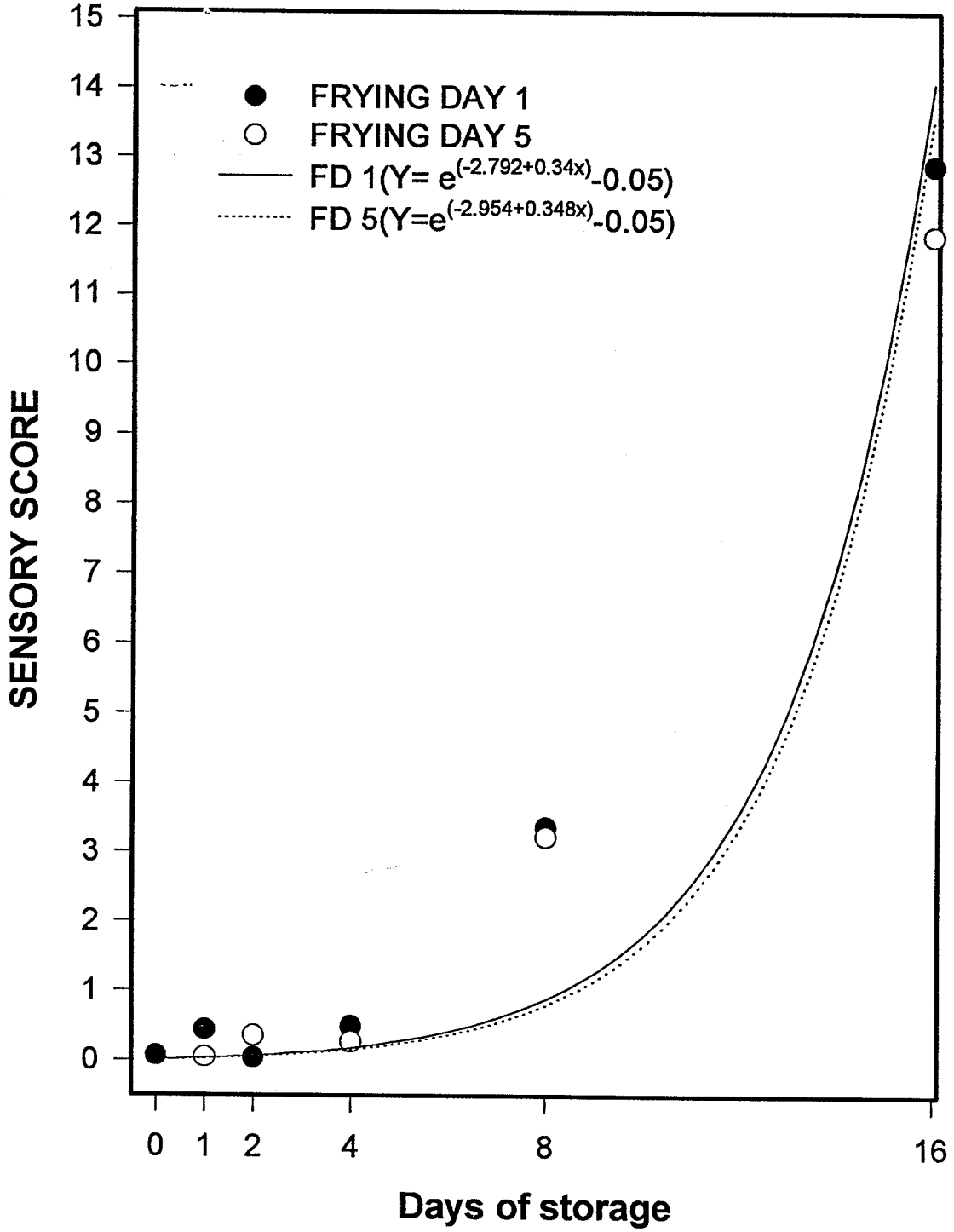


Figure 4.3. Painty Odour in Potato Chips Fried in Regular Canola Oil.

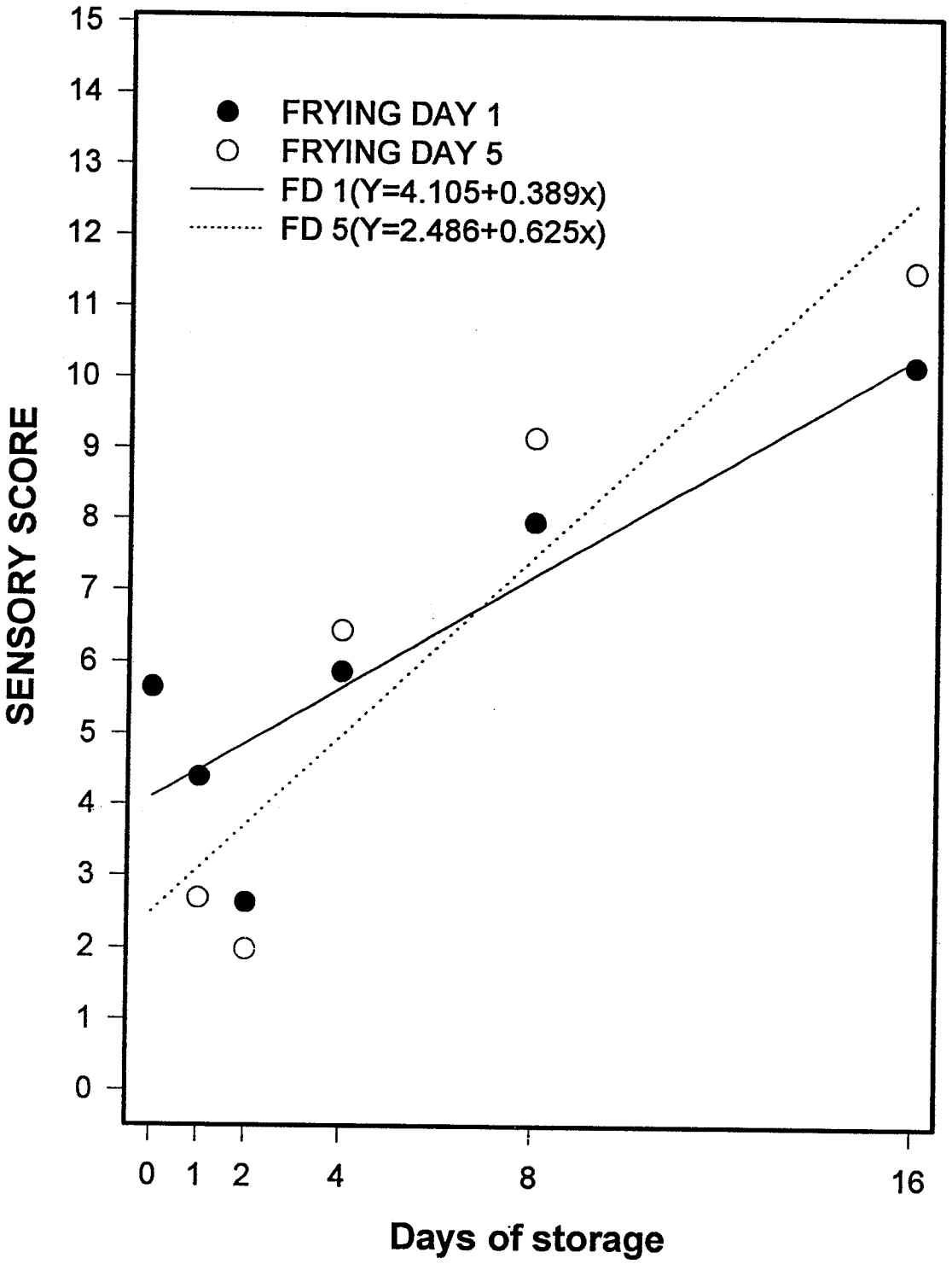


Figure 4.4. Stale/Musty Odour in Potato Chips Fried in Hydrogenated Canola Oil.

significant improvement over the null model (LRT G^2 = 13.3626, df=1, p=0.0003). The test of the fixed effects indicated that only storage day had a significant effect on the intensity of the fresh potato chip/frying oil odour (Table 4.3), and it decreased as storage increased (Figure 4.5). There was no difference between the rate of reduction in odour intensity between day 1 and day 5 of frying (Table 4.3 and Figure 4.5).

The reduced model for analysis of painty/rancid odour in chips fried in LLCO included panelist and storageday*panelist terms (Table 4.2). There was no significant loss of information after the mixed model was reduced (LRT G^2 = 0.708 (df=3), p=0.8713) and this reduced model was also a significant improvement over the null model (LRT G^2 = 26.009 (df=2), p<0.0001). The test of the fixed effects indicated that only storage day had a significant effect on the intensity of the painty/rancid odour (Table 4.3). The intensity of the odour increased with increased storage time (Figure 4.6). There was no difference in the rate of increase in painty/rancid odour intensity between day 1 and day 5 of frying (Table 4.3 and Figure 4.6).

The reduced model for analysis of fresh frying oil/potato chip odour of chips fried in HOCO included only panelist term (Table 4.2). There was no significant loss of information (LRT G^2 = 0.002 (df=10), p>0.9999) (Table 4.2) and the reduced model was a significant improvement over the null model (LRT G^2 =6.304 (df=1), p=0.0121). The test of the fixed effects

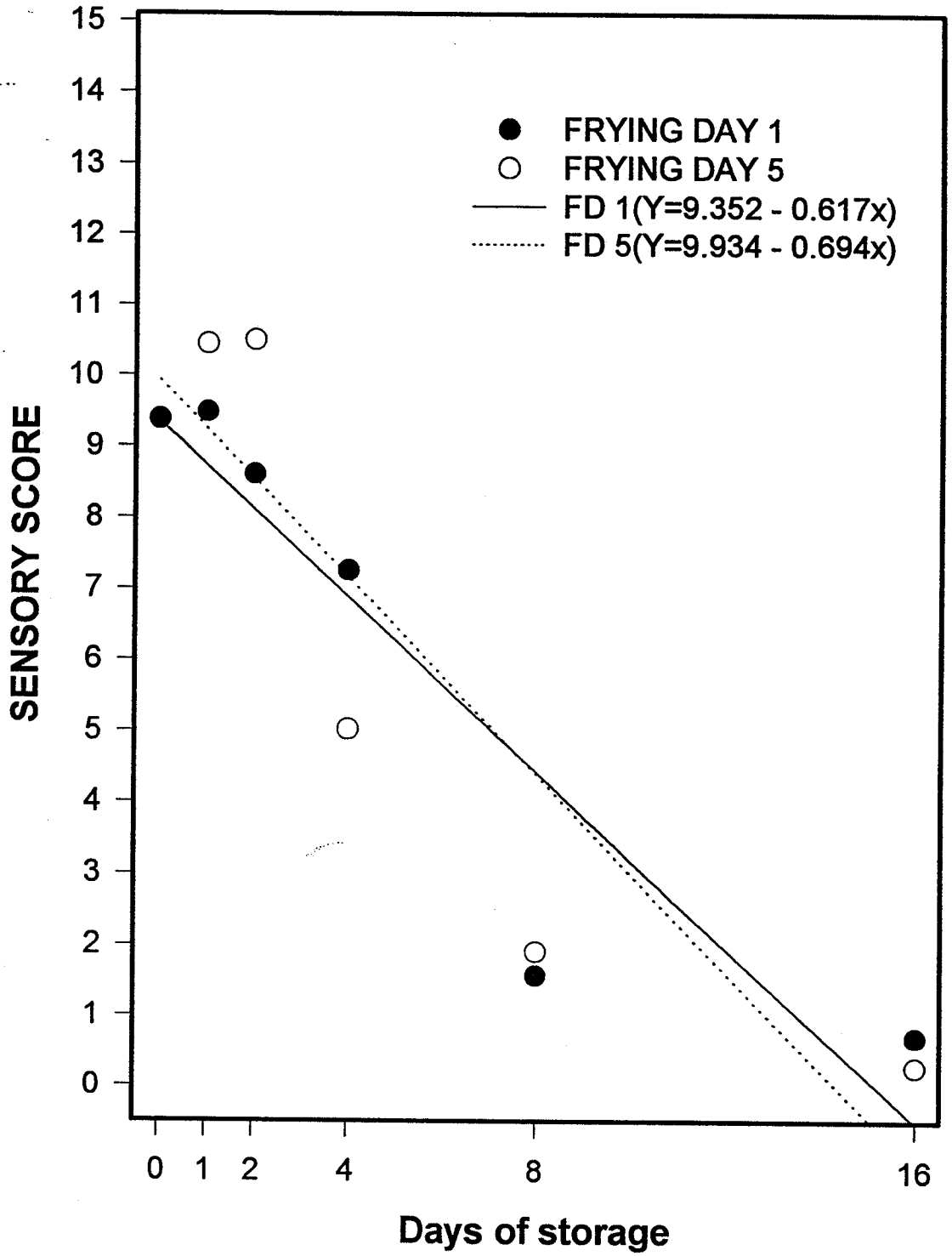


Figure 4.5. Fresh Potato Chip/Frying Oil Odour in Potato Chips Fried in Low Linolenic Canola Oil.

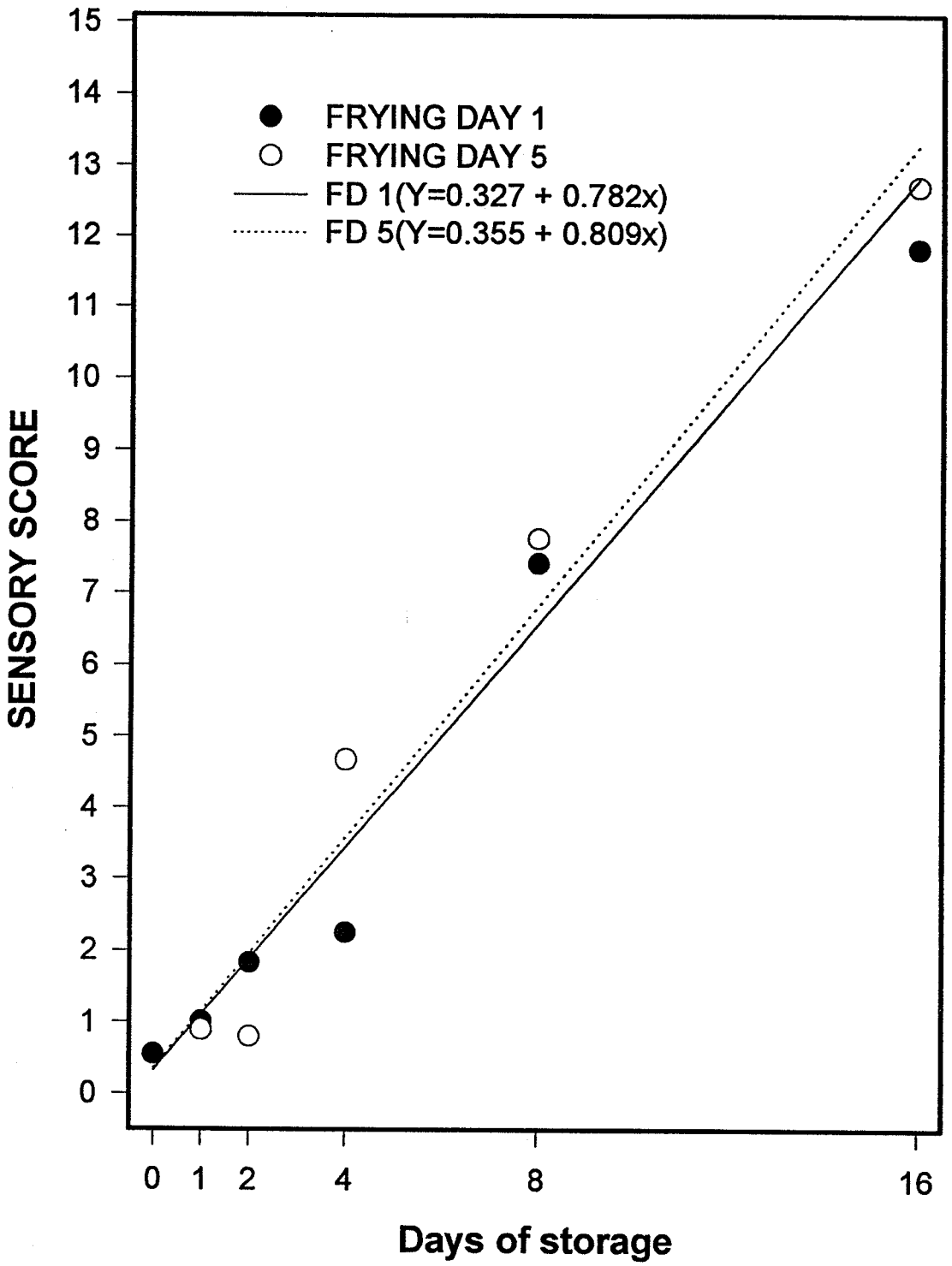


Figure 4.6. Painty/Rancid Odour in Potato Chips Fried in Low Linolenic Canola oil.

showed that only storage day had a significant effect on the fresh frying oil/potato chip odour (Table 4.3), and it decreased as the storage increased (Figure 4.7). There was no significant difference in the rate of reduction in odour intensity between frying day 1 and frying day 5 (Table 4.3 and Figure 4.7).

The reduced model for analysis of painty odour in potato chips fried in HOCO included panelist and storageday*panelist*rep terms (Table 4.2). There was no significant loss of information (LRT G^2 = 17.4305 (df=10), p=0.6536) and the reduced model was a significant improvement over the null model (LRT G^2 = 14.2377 (df=2), p=0.0008). The tests of the fixed effects indicated that only storage day had a significant effect on painty odour intensity (Table 4.3), and it increased as storage increased (Figure 4.8). There was no difference in the rate of odour accumulation between day 1 and day 5 of frying (Table 4.3 and Figure 4.8).

4.3.2 Chemical and Instrumental Results

The relationship between the two methods used to measure PV, AOCS titration method and the colorimetric method, is illustrated in Figure 4.9 with a correlation coefficient of 0.95. Thus, the colorimetric method can be used with confidence to measure the accumulation of peroxides in oil.

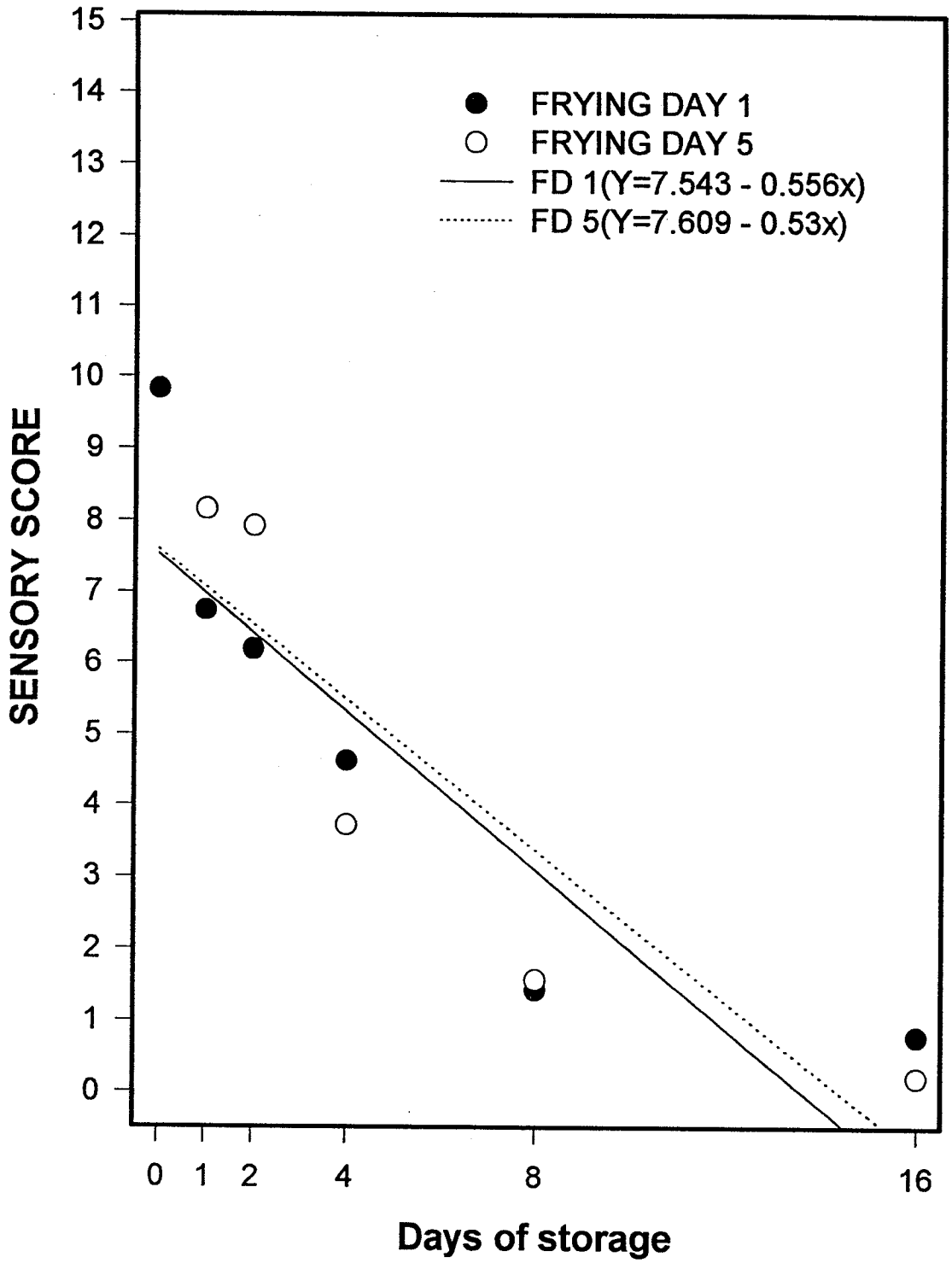


Figure 4.7. Fresh Frying Oil/Potato Chip Odour in Potato Chips Fried in High Oleic Canola Oil.

