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# Stabilizing soybean oil for industrial use

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Signatures have been redacted for privacy

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## ABSTRACT

There is considerable interest in using vegetable oils, such as soybean oil, as lubricants and hydraulic fluids because of their biodegradability. The primary factor limiting the use of vegetable oils in these applications is an increase in viscosity caused by oxidation. Oxidation rates are determined by fatty acid composition, metal ions, antioxidants, and temperature. To test antioxidants, the Active Oxygen Method of the American Oil Chemists' Society was modified. Twenty milliliters of soybean oil was heated to 105°C, about 10 mg each of colloidal copper and iron were added, and air was bubbled through the oil at 2.33 