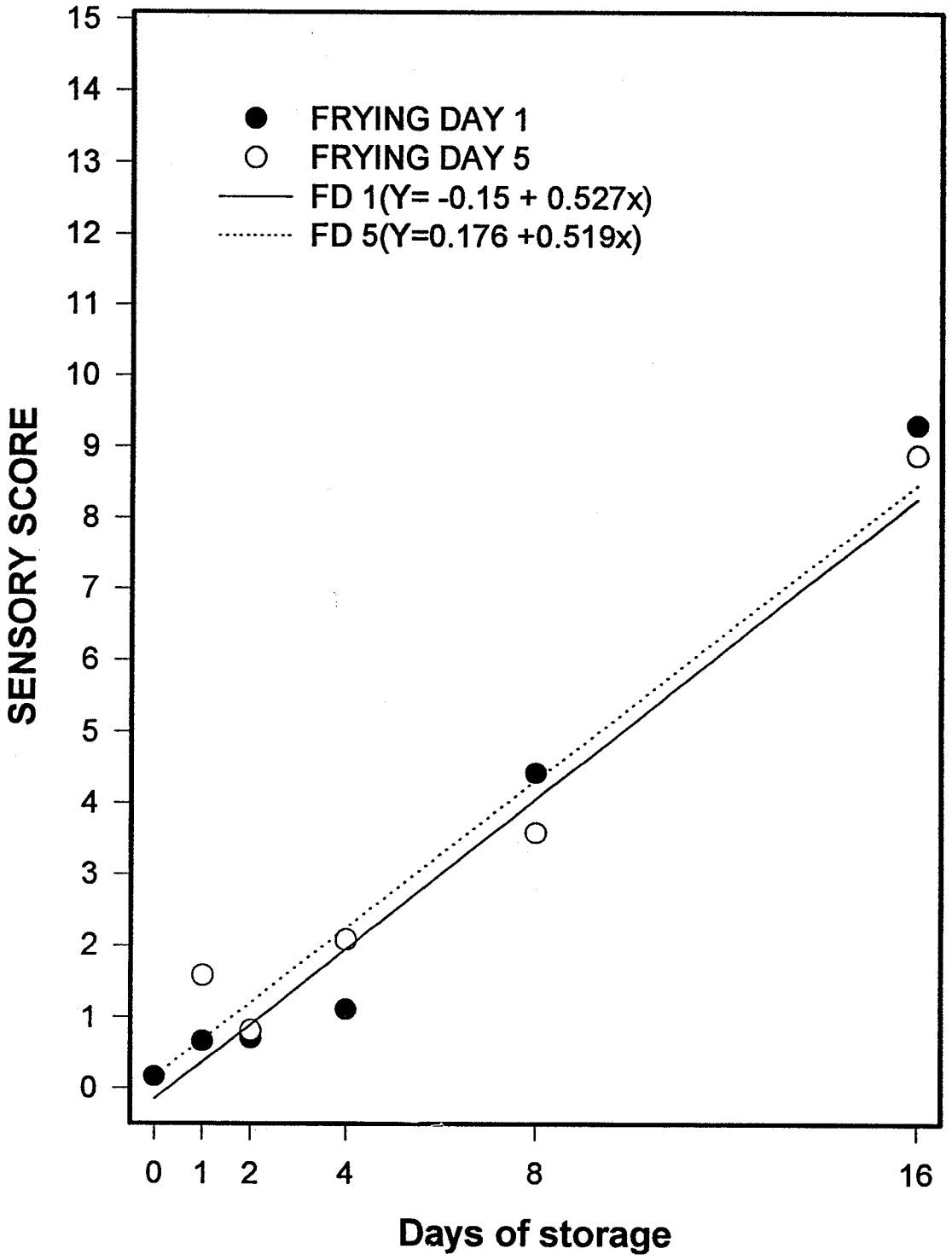


Figure 4.8. Painty Odour in Potato Chips Fried in High Oleic Canola Oil.

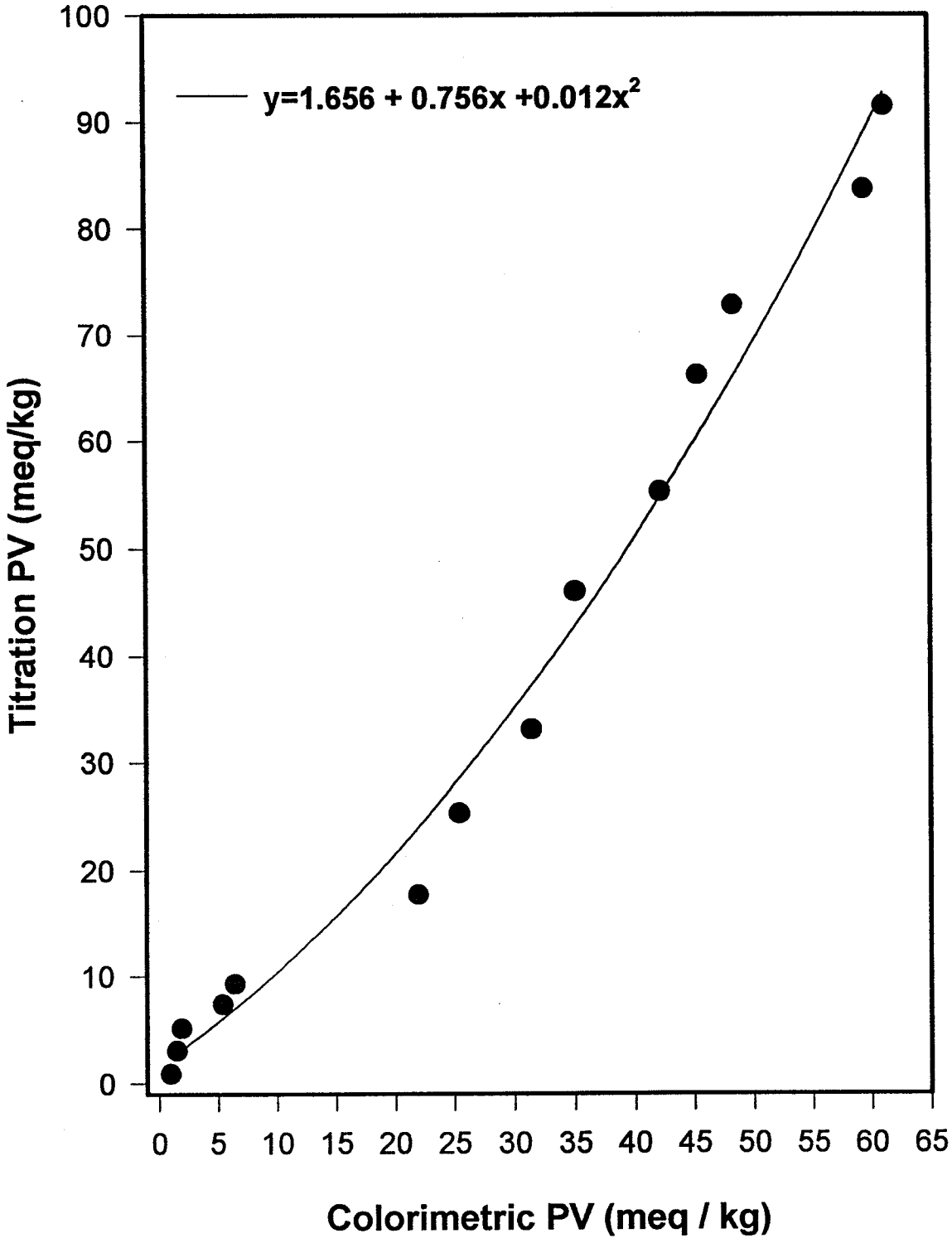


Figure 4.9. Relationship between Colorimetric and Titrated Peroxide Values in Canola Oils.

The PVs of the extracted from potato chips oils were statistically analyzed using a ln transformation. The results of analysis are presented in Figures 4.10 and 4.11. For all oils, the accumulation of peroxides in potato chips increased as storage days increased for both frying days 1 and 5 except for chips fried in HYCO. For frying day 1, potato chips fried in HYCO had a significantly lower initial level of peroxides than chips fried in the other three oils (RCO: t_{28}=5.65, p=0.0001; LLCO: t_{28}=7.16, p=0.0001; HOCO: t_{28}=6.87, p=0.0001). During the whole storage period, chips fried in HYCO had a significantly lower rate of accumulation of peroxides than chips fried in RCO (t_{28}=6.62, p=0.0001), LLCO (t_{28}=4.77, p=0.0001) and HOCO (t_{28}=2.69, p=0.0119). Potato chips fried in HOCO displayed the second lowest rate of peroxides production which was significantly lower than chips fried in RCO (t_{28}=3.93, p=0.0005) and LLCO (t_{28}=2.09, p=0.0463).

Similarly, for frying day 5 the chips fried in HYCO also had a significantly lower initial level of peroxides compared to chips fried in RCO (t_{28}=4.08, p=0.0003), LLCO (t_{28}=2.92, p=0.0068) and HOCO (t_{28}=5.84, p=0.0001). The rate of accumulation of peroxides was significantly slower for chips fried in HYCO than for those fried in RCO (t_{28}=5.05, p=0.0001) and LLCO (t_{28}=3.86, p=0.0006). The accumulation of peroxides in potato chips fried in HOCO occurred at a slower rate than in chips fried in RCO (t_{28}=5.35, p=.0001) and LLCO (t_{28}=4.16, p=0.0003). Unlike frying day 1, the starting levels of

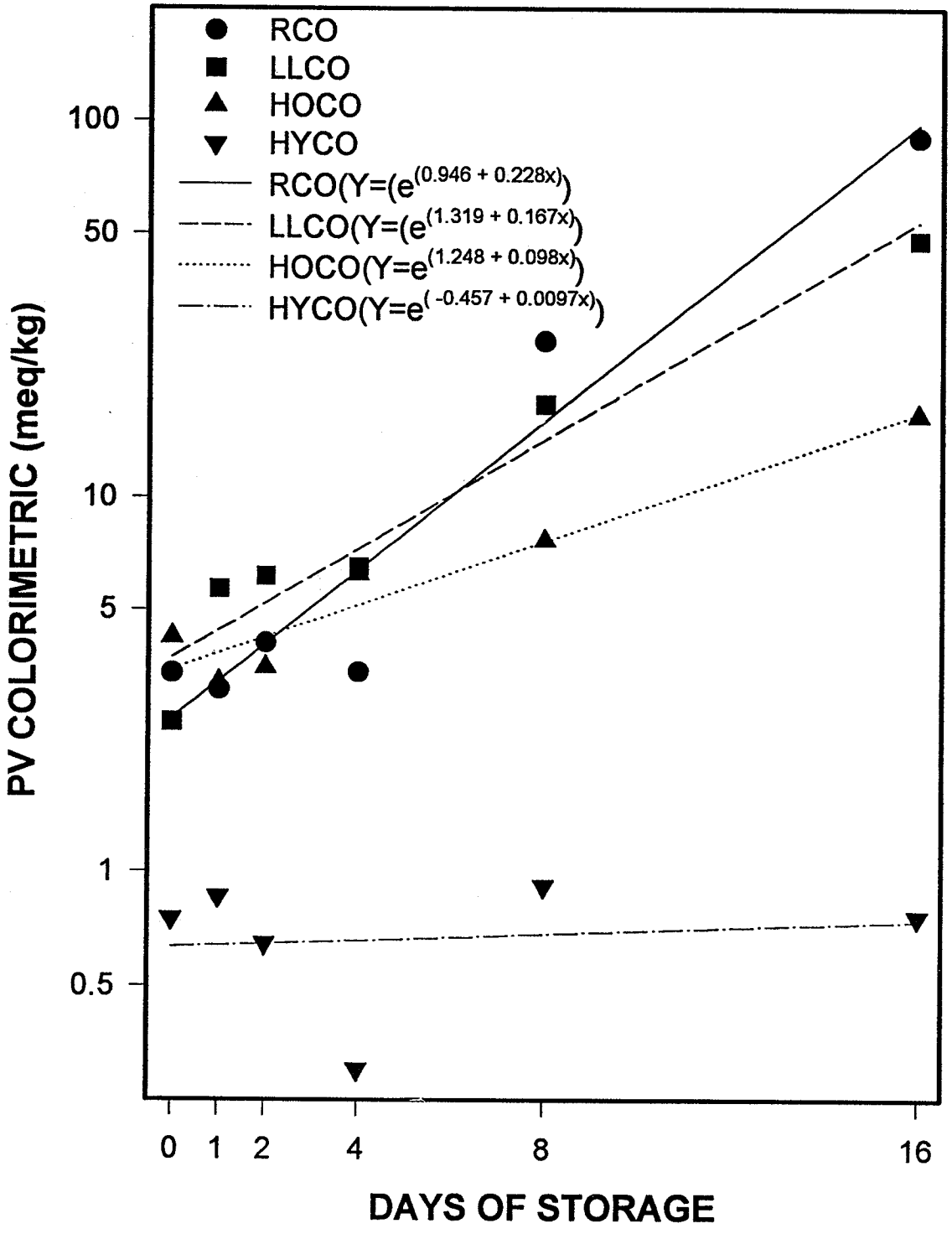


Figure 4.10. Peroxide Values in Canola Oils Extracted from Potato Chips (Frying Day 1).

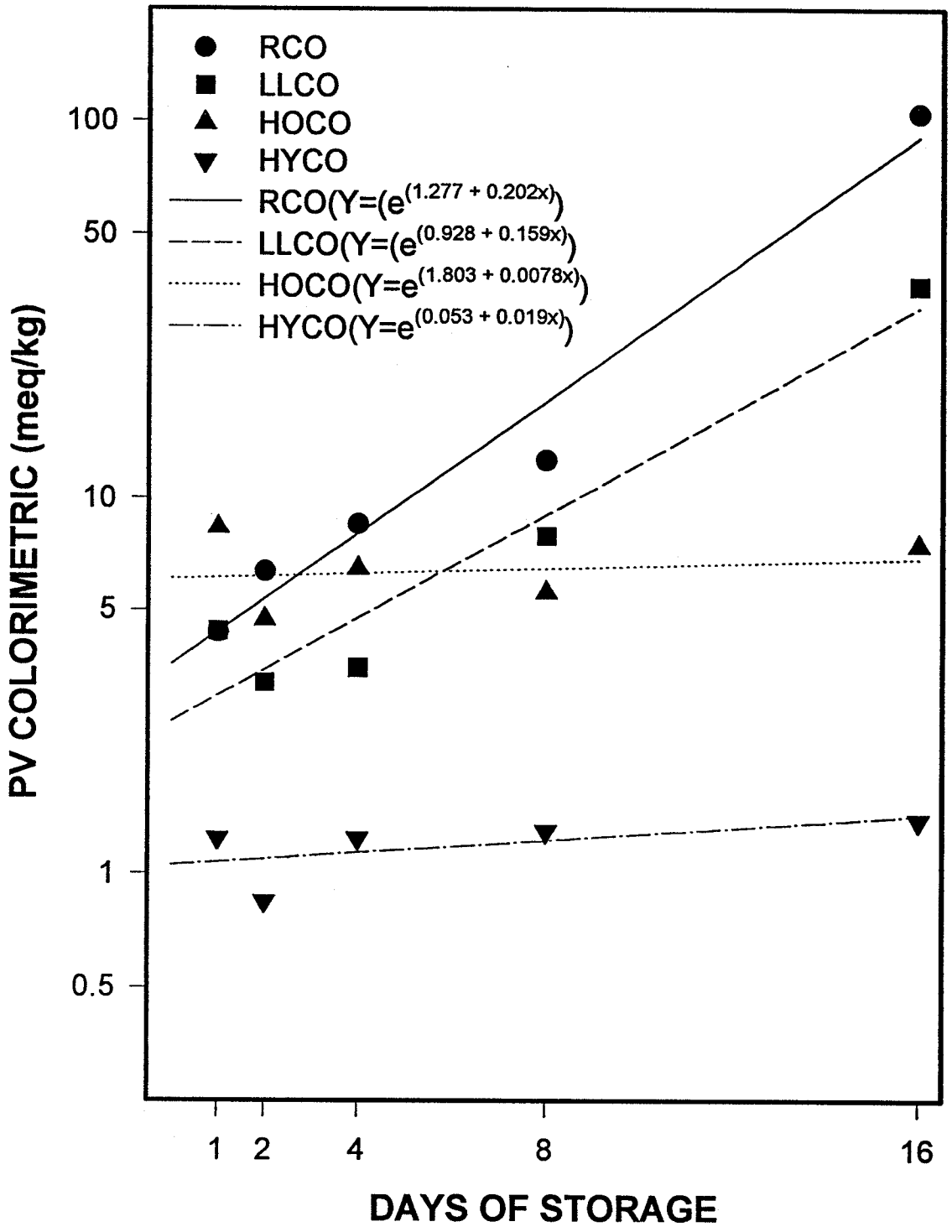


Figure 4.11. Peroxides Values in Canola Oils Extracted from Potato Chips (Frying Day 5).

peroxides were found to be significantly lower in chips fried in LLCO than in HOCO (t_{28}=2.92, p=0.0069).

When the initial amounts and accumulation rates of peroxides in potato chips were compared between frying day 1 and day 5 within each oil it was found that there was no significant difference between frying day 1 and frying day 5 for all chips except for those fried in HOCO. For these potato chips the starting levels of peroxides for frying day 1 were significantly lower than on frying day 5 (t_{28}=2.61, p=0.0145) and peroxides accumulated faster on day 1 than on day 5 (t_{28}=-2.02, p=0.0536).

The FFA data were statistically analyzed using a square root transformation. The results are presented in Figures 4.12 and 4.13. There was an increase observed in FFA accumulation for potato chips fried in RCO, LLCO and HYCO for frying day 1 with increased storage. The initial amounts of FFA in the chips were not significantly different among the oils. However, the rates of accumulation of FFA were significantly higher in chips fried in RCO than the other three oils (HYCO: t_{28}=-6.18, p=0.0001; LLCO: t_{28}=-2.89, p=0.0073; HOCO: t_{28}=-5.73, p=0.0001), and chips fried in LLCO had a significantly faster rate of FFA production than both HYCO (t_{28}=-3.28, p=0.0027) and HOCO (t_{28}=-2.84, p=0.0083) (Figure 4.12).

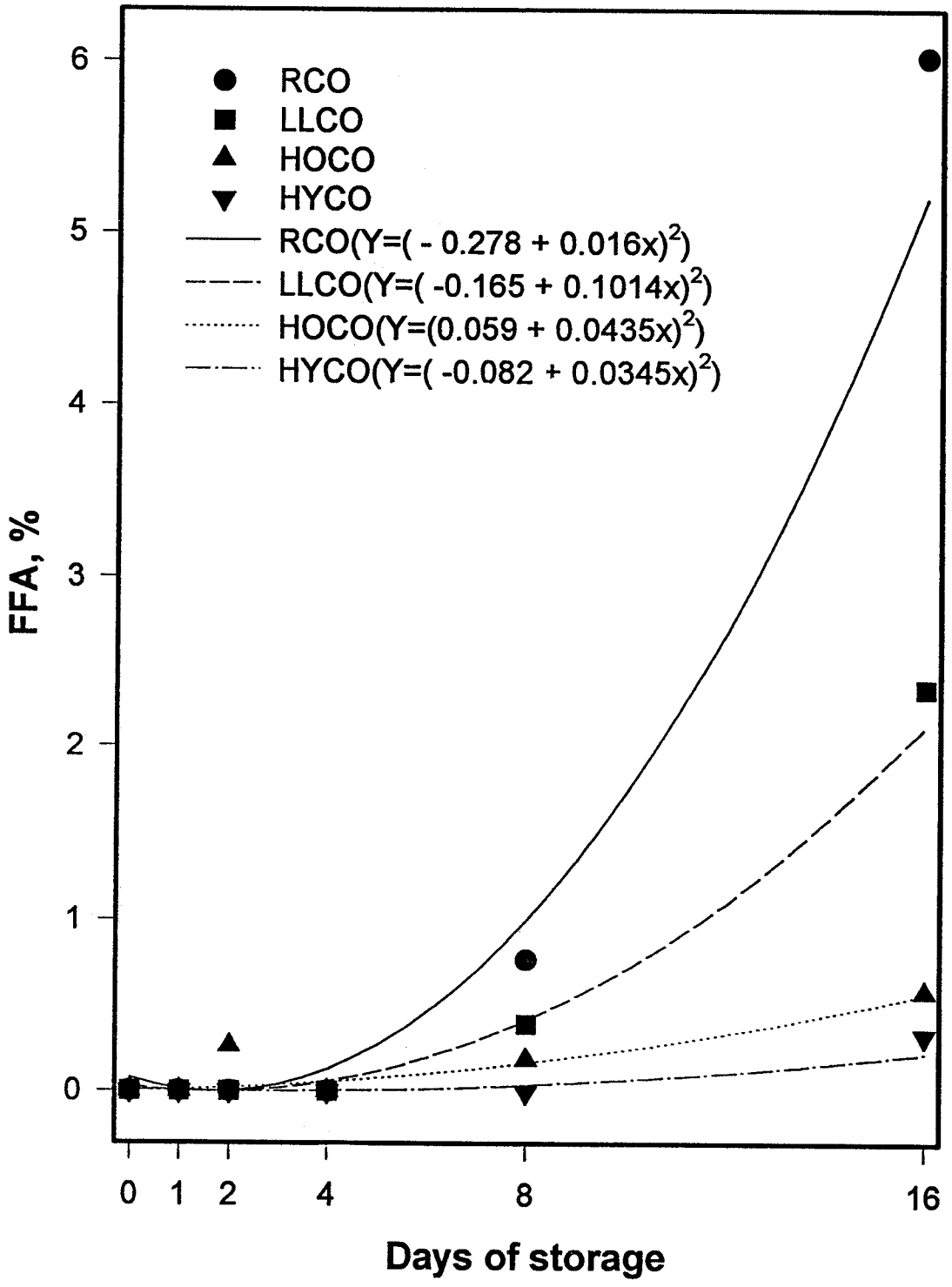


Figure 4.12. Free Fatty Acids in Canola Oils Extracted From Potato Chips (Frying Day 1).

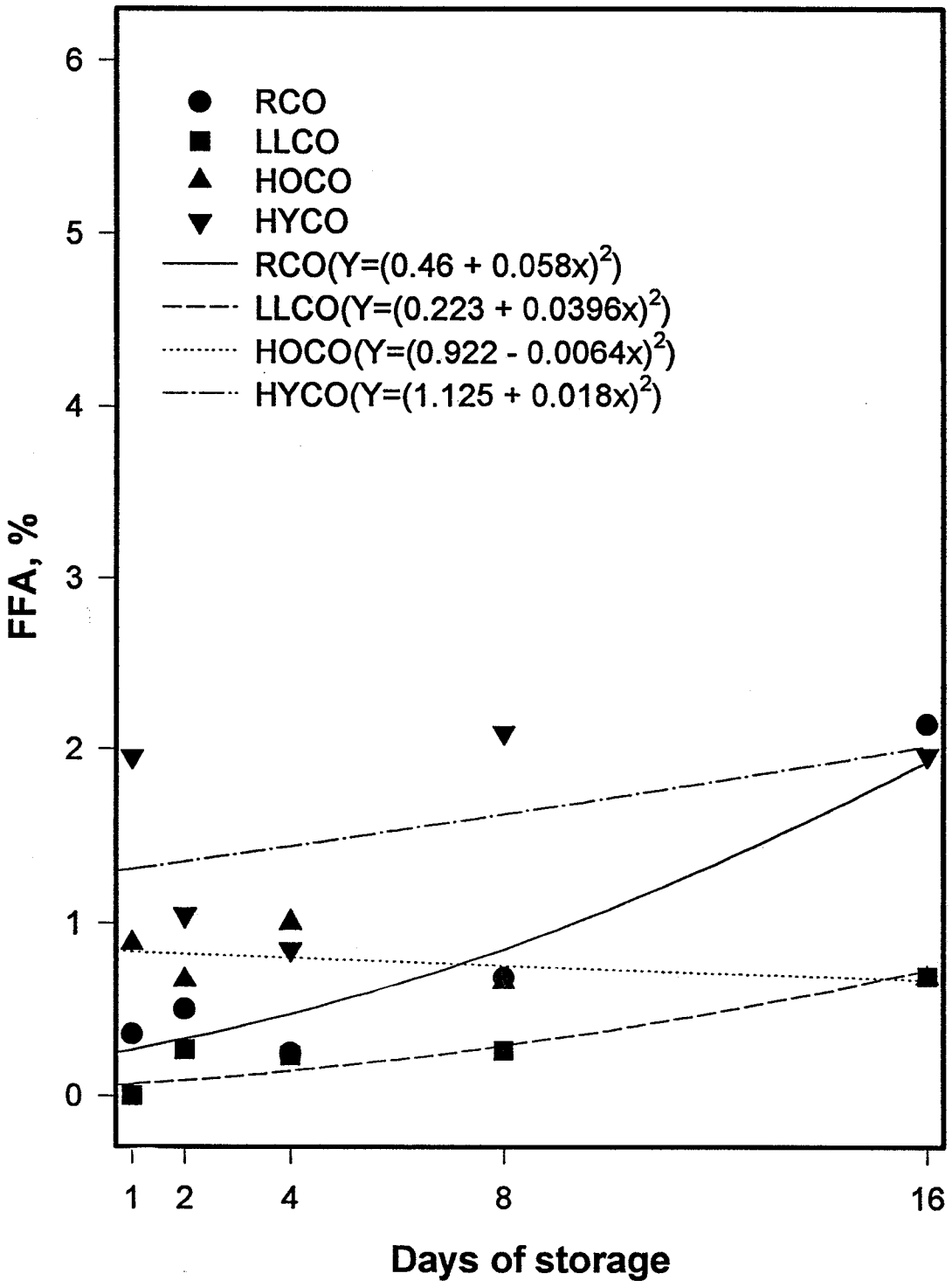


Figure 4.13. Free Fatty Acids in Canola Oils Extracted from Potato Chips (Frying Day 5).

Potato chips fried in RCO from day 5 of frying had a significantly lower initial levels of FFA than potato chips fried in both HYCO (t_{28}=3.58, p=0.0013) and HOCO (t_{28}=2.49, p=0.019). This was also true for chips fried in LLCO. They were found to have significantly lower initial levels of FFA than HYCO (t_{28}=4.86, p=.0001) and HOCO (t_{28}=3.77, p=0.0008). The rate of FFA accumulation in potato chips fried in RCO was significantly faster than in chips fried in HOCO (t_{28}=-2.87, p=0.0077) and HYCO (t_{28}=-1.77, p=0.0883), and chips fried in LLCO accumulated FFA at a faster rate than chips fried in HOCO (t_{28}=-2.05, p=0.0502).

The accumulation of FFA in chips from frying day 5 followed a different pattern than chips from frying day 1. Unlike frying day 1, where all chips did not have any FFA initially, the frying day 5 chips had starting levels similar to the levels found in the frying oils after 5 days of frying. This could be observed by comparing Figures 3.2 and 4.13. Within each oil, the effect of frying day revealed that there was a significantly higher initial level of FFA for all chips fried on day 5 compared to day 1 RCO (t_{28}=-4.33, p=0.0002), HYCO (t_{28}=-7.09, p=0.0001), LLCO (t_{28}=-2.28, p=0.0307) and HOCO (t_{28}=-5.07, p=0.0001). This would indicate that potato chips from frying day 5 absorbed degradation products at the levels already present in the frying oils. The effect of frying day within a given oil showed that there were significantly lower accumulation rates of FFA for frying day 5 compared to frying

day 1 for chips fried in RCO (t_{28}=4.77, p=0.0001), LLCO (t_{28}=2.89, p=0.0074) and HOCO (t_{28}=2.33, p=0.0273).

The CDA data were statistically analyzed using a ln transformation. The results of the analysis showed that potato chips fried in both RCO and LLCO had a pronounced increase in CDA amounts after storage for both frying day 1 and 5 (Figures 4.14 and 4.15, respectively). The potato chips fried in HOCO from frying day 1 had a slight increase in CDA accumulation with increased storage time, but this was not evident for frying day 5. For both frying days, the level of CDA in potato chips fried in HYCO did not change over storage time.

The potato chips fried in LLCO from frying day 1 had significantly lower initial levels of CDA compared to chips fried in RCO (t_{28}=4.14, p=0.0003), HYCO (t_{28}=9.97, p=0.0001) and HOCO (t_{28}=9.04, p=0.0001). The potato chips fried in RCO had the second lowest initial levels of CDA which were significantly lower those found in chips fried in HYCO (t_{28}=5.83, p=0.0001) and HOCO (t_{28}=4.90, p=0.0001) chips. The pairwise comparison of the rates of CDA accumulation in the potato chips fried in four oils during storage showed that they were all significantly different from each other. Chips fried in RCO accumulated CDA faster than chips fried in HYCO (t_{28}=-12.04, p=0.0001), LLCO (t_{28}=-3.21, p=0.0033) and HOCO (t_{28}=-9.27, p=0.0001); potato chips fried in LLCO had greater rates of CDA accumulation than chips fried in HYCO (t_{28}=-6.05, p=0.0001) and HOCO (t_{28}=-8.83, p=0.0001); and chips fried in

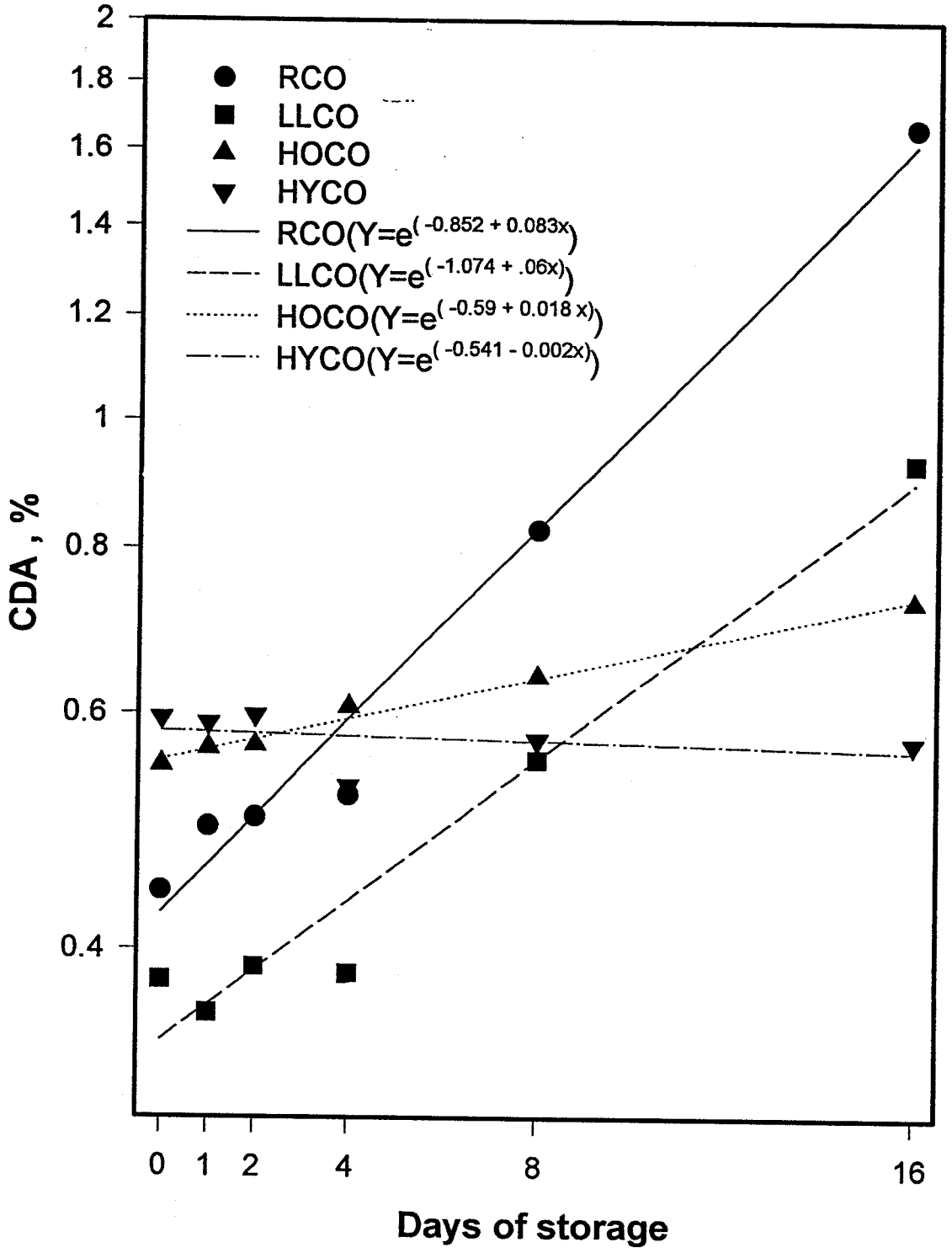


Figure 4.14. Conjugated Dienoic Acids in Canola Oils Extracted from Potato Chips (Frying Day 1).

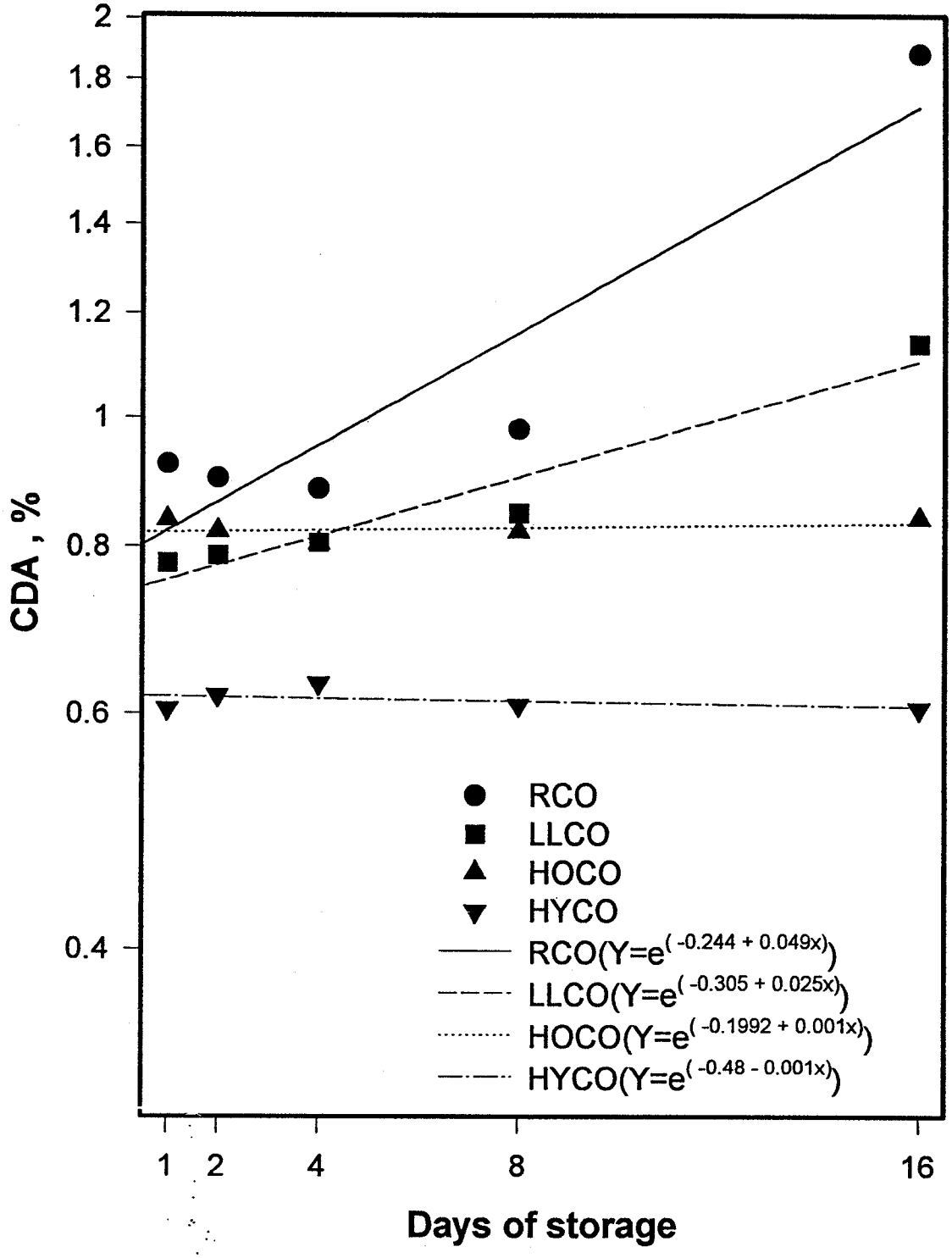


Figure 4.15. Conjugated Dienoic Acids in Canola Oils Extracted from Potato Chips (Frying Day 5).

HOCO accumulated CDA faster than chips fried in HYCO (t_{28}=-2.77, p=0.0098).

The stored potato chips fried in HYCO after frying day 5 had a significantly lower initial level of CDA than chips fried in RCO (t_{28}=3.67, p=0.001), LLCO (t_{28}=2.71, p=0.0112) and HOCO (t_{28}=4.36, p=0.0002). A significantly greater rates of CDA accumulation were found for chips fried in RCO compared to those fried in HYCO (t_{28}=-6.43, p=0.0001), LLCO (t_{28}=-3.04, p=.005) and HOCO (t_{28}=-6.14, p=0.0001). Potato chips fried in LLCO also had a significantly greater rate of CDA accumulation than chips fried in HYCO (t_{28}=-3.38, p=0.0021) and HOCO (t_{28}=-3.09, p=0.0044) chips.

Within each oil, the initial amounts of CDA in chips were found to be significantly higher in frying day 5 compared to frying day 1 for chips fried in LLCO (t_{28}=-12.97, p=0.0001) and HOCO (t_{28}=-3.95, p=0.0005). Again as in FFA, the initial levels of CDA for frying day 5 seem to follow the order found after five days of frying in the oil (compare Figures 3.4 and 4.15). This would indicate that potato chips from frying day 5 absorbed degradation products at the levels already present in the frying oil. A significantly greater rate of CDA accumulation was found in chips stored from frying day 1 than frying day 5 for RCO (t_{28}=-4.58, p=0.0001), LLCO (t_{28}=-4.72, p=0.0001) and HOCO (t_{28}=-2.21, p=0.03574).

The data from polar components measurements were statistically analyzed using a ln transformation. The results

of analysis are presented in Figures 4.16 and 4.17. Potato chips from both frying days showed a slight increase in the amount of polars over the 16 day storage period except for chips fried in RCO which showed a pronounced increase. For frying day 1, the initial level of polar components in chips fried in RCO was significantly lower than in potato chips fried in HYCO (t_{28}=2.12, p=0.0427), LLCO (t_{28}=3.40, p=0.0021) and HOCO (t_{28}=3.32, p=0.0025) potato chips. Chips fried in RCO had a significantly higher rate of accumulation of polars compared to potato chips fried in HYCO (t_{28}=-5.35, p=0.0001), LLCO (t_{28}=-4.52, p=0.0001) and HOCO (t_{28}=-4.70, p=0.0001).

The frying day 5 chips fried in RCO had a significantly lower initial level of polars than chips fried in HYCO (t_{28}=3.75, p=0.008) and HOCO (t_{28}=3.40, p=0.0021), chips fried in LLCO had significantly lower initial levels of polars than chips fried in HYCO (t_{28}=2.33, p=0.0273) and HOCO (t_{28}=1.98, p=0.0576). Chips fried in RCO exhibited the highest rate of accumulation of polars compared to the chips fried in HYCO (t_{28}=-4.16, p=0.0003), LLCO (t_{28}=-4.06, p=0.0004) and HOCO (t_{28}=-4.24, p=0.0.0002).

Within a given oil, there was a significantly higher initial amount of polars for frying day 5 compared to frying day 1 for chips fried in RCO (t_{28}=-2.58, p=0.0154), HYCO (t_{28}=-4.75, p=0.0001) and HOCO (t_{28}=-3.29, p=0.0576). There were no significant differences observed in the rates of polars

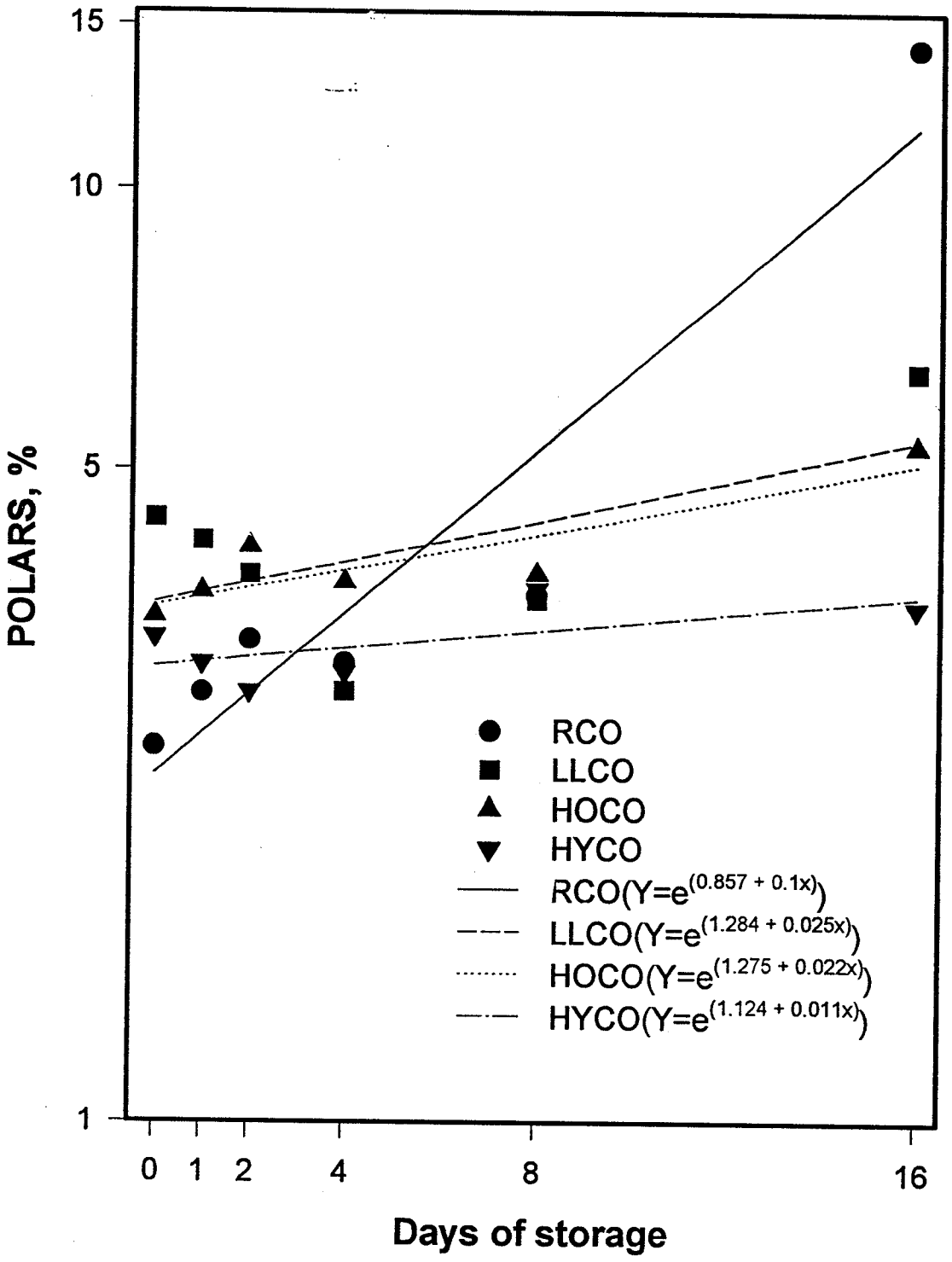


Figure 4.16. Polar Components in Canola Oils Extracted from Potato Chips (Frying Day 1).

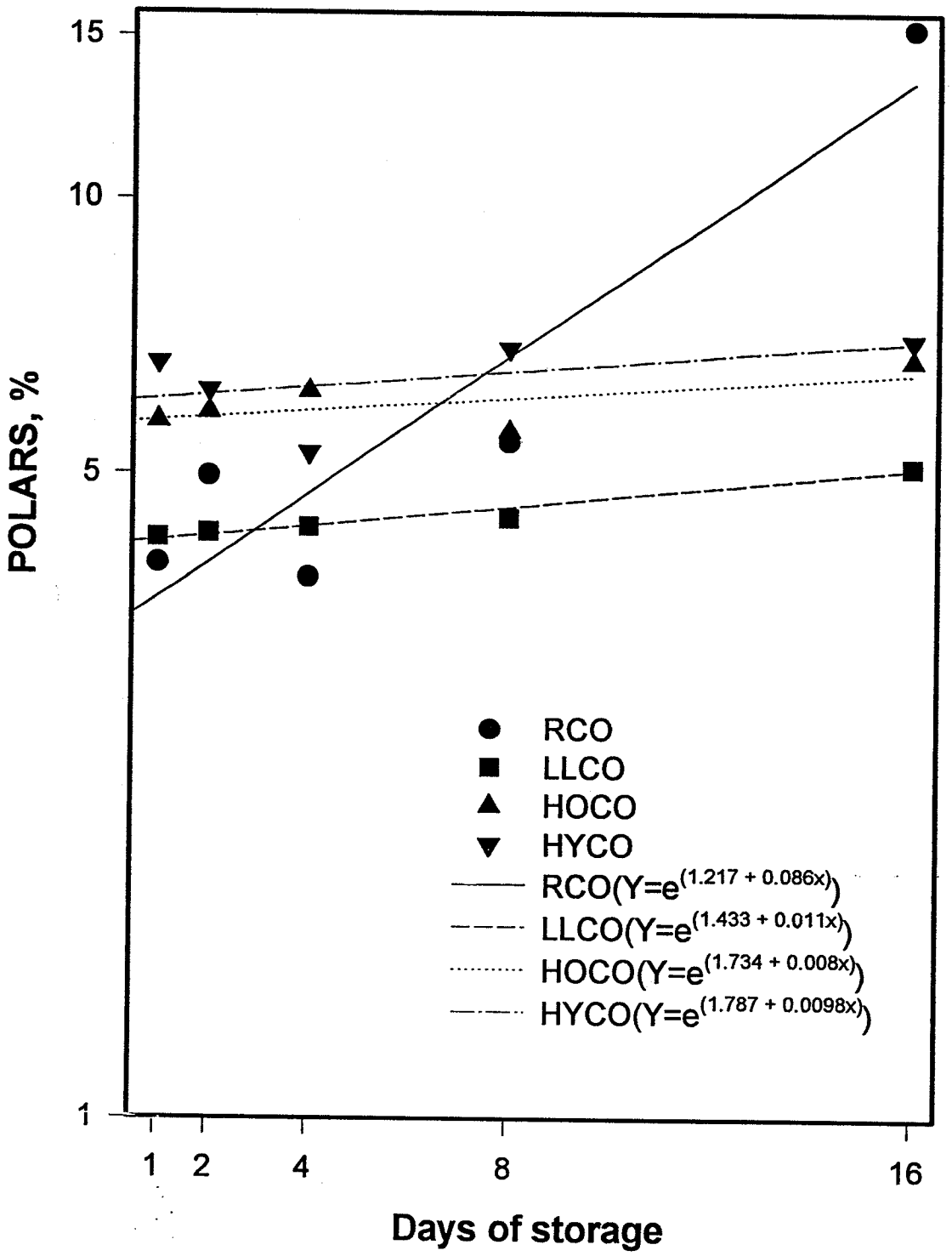


Figure 4.17. Polar Components in Canola Oils Extracted from Potato Chips (Frying Day 5).

accumulation between frying day 1 and day 5 for chips fried in all four oils.

The analysis of volatile odour components in potato chips permitted identification of 25 compounds. More than 15 components could not be identified. For purposes of simplifying the interpretation of the results, the volatiles were grouped according to their chemical structure as follows: hydrocarbons, saturated and unsaturated carbonyls, dienals and pyrazines. Total volatiles included both identified and unidentified components.

For frying day 1 and 5, the amount of hydrocarbons increased for chips fried in all oils except in HYCO which remained unchanged (Figures 4.18 and 4.19). In chips from frying day 1, the greatest change occurred in chips fried in RCO which had the hydrocarbons amounts about nine times greater after 16 days of storage compared to chips fried in LLCO and HOCO. Chips fried in LLCO and HOCO had similar amounts of hydrocarbons. For frying day 5, the hydrocarbons in chips fried in RCO increased about 4 times after 16 days of storage compared to chips fried in HOCO. The accumulation of hydrocarbons in chips fried in LLCO was only about half the level that occurred for the chips fried in HOCO. There was about twice as much hydrocarbons in potato chips fried in RCO from frying day 1 than from frying day 5 after 16 days of storage.

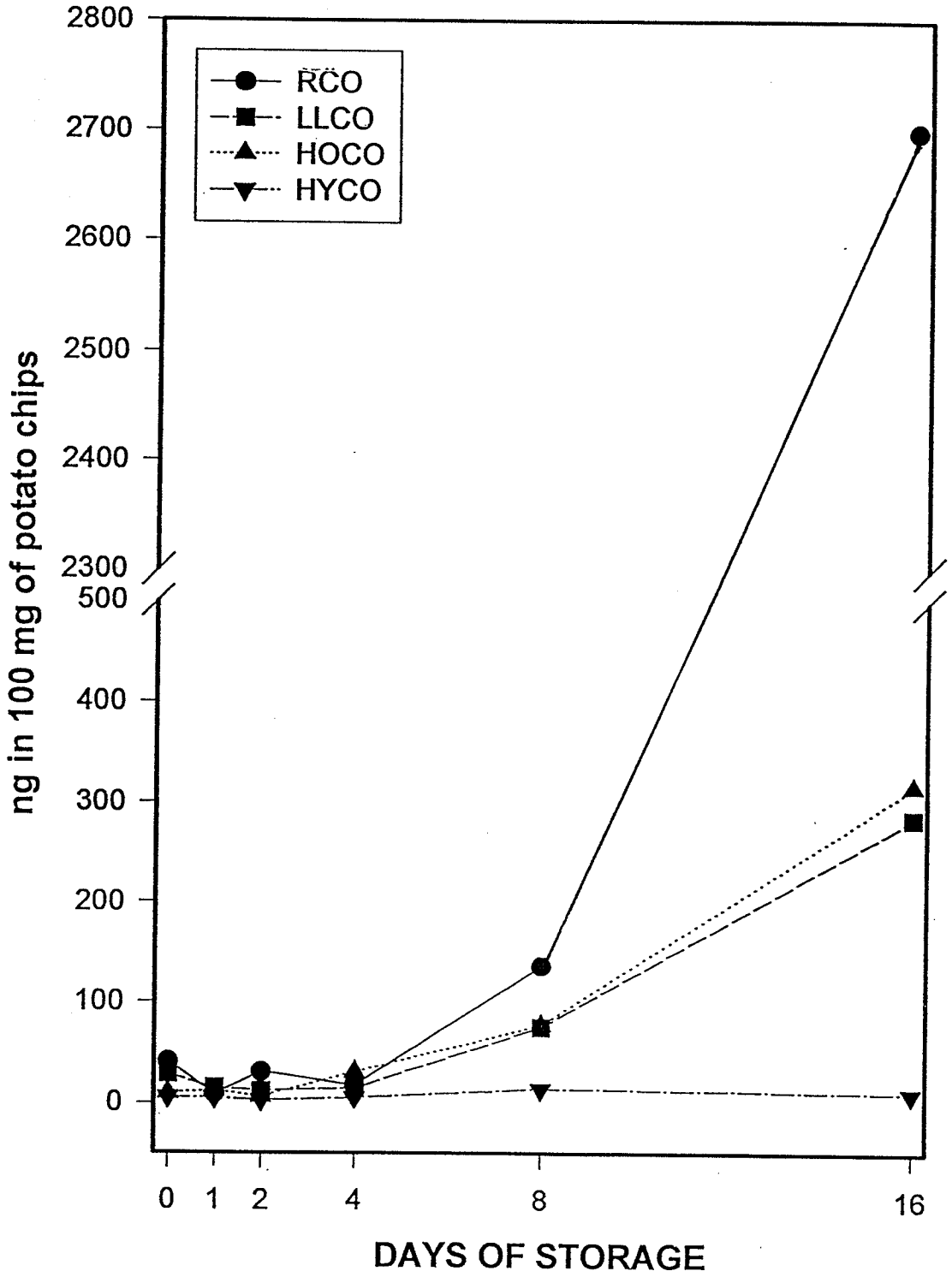


Figure 4.18. Hydrocarbons in Potato Chips (Frying Day 1).

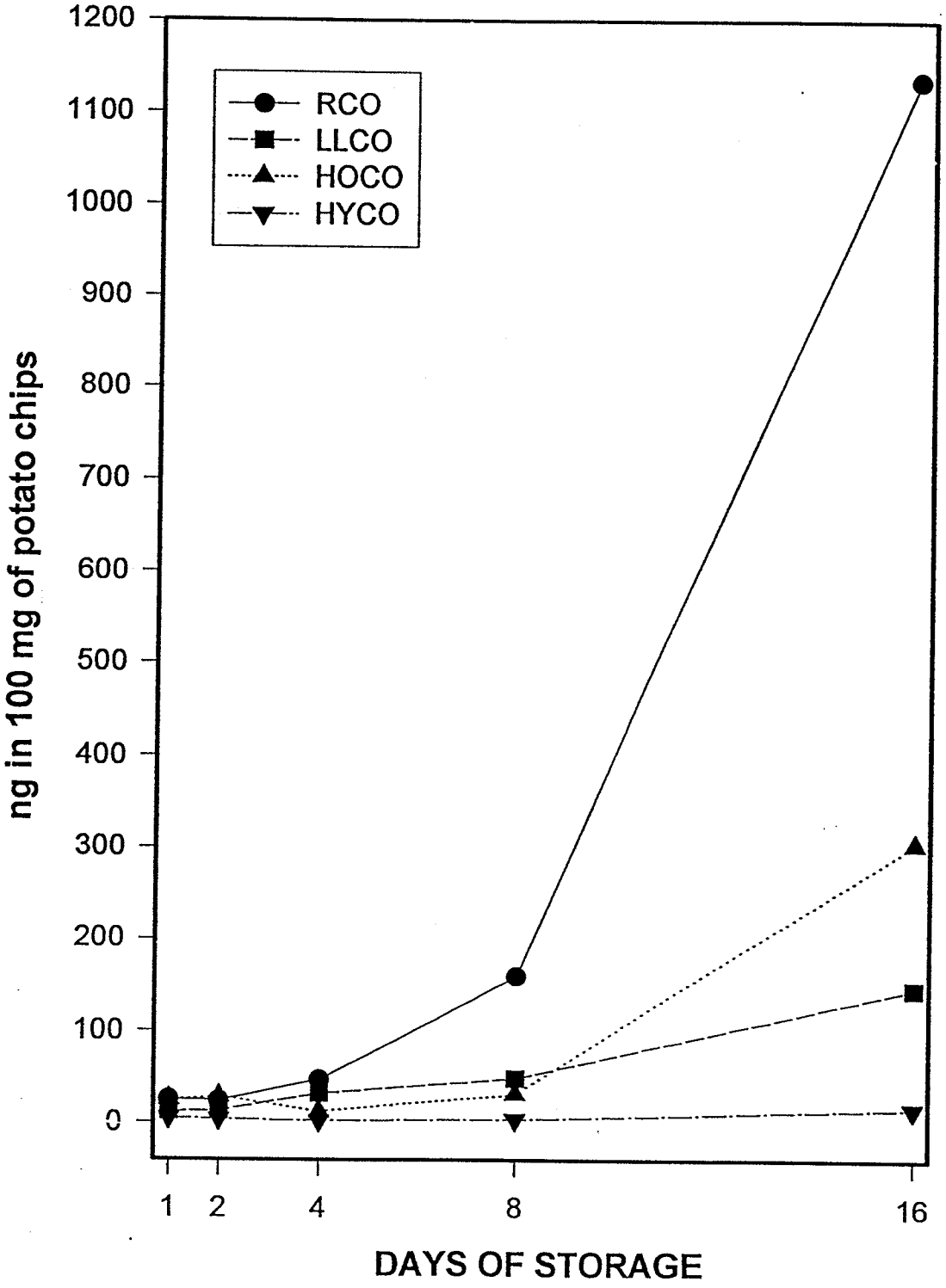


Figure 4.19. Hydrocarbons in Potato Chips (Frying Day 5).

The saturated carbonyls level increased dramatically for all potato chips after day 8 of storage for frying day 1 except for chips fried in RCO which showed a large increase after day 4 of storage Figure 4.20). The lowest accumulation of saturated carbonyls happened in chips fried in HYCO followed by potato chips fried in LLCO and HOCO. For frying day 5, the chips fried in HYCO and HOCO showed very little change over the whole storage period (Figure 4.21). In contrast, chips fried in RCO and LLCO had a sharp increase in saturated carbonyls after 8 days of storage, with chips fried in RCO having about 3.5 times more saturated carbonyls than chips fried in LLCO after 16 days of storage. The level of saturated carbonyls in potato chips fried in RCO from frying day 1 was approximately 3.5 times greater after 16 days of storage than from frying day 5. Both saturated and unsaturated carbonyls have been found to be responsible for the oxidized flavours of oils and fried foods (Dixon and Hammond, 1984; Mookherjee *et al.*, 1965).

There was little change in unsaturated carbonyls levels in potato chips fried in HYCO, LLCO and HOCO from frying day 1 (Figure 4.22). The potato chips fried in RCO showed a steady increase in unsaturated carbonyls with a sharp increase after 8 days of storage. Chips fried in HYCO and HOCO from frying day 5 showed a slight decrease in unsaturated carbonyls amounts over 16 days of storage (Figure 4.23). In contrast,

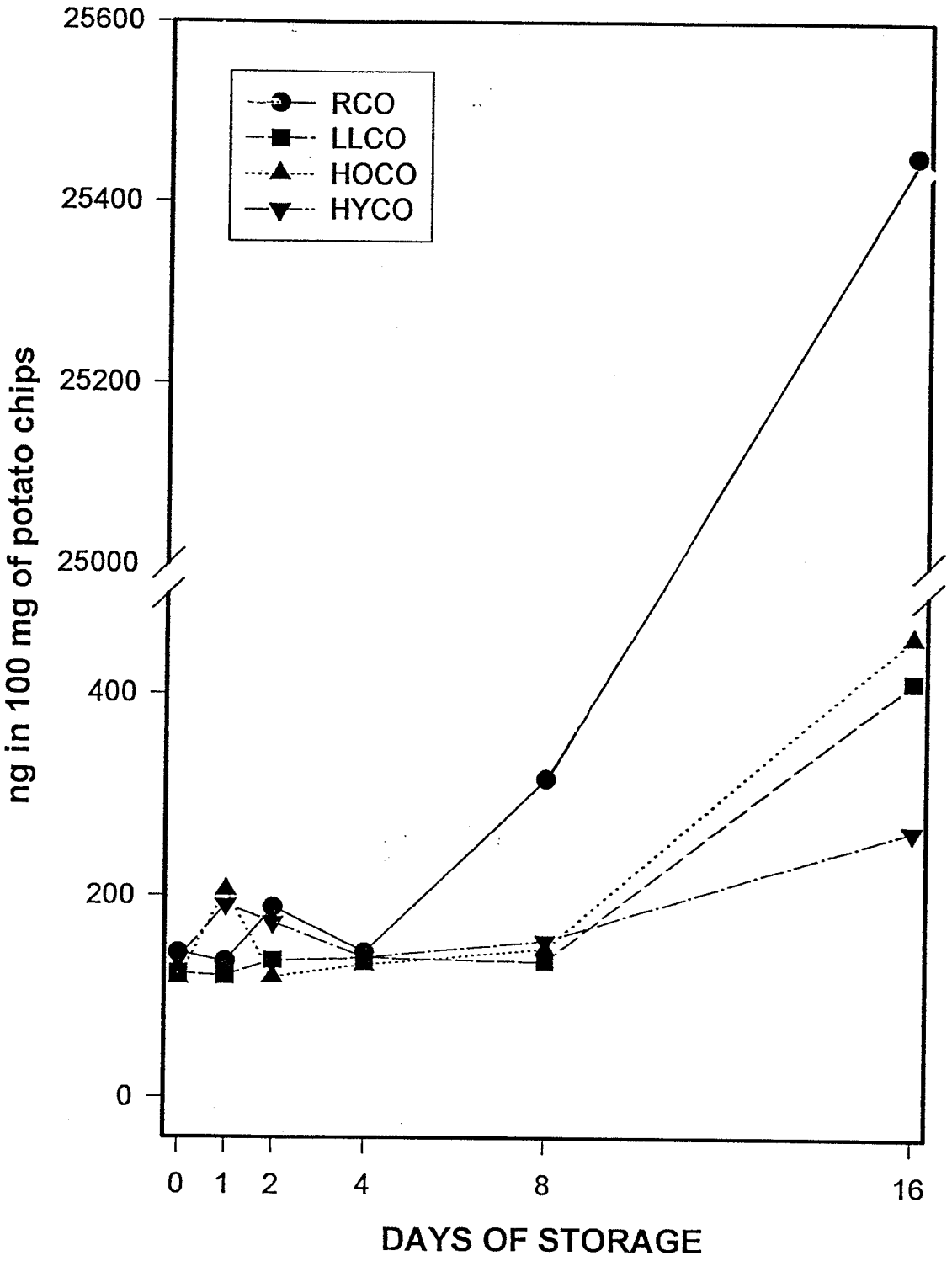


Figure 4.20. Saturated Carbonyls in Potato Chips (Frying Day 1).

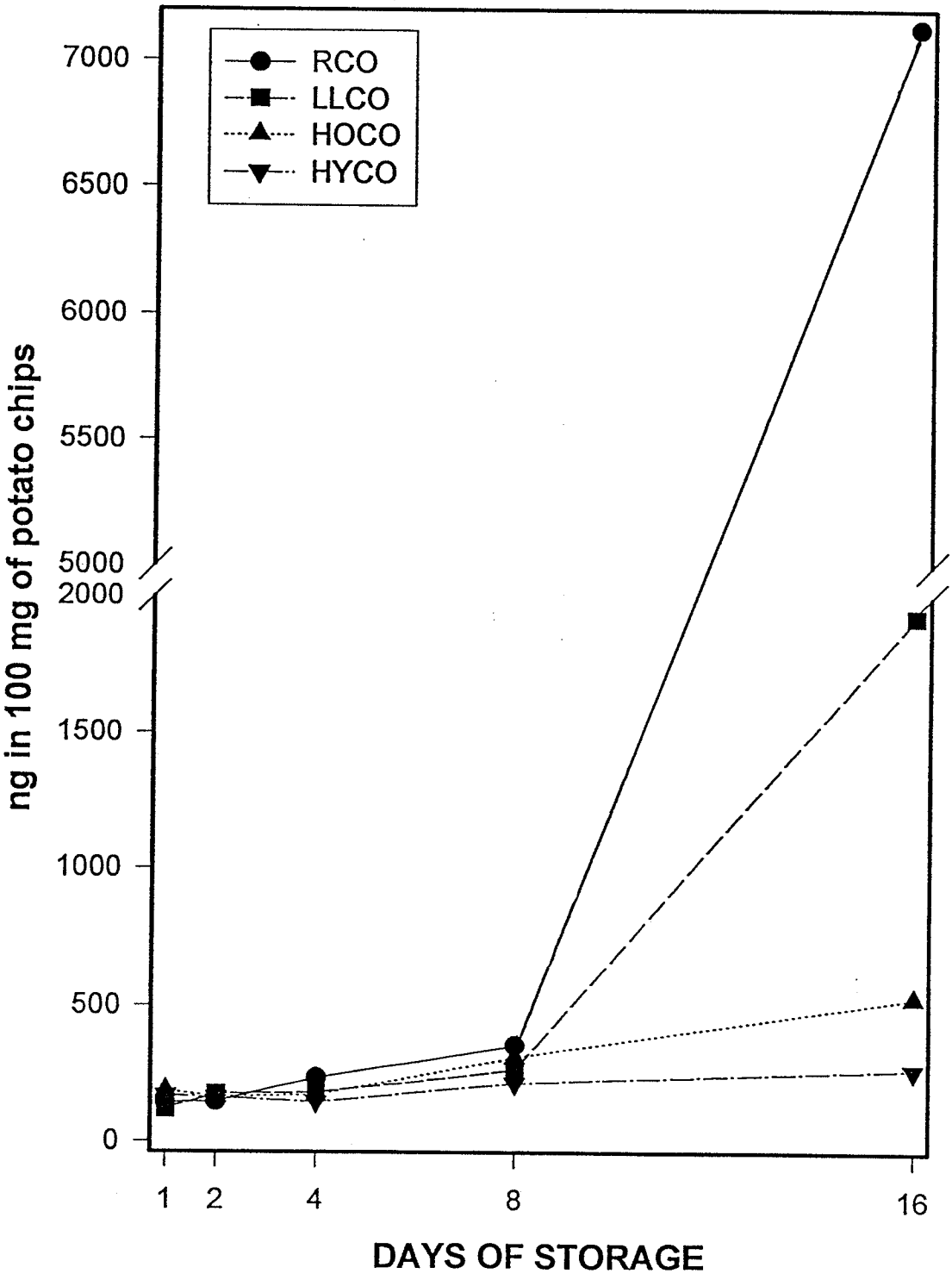


Figure 4.21. Saturated Carbonyls in Potato Chips (Frying Day 5).

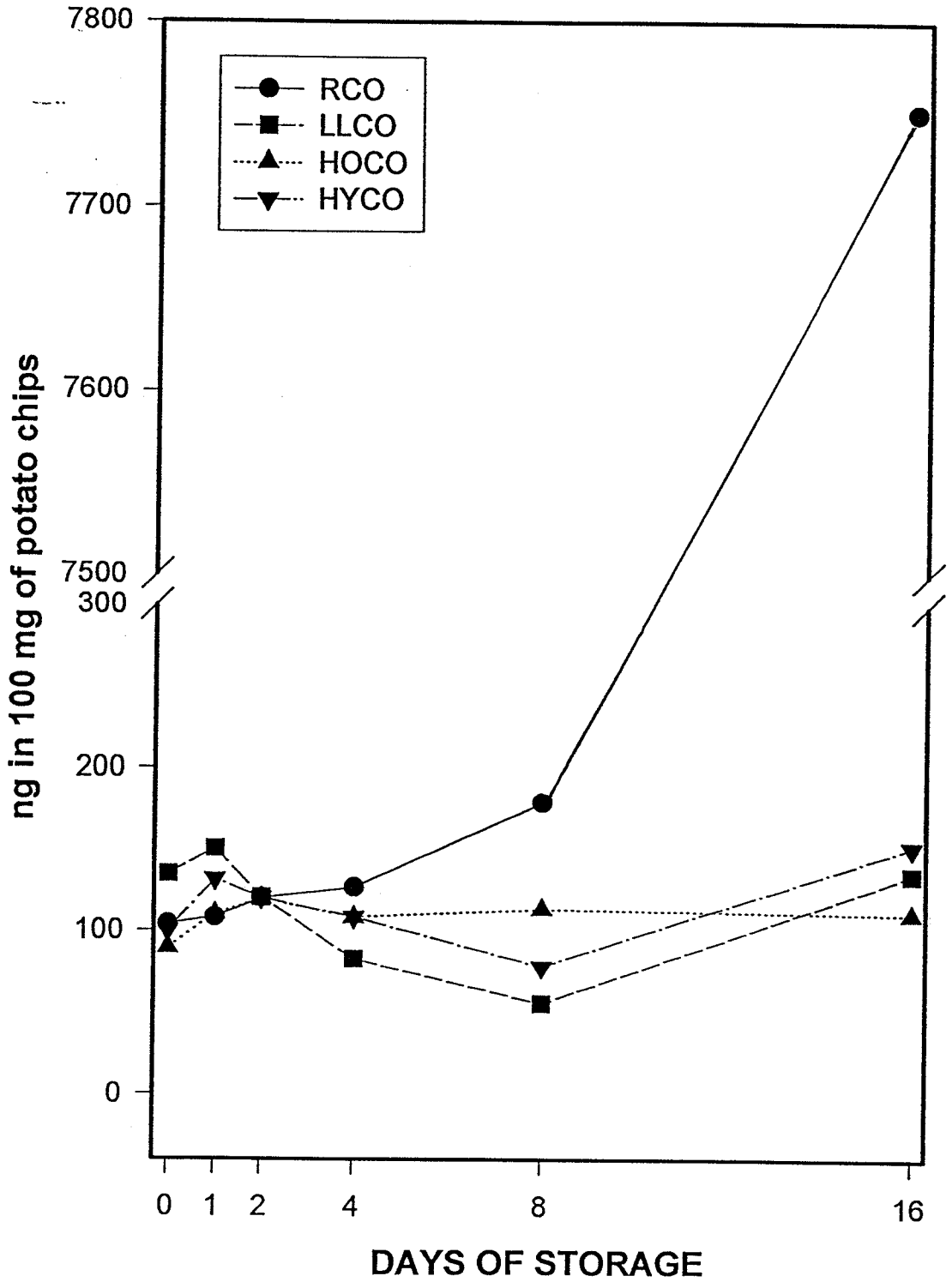


Figure 4.22. Unsaturated Carbonyls in Potato Chips (Frying Day 1).

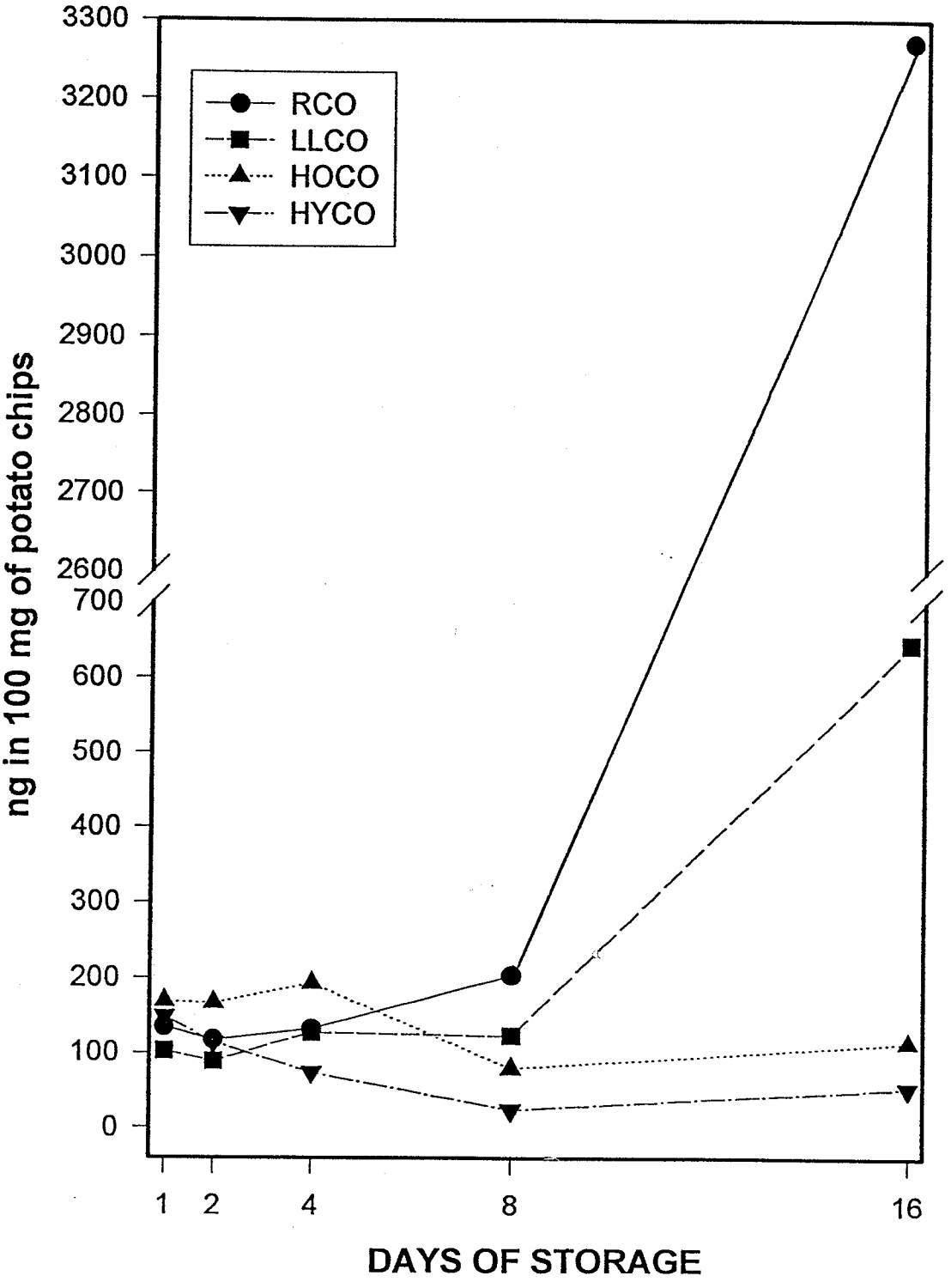


Figure 4.23. Unsaturated Carbonyls in Potato Chips (Frying Day 5).

chips fried in RCO and LLCO had a sharp increase in the unsaturated carbonyls amounts after 8 days of storage. The level of unsaturated carbonyls in the chips fried in RCO was roughly 5 times higher than in the chips fried in LLCO after 16 days of storage. The potato chips fried in RCO from frying day 1 showed higher accumulation of unsaturated carbonyls compared to frying day 5 after 16 days of storage. In contrast, chips fried in LLCO from frying day 5 had a higher levels of unsaturated carbonyls than frying day 1 after 16 days of storage.

The amount of dienals in the potato chips fried in HYCO remained around zero throughout the storage period for both frying days (Figures 4.24 and 4.25). The potato chips fried in LLCO showed an initial decrease in dienals followed by an increase for both frying days. The dienals in chips fried in HOCO from frying day 1 increased over storage whereas they decreased in chips from frying day 5. The greatest change in dienals was observed for chips fried in RCO with a sharp increase being observed after 8 days of storage for both frying days. The accumulated amount of dienals for chips fried in RCO from frying day 1 was approximately 2.5 times greater than for frying day 5 after 16 days of storage.

The amount of pyrazines in chips from frying day 1 showed an increase initially for all oils followed by a decrease (Figure 4.26). Then potato chips fried in LLCO and HOCO had the lowest levels of pyrazines at the end of the storage

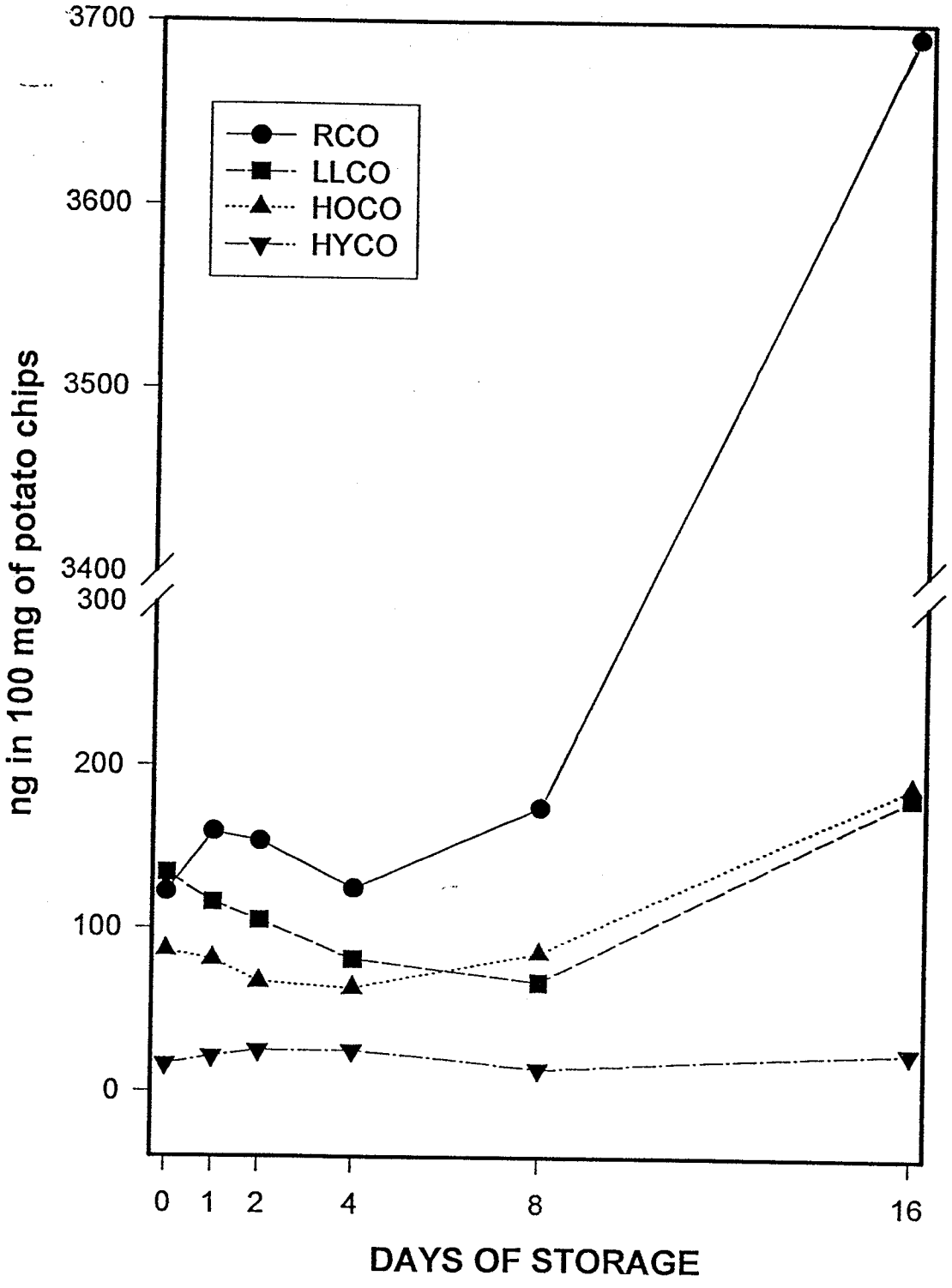


Figure 4.24. Dienals in Potato Chips (Frying Day 1).

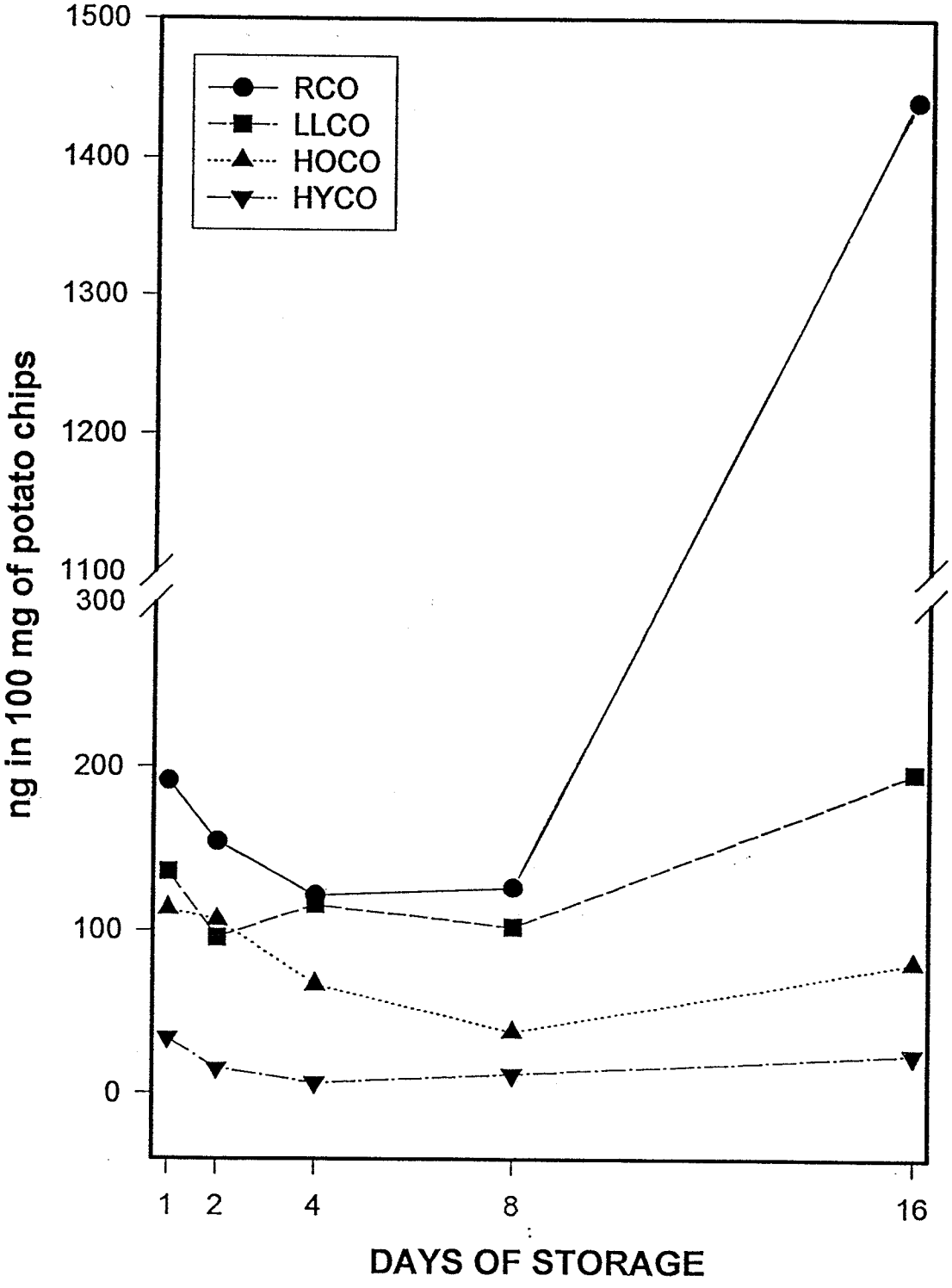


Figure 4.25. Dienals in Potato Chips (Frying Day 5).

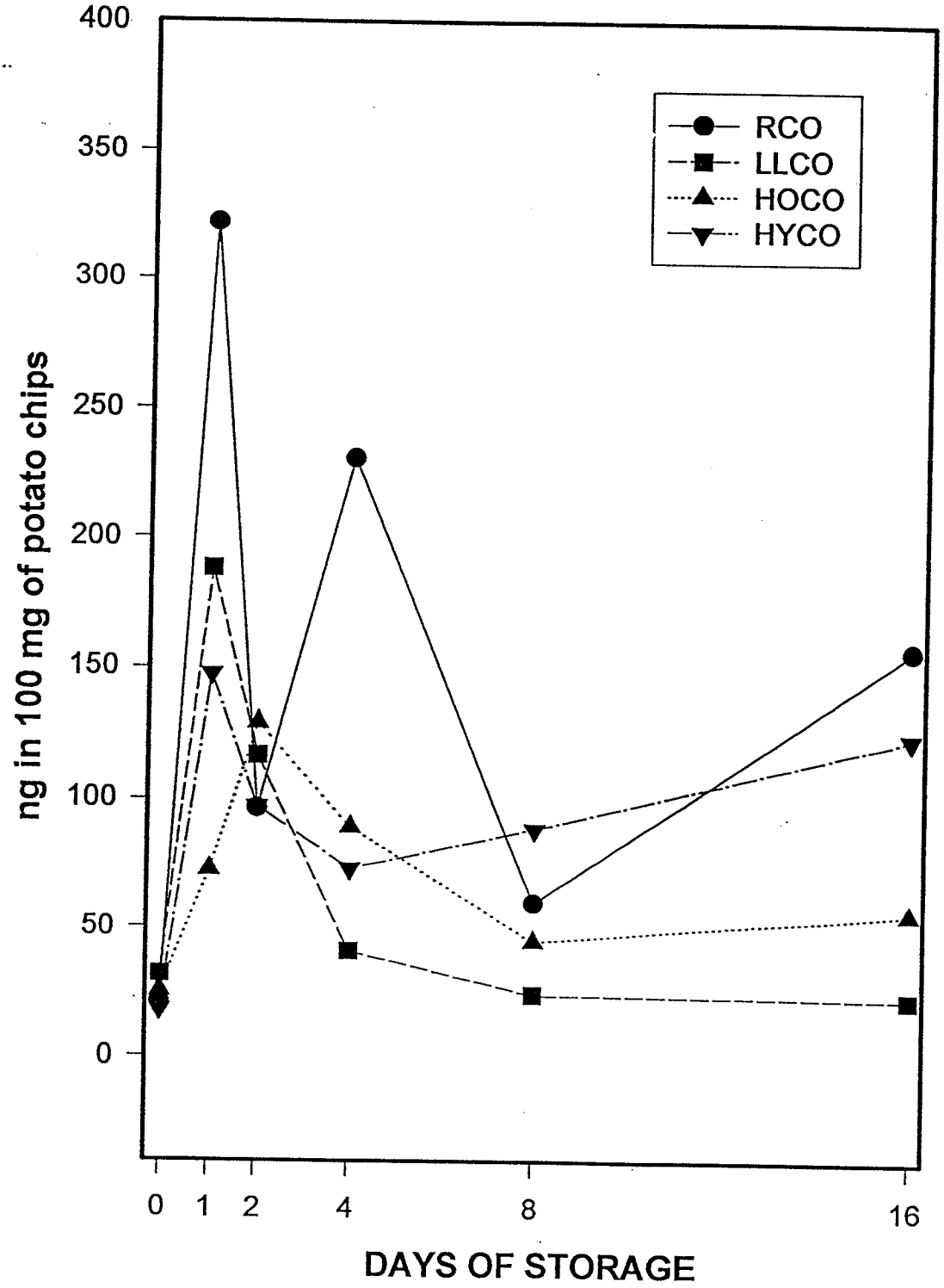


Figure 4.26. Pyrazines in Potato Chips (Frying Day 1).

period. Chips fried in RCO showed the greatest fluctuations in pyrazines amounts over storage. For frying day 5, pyrazines demonstrated a steady decrease in chips fried in HYCO and LLCO, whereas for chips fried in RCO and HOCO there was an increase at storage day 2 followed by a decrease (Figure 4.27). The total amount of pyrazines after 16 days of storage for both frying days was similar in potato chips fried in HOCO and LLCO, whereas potato chips fried in RCO and HYCO had lower levels for frying day 5 compared to frying day 1. Pyrazines in potato chips are associated with potato flavour (Melton *et al.*, 1993).

The amount of total volatiles in frying day 1 potato chips showed a gradual increase for potato chips fried in HYCO, LLCO and HOCO, whereas there was a rapid increase in volatiles for chips fried in RCO (Figure 4.28). The amount of total volatiles in potato chips fried in RCO was about 25-40 times greater than in chips fried in the other oils. For the chips fried in HYCO from frying day 5, there was a slight decrease in total volatiles over 16 days of storage (Figure 4.29). In contrast, chips fried in HOCO and LLCO demonstrated a modest increase in total volatiles whereas the potato chips fried in RCO had a dramatic increase which was about 2.5 times smaller than it was for frying day 1. The amounts of total volatiles in potato chips fried in HYCO and HOCO were lower for frying day 5 as compared to frying day 1. In contrast, potato chips fried in LLCO had a higher accumulation of total

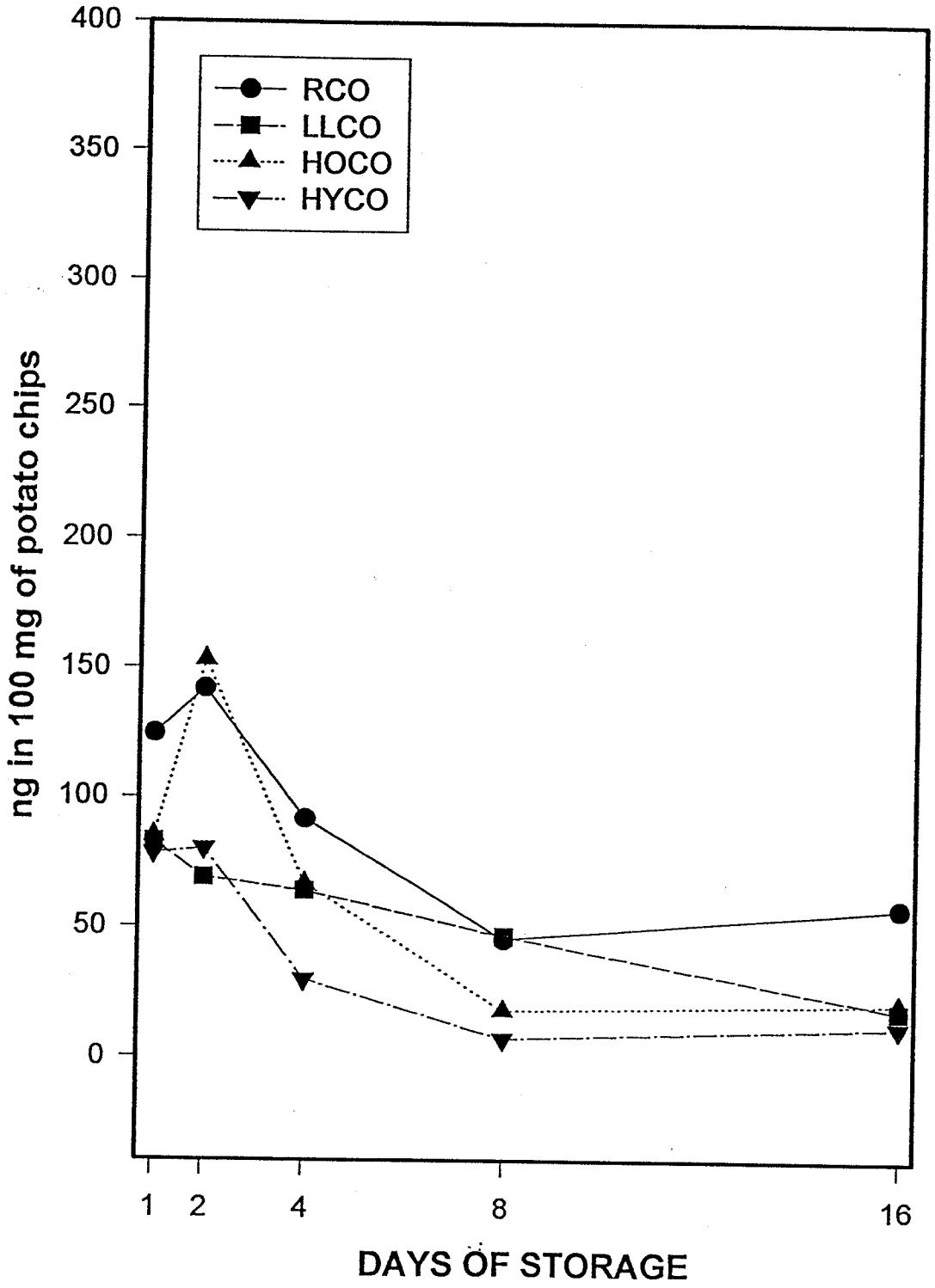


Figure 4.27. Pyrazines in Potato Chips (Frying Day 5).

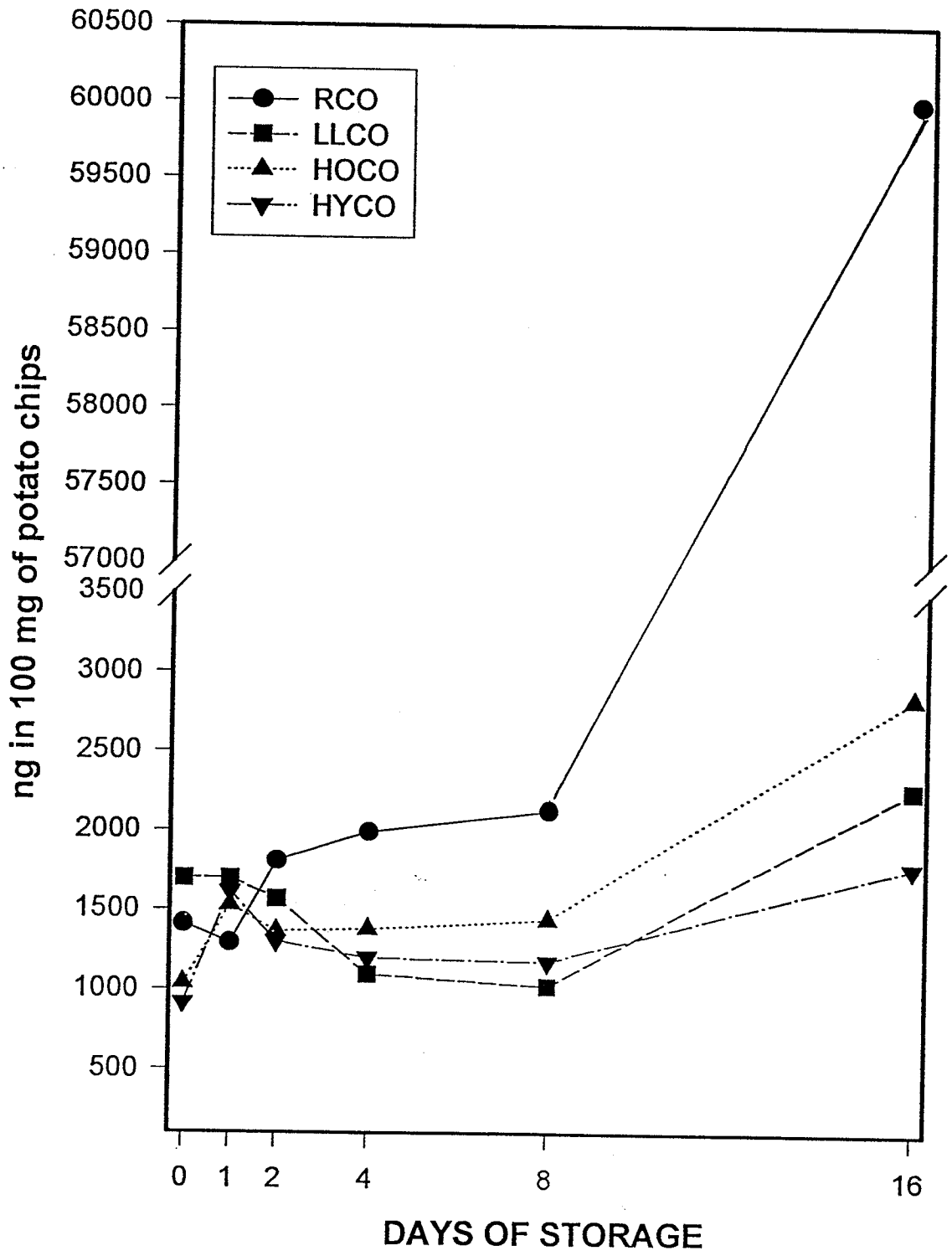


Figure 4.28. Total Volatiles in Potato Chips (Frying Day 1).

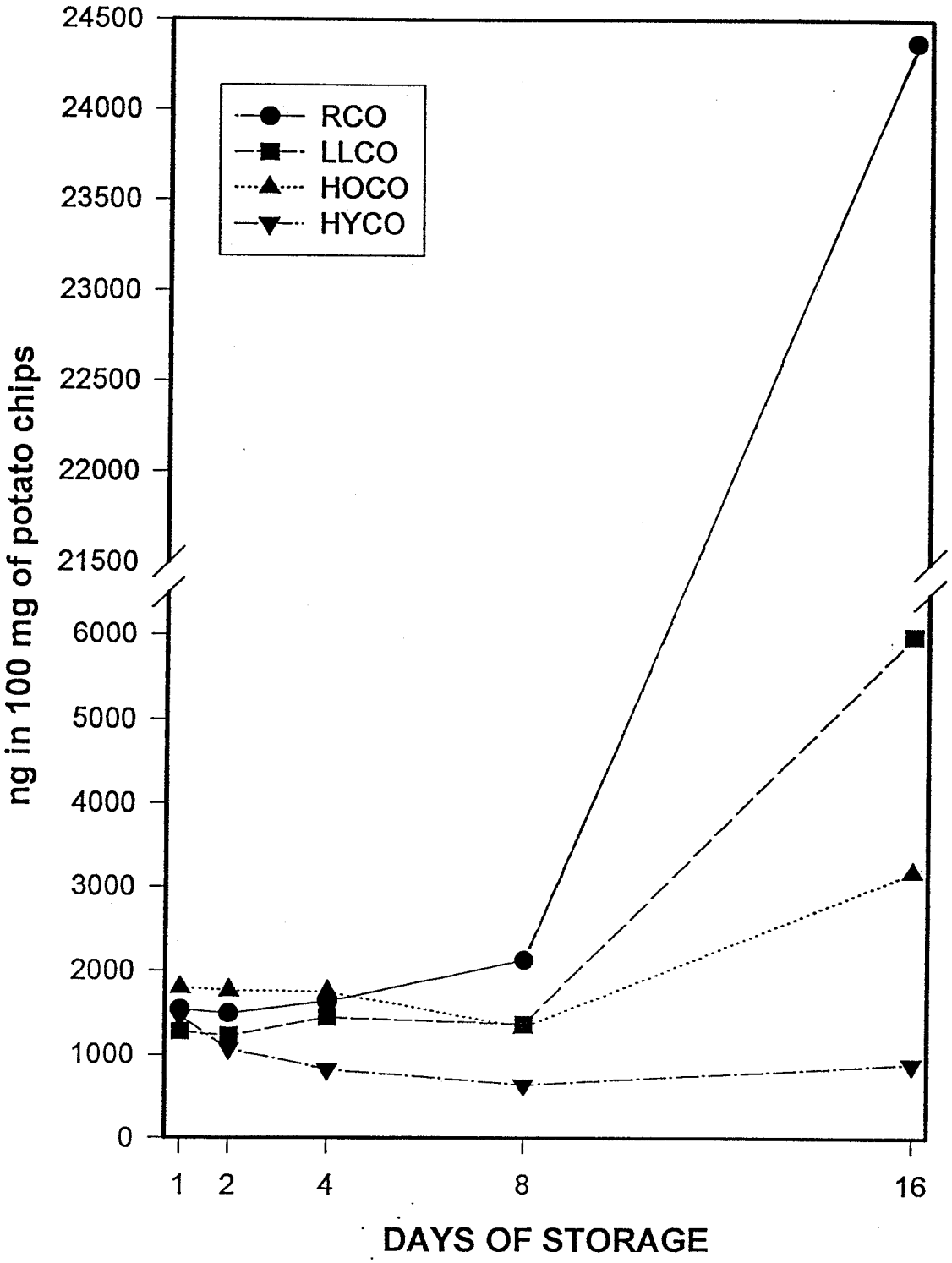


Figure 4.29. Total Volatiles in Potato Chips (Frying Day 5).

volatiles after 16 days of storage for frying day 5 than day 1.

The analyses of volatiles in the references used for the sensory evaluation of oxidized odours are presented in Table 4.4. The RCO reference had the highest amounts of total volatiles, especially saturated and unsaturated carbonyls which are associated with the intensity of oxidized odours. The least accumulation of volatiles in each group was found in HYCO reference. Both LLCO and HOCO references had similar levels of total volatiles, with LLCO reference having higher accumulation of saturated carbonyls and HOCO having higher accumulation of unsaturated carbonyls.

4.3.3 Summary of Results

In summary, the RCO potato chips had:

- a significant storage day effect with painty odour increasing as storage increased,
- significantly greater rates of accumulation of peroxides than HYCO and HOCO chips for both frying days,
- significantly greater rates of accumulation of FFA than HYCO, LLCO and HOCO chips on frying day 1, and HYCO and HOCO chips on frying day 5,
- significantly greater rates of accumulation of CDA and polar components than HYCO, LLCO and HOCO chips for both frying days,
- higher amounts of total volatiles after 16 days of storage compared to HYCO, LLCO and HOCO chips for both frying days.

Table 4.4. Volatiles Analyses in Sensory References of Oxidized Odours (ng/100mg).

Groups of Volatiles	RCO painty	HYCO stale/ musty	LLCO painty/ rancid	HOCO painty
HC[1]	1897.0	3.1	253.7	159.6
SC[2]	14576.0	93.9	315.5	163.8
UC[3]	5264.1	62.7	115.8	225.7
Dienals	2799.8	14.7	128.3	105.9
Pyrazines	72.8	19.5	10.0	14.6
Total	42472.9	835.6	1689.1	1889.2

[1] - Hydrocarbons,

[2] - Saturated Carbonyls,

[3] - Unsaturated Carbonyls.

The HYCO potato chips had:

- a significant storage day effect with stale/musty odour increasing as storage increased;
- significantly greater rate of stale/musty odour accumulation for frying day 5 than frying day 1,
- significantly lower rates of accumulation of FFA than RCO and LLCO chips for both frying days
- significantly lower rates of accumulation of peroxides and CDA than RCO, LLCO and HOCO chips on frying day 1 and RCO and LLCO chips on frying day 5,
- significantly lower rates of accumulation of polar components than RCO chips for both frying days,
- lower amounts of total volatiles after 16 days of storage compared to RCO, LLCO and HOCO chips for both frying days.

The LLCO potato chips had:

- a significant storage day effect with fresh potato chip/frying oil odour decreasing and painty/rancid odour increasing as storage increased,
- significantly greater rates of accumulation of peroxides than HYCO and HOCO chips for both frying days,
- significantly lower rates of accumulation of FFA than RCO chips on frying day 1, significantly greater rates of accumulation of FFA than HYCO and HOCO chips on frying day 1 and HOCO potato chips on frying day 5,
- significantly lower rates of accumulation of CDA than RCO chips and significantly greater rates of accumulation of CDA than HYCO and HOCO chips for both frying days,
- significantly lower rates of accumulation of polars than RCO chips for both frying days,
- intermediate amounts of total volatiles for both frying days.

The HOCO potato chips had:

- a significant storage day effect with fresh frying oil/potato chip odour decreasing and painty odour increasing as storage increased,
- significantly lower rates of accumulation of peroxides than RCO and LLCO chips for both frying days, and significantly greater rates of accumulation of peroxides than HYCO chips on frying day 1,
- significantly lower rates of accumulation of FFA than RCO and LLCO chips for both frying days,
- significantly lower rates of accumulation of CDA than RCO and LLCO chips for both frying days, and significantly greater rates of accumulation of CDA than HYCO chips on frying day 1,
- significantly lower rates of accumulation of polar components than RCO chips for both frying days,
- intermediate amounts of total volatiles for both frying days.

4.4 DISCUSSION AND CONCLUSIONS

The sensory evaluation of potato chips stored for 16 days at 60°C showed that there was a decrease in the intensity of fresh potato chip/frying oil odour for chips fried in LLCO and fresh frying oil/potato chip odour for chips fried in HOCO as storage increased. Similar results were demonstrated by Hawrysh *et al.*(1995) when tortilla chips were stored for 16 days at 60°C where the intensity of characteristic tortilla chip odour decreased slightly in chips fried in low linolenic and partially hydrogenated canola oils, and dropped dramatically in chips fried in RCO. A similar trend in the reduction of characteristic potato chip odour was observed by Hawrysh (1992) in potato chips that had been fried in regular and partially hydrogenated canola oils which had been stored for 12 days under similar conditions. In the present study, painty odour intensity increased in chips fried in RCO and HOCO, painty/rancid odour intensity increased in chips fried in LLCO and stale/musty odour intensity increased in chips fried in HYCO as storage time increased. These findings are supported by Hawrysh (1992) who reported that RCO potato chips increased in intensity of off, rancid and painty odour over 12 days of storage. Both RCO and LLCO tortilla chips were shown by Hawrysh *et al.* (1995) to have higher intensity of off, rancid and painty odour during 16 days of storage. However, tortilla chips fried in LLCO only had a slight increase in these odours, whereas chips fried in RCO had a substantial

increase in intensity of these odours. In the latest review of sensory evaluation methods for oils and fried products, Warner (1995) reported that during early stages of oxidation the stale odour is present in fried products. Later, in more advanced stages, oxidation is expressed in rancid and painty odours. This could means that during 16 days of storage in the present study, chips fried in HYCO were in the early stages of oxidation, since their odour was described as stale/musty. The potato chips fried in the other three oils had undergone further oxidation, since they had achieved painty and rancid odours during storage.

In the present study, potato chips fried in the four oils could not be compared directly since different odour attributes and references were used. However, some indication of the potential relationship among the odour attributes can be found by examining the volatiles present in the references used for the sensory evaluation of oxidized odours. Since the reference that was used to evaluate the chips fried in RCO had the highest level of degradation as indicated by elevated amounts of total volatiles, especially saturated and unsaturated carbonyls, it is reasonable to assume that it had the highest intensity of painty odour. Similarly, the reference that was used to evaluate the potato chips fried in HYCO had the lowest level of deterioration as exhibited by reduced amounts of total volatiles. This is consistent with

the panelists using the term stale/musty for chips fried in HYCO.

The results of chemical analysis of the oils extracted from potato chips fried in all four oils showed an increased accumulation of peroxides, FFA, CDA, polar components and total volatiles with increased storage. The potato chips fried in RCO were the least stable among the tested chips because they developed all components at the fastest rate, and the accumulation of volatiles was the highest among all chips at the end of the storage period. The potato chips fried in HYCO were the most stable since they developed the degradation compounds at the slowest rate and accumulation of volatiles was the lowest among all potato chips after 16 days of storage. Potato chips fried in LLCO and HOCO had better storage stability than chips fried in RCO since they had lower rates of accumulation of peroxides, FFA, CDA and polars. Chips fried in LLCO and HOCO had lower amounts of total volatiles after 16 days of storage than RCO chips. Among potato chips fried in LLCO and HOCO, the chips fried in HOCO showed better stability to oxidation than potato chips fried in LLCO because they had lower rates of accumulation of peroxides, FFA and CDA. Only for frying day 5, the total amount of volatiles in potato chips fried in HOCO was smaller than in chips fried in LLCO after 16 days of storage. For frying day 1 the results for potato chips fried in both oils were similar.

These findings are in agreement with results by Hawrysh (1992) who found that potato chips fried in RCO had much greater accumulation of peroxides and CDA than those fried in partially hydrogenated canola oil. The amount of total volatiles was dramatically increased in potato chips fried in RCO as compared to those fried in partially hydrogenated canola oil. In a later study by Hawrysh (1993) and Hawrysh *et al.* (1995) tortilla chips fried in RCO were found to produce a considerably higher levels of peroxides after 16 days of storage at 60°C than chips fried in partially hydrogenated and LLCO. The tortilla chips fried in partially hydrogenated canola oil accumulated a significantly lower level of peroxides than chips fried in LLCO after 16 days of storage. The amount of FFA, CDA and total volatiles after 16 days of accelerated storage at 60°C was the highest in tortilla chips fried in RCO, followed by those fried in LLCO and partially hydrogenated canola oils.

The results of the present study are also in agreement with the findings of Liu and White (1992) who reported increased accumulation of peroxides in bread cubes fried in regular soybean oil compared to cubes fried in two low linolenic oils and stored for 7 days at 60°C. In contrast to the present study, Hawrysh (1992) did not find the amounts of FFA to be different for potato chips fried in RCO compared to partially hydrogenated canola oil during 12 days of storage at 60°C.

Observed changes in perceived odour properties were supported in the present study by the data from volatile analysis. The decrease in intensity of fresh potato chip/frying oil odour in potato chips fried in LLCO and fresh frying oil/potato chip odour in potato chips fried in HOCO during 16 days of storage was accompanied with a decrease in the amount of pyrazines. Similarly, the increase in painty, stale/musty, painty/rancid and painty odour in chips fried in RCO, HYCO, LLCO and HOCO respectively corresponded to an increase in the amounts of total volatiles in the chips, especially saturated and unsaturated carbonyls which are associated with oxidized odours, as storage progressed.

The effect of oil degradation products on potato chip storage stability was shown in chips fried in HYCO when the rate of stale/musty odour accumulation increased on frying day 5 compared to frying day 1. This supports the findings of Asap and Augustin (1986) who reported that the longer the oil was used for frying the faster rancid odour developed in potato chips stored at 60°C. The increased oxidation in the presence of degradation products is expected to induce the rise in the amounts of total volatiles (Yoon *et al.*, 1988). However, in the present study a decrease in levels of total volatiles in chips fried in RCO and HYCO from frying day 5 as compared to frying day 1 was observed. The levels of total volatiles were similar in the potato chips fried in HOCO from both frying days. Only the potato chips fried in LLCO from frying day 5

showed a higher accumulation of volatiles after storage than chips from frying day 1. The rate of accumulation of peroxides in chips fried in HOCO was significantly greater for frying day 1 than for day 5. Similarly, the accumulation rates of FFA and CDA in chips fried in RCO, LLCO and HOCO were significantly greater for frying day 1 than for day 5.

The results clearly showed that the oxidation of potato chips during storage is accompanied by increased accumulation of primary and secondary oxidation products as measured by PV, CDA, FFA and polars, as well as total volatiles. The sensory results also showed that storage had a significant effect on increasing the intensity of oxidised odours in the chips. Potato chips fried in RCO containing the highest level of 18:3 (10.1%) were found to be the least stable, whereas potato chips fried in HYCO containing no 18:3 were found to be the most stable. Potato chips fried in HOCO were found to have similar storage stability to potato chips fried in HYCO, and potato chips fried in LLCO were slightly better in stability than chips fried in RCO.

The effect of oil degradation products on the storage stability of chips requires more study.

CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 GENERAL CONCLUSIONS

The overall objective of this research was to evaluate the frying performance of two genetically modified canola oils, LLCO and HOCO, and to determine the storage stability of potato chips fried in these oils.

The initial evaluation of fresh RCO, HYCO, LLCO and HOCO showed that the genetically modified oils had decreased levels of 18:3 (LLCO had levels of 3.7% and HOCO had levels of 5.5%) compared to RCO with levels of 10.8%. All oils were of good initial quality as measured by PV and FFA, however, increased levels of linolenic acid *trans* isomers were found in the HOCO suggesting the use of deodorization temperatures above 245°C during processing.

Determination of the frying performance of the oils indicated that all oils showed signs of degradation after 40 hours of frying. LLCO and HOCO had similar frying stability to HYCO, but HYCO had increased accumulation rates of FFA and polymers which can be attributed to the additional processing step, i.e hydrogenation. Both LLCO and HOCO showed some improvement over RCO in frying stability as measured by FFA, CDA and polars, but it was not as pronounced as expected. There are several explanations for this finding. The use of an antifoaming agent which can exhibit antioxidant properties when added to an oil, will retard the degradation of the oil.

Although, the antifoaming agent was added to all oils, it is possible that the antioxidant effect may have differed depending on the oil. Secondly, the high initial levels of tocopherols found in RCO compared to HOCO and LLCO may have slowed down the degradation of the RCO (Li, 1996). Thirdly, the addition of 10-15% fresh oil to the used oil each morning prior to frying may have slowed down the decomposition of the oil by diluting the amount of degradation products in the oil. For RCO and HYCO, which had high levels of tocopherols in the fresh oil, the addition of fresh oil would have introduced additional levels of tocopherol. With regards to the performance of the HOCO, it appears that the initial quality of the oil was somewhat impaired by the use of a high deodorization temperature during processing as suggested by the presence of 18:3 geometrical isomers and elevated initial levels of polar components.

The investigation on the storage stability of potato chips fried in these oils revealed that all chips accumulated primary and secondary oxidation products and had increased intensity scores for oxidation odours. Potato chips fried in RCO had higher rates of accumulation of peroxides, FFA, CDA and polars, and higher amounts of total volatiles compared to chips fried in HYCO, LLCO and HOCO, indicating lower storage stability of chips fried in RCO. Chips fried in HYCO had lower rates of accumulation of peroxides and CDA than chips fried in LLCO and HOCO, lower rates of FFA accumulation than chips

fried in LLCO, and no difference in the rates of polars accumulation during 16 days of storage compared to chips fried in LLCO and HOCO. Chips fried in HYCO and HOCO had the lowest amounts of total volatiles during storage. All chips showed an increase in intensity of oxidized odours over the storage period. However, it was not possible to compare results directly from the sensory evaluation of potato chips fried in the different oils since different odour attributes were measured. However, some indication of potential relationship was obtained from the analysis of volatiles in the stored sensory references that represented the end points on the line scales used to rate the intensity of oxidized odours in potato chips. The reference for chips fried in RCO had the highest accumulation of total volatiles among the four different references used. This suggests that the odour intensity in the reference used to evaluate the potato chips fried in RCO was likely higher than in the other references and consequently the potato chips fried in RCO had much stronger oxidized odours than the potato chips fried in the other three oils.

The changes in sensory properties of potato chips are supported in the present study by the results of chemical and instrumental measurements. For example, the decrease in intensity of fresh potato chip/frying oil odour in chips fried in LLCO and fresh frying oil/potato chip odour in chips fried in HOCO during 16 days of storage was accompanied with a decrease in the amount of pyrazines, which are associated with

potato odour. Similarly, the increase in the intensity of painty, stale/musty, painty/rancid and painty odour in potato chips fried in RCO, HYCO, LLCO and HOCO, respectively, corresponded to an increase in the amounts of peroxides, FFA, CDA polars and total volatiles in the potato chips as storage progressed.

The prooxidative effect of frying oil degradation products on the storage stability of potato chips was demonstrated only for HYCO chips by an increased rate of stale/musty odour for frying day 5 compared to frying day 1. In contrast to studies that have found that oil degradation products have a prooxidative effect on storage stability, the results from this study showed that chips from frying day 5 usually had a slower accumulation of degradation products during storage compared to chips from frying day 1.

5.2 RECOMMENDATIONS FOR FUTURE RESEARCH

In the present study all oils except HOCO were industrially processed. Comparison of the initial quality of fresh oils used in the study revealed that HOCO had increased amounts of 18:3 isomers which is indicative of a deodorization temperature higher than 245°C being applied during processing. Thus, further investigation is needed which compares the frying performance of all industrially processed canola oils.

The odour of the potato chips fried in the various oils could not be compared directly since different odour attributes were used by the panel to monitor changes in the chips during storage. During training, panelists were not able to identify common attributes to describe the odour of the potato chips fried in the four oils. In future studies, more effort will be needed to train the panel to use common attributes and references to evaluate the storage stability of potato chips fried in the various oils. This is necessary so that direct comparisons can be made across all chips.

Potato chips from frying day 1 showed more accelerated oxidation compared to frying day 5 as indicated by most of the sensory, chemical and instrumental data. However, higher rates of accumulation of stale/musty odour were found in HYCO chips, and increased levels of total volatiles for LLCO chips were observed for frying day 5 compared to frying day 1. Therefore, additional investigation is needed to determine the effect of

frying oil degradation products on storage stability of fried foods.

As a guideline for future studies on frying performance of the oils, the five day frying protocol proved to be sufficient to observe differences between the oils. Future studies on the storage stability of the fried foods could be done using chips from frying day 1 since both frying day 1 and frying day 5 samples showed similar trends.

Among the chemical and instrumental tests for evaluation of frying performance of the oils, the determination of polars by TLC-FID has potential because it measures individual components separately and takes into account differences in chemical structures of the degradation products. It is also a rapid method when a large number of the samples need to be analyzed. The use of FA composition analysis may be omitted in future studies because the results did not provide any additional information about the degree of oil degradation. Polymers determination from FA composition analysis is not recommended since better results may be obtained if the direct method for polymers is used.

The colorimetric PV method is rapid, easy and showed high correlations with the titration method, which is currently used to measure peroxides in oils. However, more work needs to be done to determine why the amounts of peroxides differ when determined by two methods.

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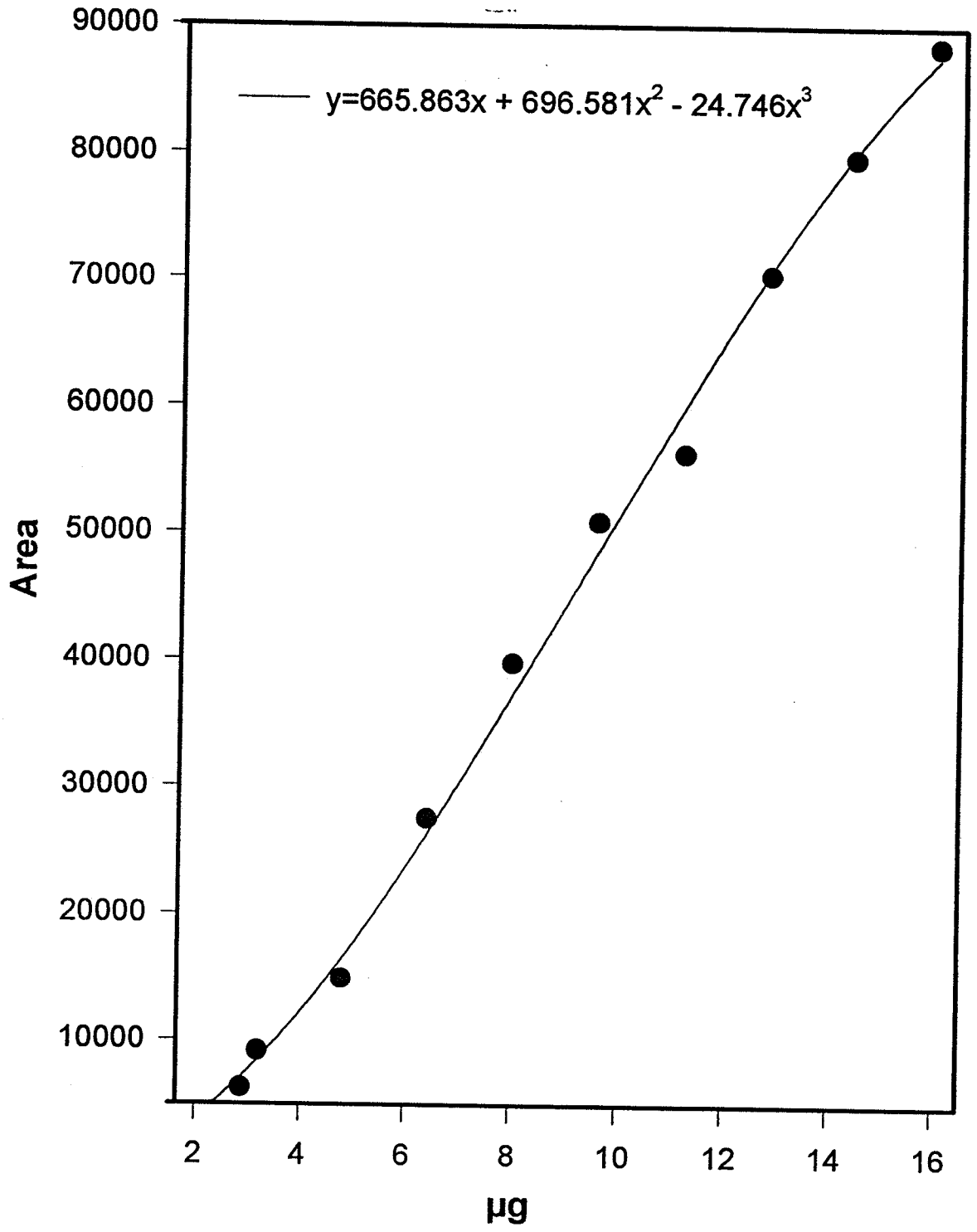
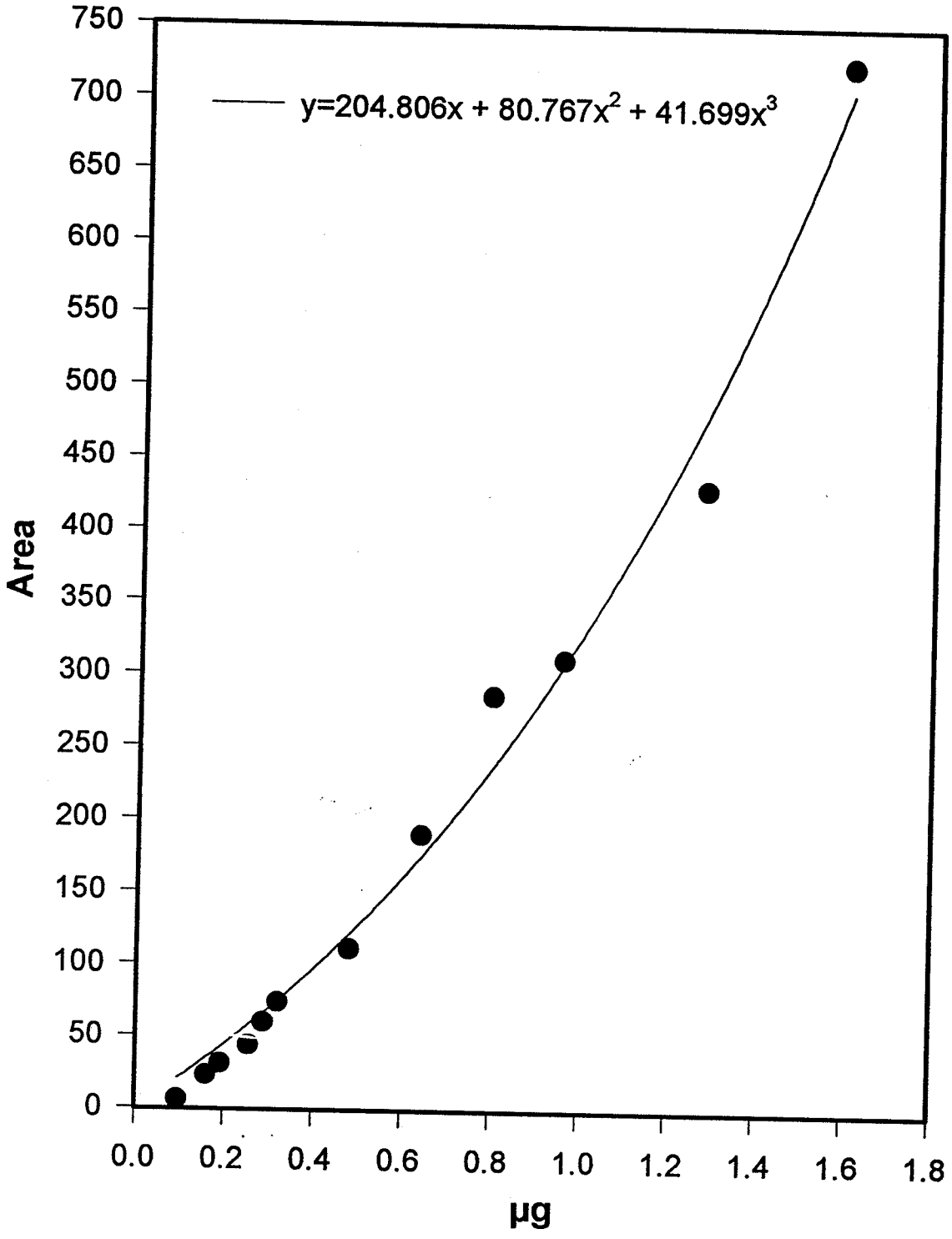
Appendix 1.1.

Calibration Curve for Triglycerides Determination for Polar Components Measurement by TLC-FID.



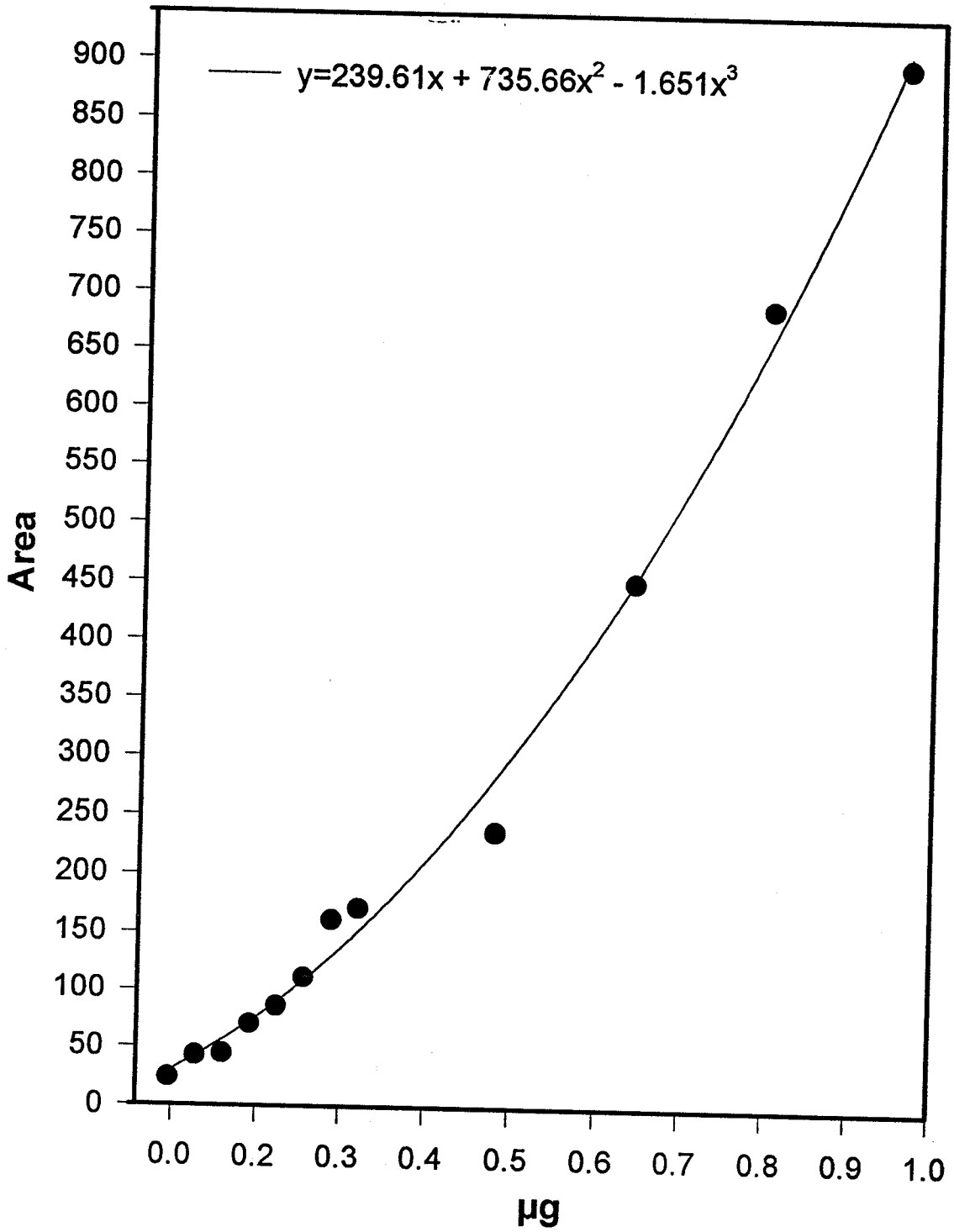
Appendix 1.2.

Calibration Curve for Free Fatty Acids Determination for Polar Components Measurement by TLC-FID.



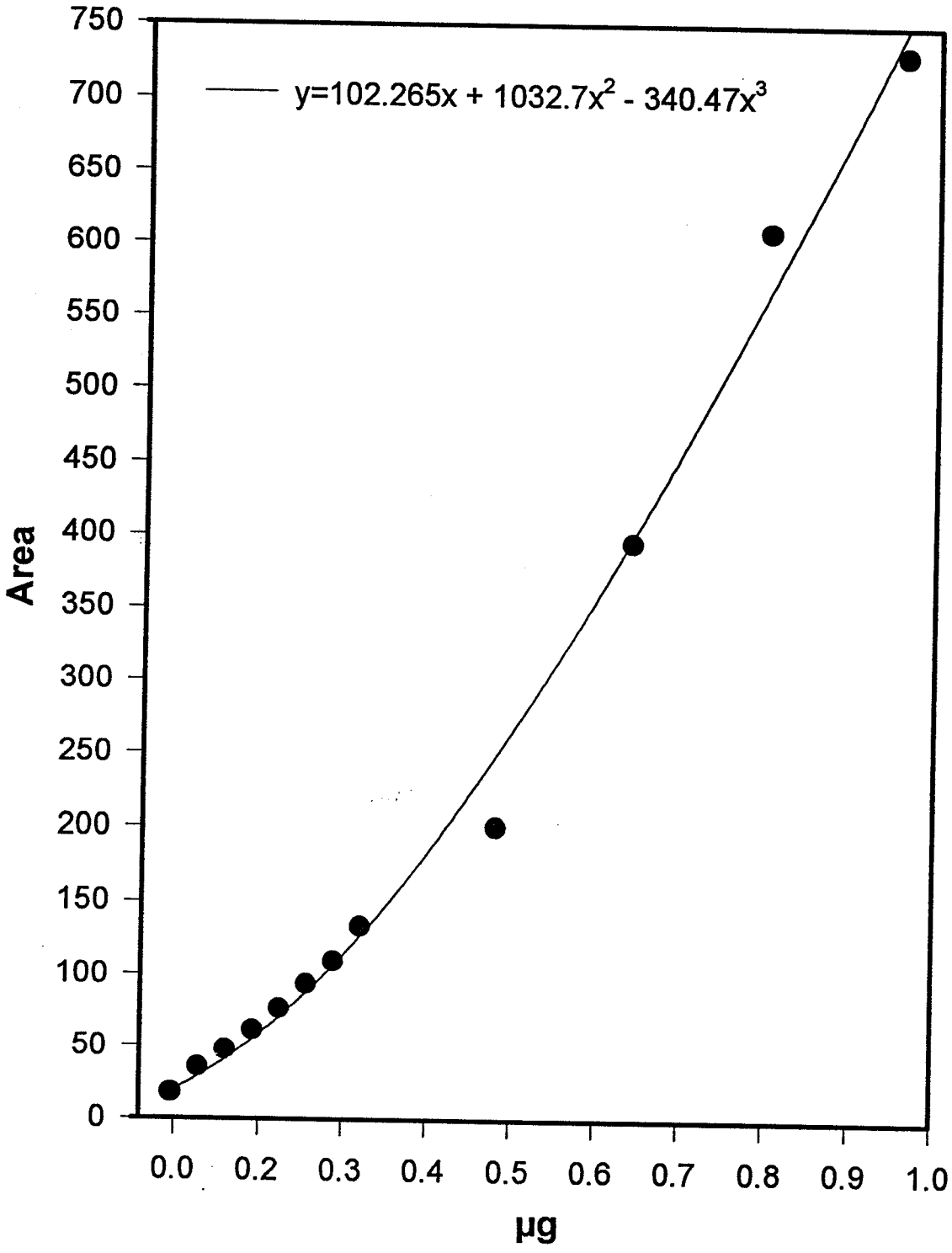
Appendix 1.3.

Calibration Curve for 1,2-Diglycerides Determination for Polar Components Measurement by TLC-FID.



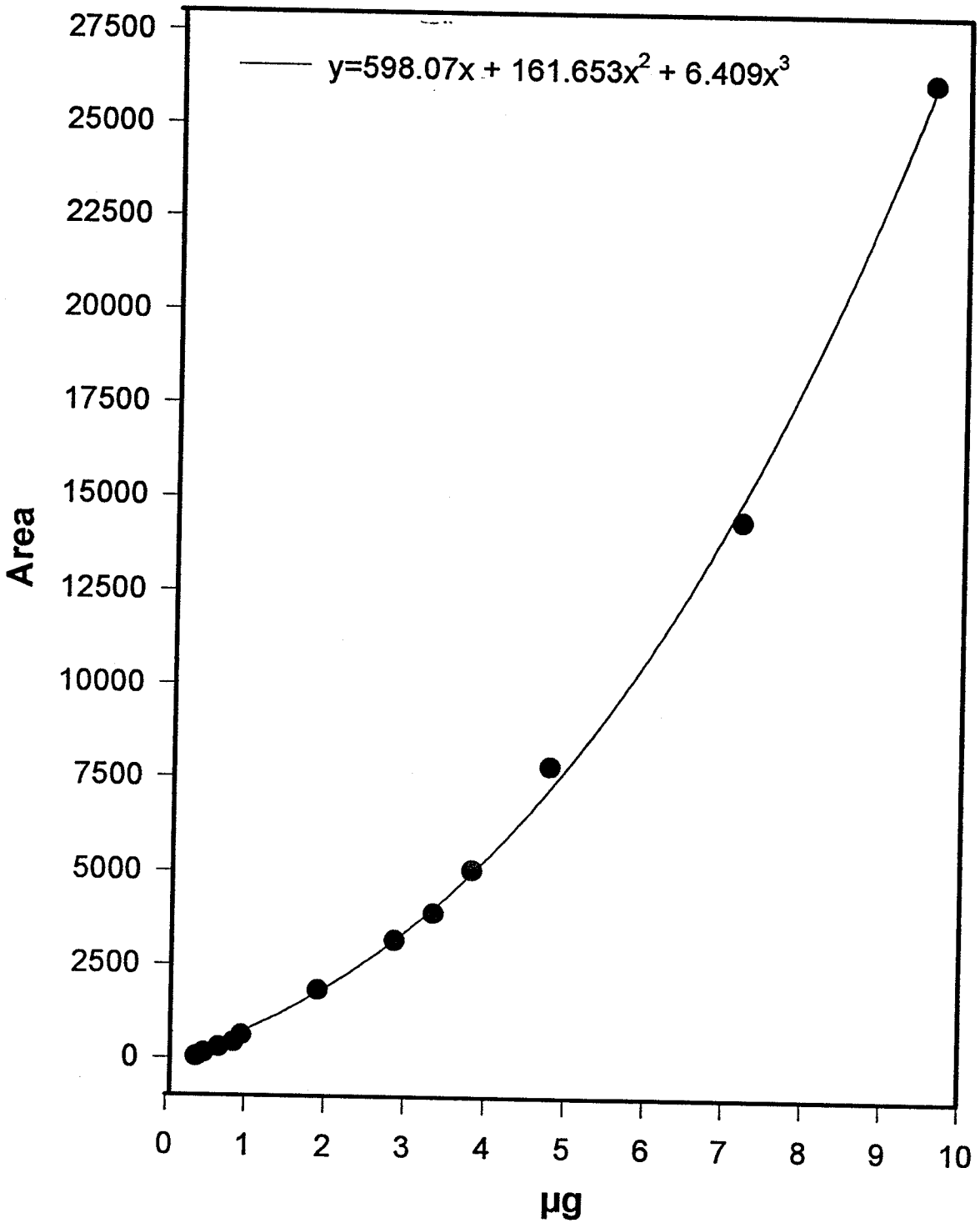
Appendix 1.4.

Calibration Curve for 1,3-Diglycerides Determination for Polar Components Measurement by TLC-FID.



Appendix 1.5.

Calibration Curve for Highly Polar Compounds Determination for Polar Components Measurement by TLC-FID.



Appendix 2

Approval for Research Proposal Involving Human Subjects

UNIVERSITY OF MANITOBA

FACULTY OF HUMAN ECOLOGY

APPROVAL FOR RESEARCH PROPOSAL INVOLVING HUMAN SUBJECTS

This is to certify that Dr. Linda Malcolmson, of the Faculty of Human Ecology, submitted a proposal for a research project entitled:

Storage Stability of Potato Chips Fried in Canola Oils with Modified Fatty Acid Composition

The Faculty of Human Ecology Ethics Review Committee is satisfied that the appropriate ethical criteria for research involving human subjects have been met.

Members of the Committee:

Name	Position	Department
J. Bond	Professor	Family Studies
M. Campbell	Associate Professor	Foods and Nutrition
W. Pelton	Associate Professor	Clothing and Textiles

Date: September 20, 1994

Rosemary Mills
Committee Chair

Appendix 3

Letter of Invitation to Participate in Sensory Panel

THE UNIVERSITY OF MANITOBA

FACULTY OF HUMAN ECOLOGY

Department of Foods and Nutrition

Human Ecology Building
Winnipeg, Manitoba
Canada R3T 2N2

(204) 474-6830
(204) 275-5299 FAX

October 17, 1994

Dear _____:

As part of my thesis research in the Foods and Nutrition department in the Faculty of Human Ecology, I am planning to use a trained sensory panel to study potato chips odour. I invite you to participate in this project. This letter will explain what your commitment will be and the tasks involved. If you have any questions please call me at 474-6874.

In order to assess your sensory capability for this food product two screening sessions of approximately 20 min each will be conducted. Your role on successful completion of the screening would be to examine the characteristic odour of a series of potato chip samples. There will be approximately 10 to 12 training sessions of 30 minutes each followed by 16 test sessions (15 minutes each).

You will receive a $20 gift certificate redeemable at the University of Manitoba Bookstore after you have completed all of the sessions.

Results will not be reported by individuals' names nor will any names be associated with the results. Participants will be identified by numbers only. All data will be kept strictly confidential and under lock and key until published, or for five years whichever is shorter.

The testing will take place during the weeks of October 24 - December 20, 1994 in the new George Weston Limited Sensory and Food Research Laboratory, Room 400 of the Human Ecology Building.

By participating in this research, you would gain experience in sensory evaluation and insight into how the sensory measurements are made.

Please confirm your participation by completing 1) the consent form, 2) the schedule listing your class times and 3) panelist questionnaire. Return these attachments to me by October 24, 1994. Once confirmations are received you will be advised of the test schedule.

I hope that you will be able to assist us in carrying out this research. Thank you.

Sincerely yours,

Iana Petukhov, Graduate Student

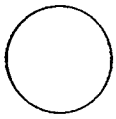
encls.
cc. L.Malcolmson
R.Przybylski

Appendix 4

USE OF LINE SCALES

Name _____ Date _____

Estimate the proportion of the circle that is shaded and place a vertical mark across the line scale to indicate that amount.



none _____ all

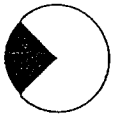
none _____ all

none _____ all

none _____ all

none _____ all

none _____ all



none _____ all

none _____ all

Appendix 5

Procedure for Colorimetric PV Determination

About 30 mg of oil was weighed accurately (to 0.0001g) into 4 ml screw top vial. Three ml of n-Propyl alcohol was added into the vial and the content mixed by inverting the vial 10 times.

The FOX 2 reagent was prepared as follows:

a. Prepare stock solution in 100 ml volumetric flask which contains 5 mMol of Xylenol Orange, 7.5 mMol Ammonium Ferrous Sulfate and 250 mMol of Sulfuric Acid (H_2SO_4).

b. Prepare a 4.4 mMol solution of BHT (Butyl Hydroxy Toluene) in Methanol.

c. Prepare the working solution of FOX 2 Reagent by mixing of one part of stock solution with 9 parts of BHT-Methanol. The final concentration of components: 500 μMol of Xylenol Orange, 1.25 m Mol Ammonium Ferrous Sulfate, 25 mMol of Sulfuric Acid, 4 mMol of BHT in 90% Methanol. The sulfuric acid in the solution is essential as the ROOH oxidizes Fe^{2+} selectively in acidic environment (Wolff, 1994).

4.5 ml of the working FOX 2 reagent (9 parts) and 0.5 ml of a sample solution in n-propanol (1 part) was pipetted into a 20 ml screw top tube. The content of the tube was mixed well using Vortex. The mixture was then incubated at room temperature for 30 min and centrifuged for 5 min at 1200 rpm.

Along with the samples a blank was prepared to calibrate the spectrophotometer. 4.5 ml of FOX 2 Reagent and 0.5 ml of n-propanol were added into the tube.

The 5 ml capacity spectrophotometric tubes were prepared. The spectrophotometer cuvette was washed with methanol 2-3 times before taking any reading. The content of the "Blank" tube was transferred into a spectrophotometric tube. The spectrophotometer SP6-300 (PYE UNICAM) was zeroed with a blank, and the reading was repeated.

The content of the tubes containing the samples was transferred into the spectrophotometric tubes using a disposable plastic pipet, taking care not to disturb any sediment at the bottom of the screw top tube. Absorbance was read at 560 nm. The absorbance reading should fall within the range of 0.1 to 0.9. If the sample did not fall within the range, the sample was diluted or its amount was increased. All the samples were run in duplicate.

At the end of the day the instrument cuvette was washed 2-3 times with methanol.

Appendix 6

Preparation of Calibration Curve for Colorimetric PV Determination

The calibration curve represents the relationship between the amount of ferric ion in the solution (Fe^{3+}) and the absorbance of this solution at wavelength 560 nm. Ferric chloride ($FeCl_3 \cdot 6H_2O$) was used as a source of ferric ion. The stock solution was prepared by dissolving 49.2 mg of ferric chloride in 100 ml of distilled water. All mixing was done in 100 ml volumetric flask. Before the level of distilled water was brought to the notch, 2.5 ml of 3.6% of hydrochloric acid (HCl) added to ensure the acidity of the solution. The stock solution was then diluted further to different concentrations which were later used to obtain points for calibration curve (Appendix 6).

The procedure for obtaining the calibration curve points is the same as described in Appendix 4, except that the content of the 25 ml flask is a ready to use in place of sample solution.

Appendix 7

Calibration curve for colorimetric PV determination

Point on calibration curve	Amount of stock solution in 25 ml volumetric flask (ml)	Concentration of Fe^{3+} in an assay (μg)
1	1.25	2.54
2	2.5	5.08
3	3.7	7.52
4	4.5	9.15
5	5.2	10.57
6	6.2	12.61
7	7.5	15.25
8	8.7	17.69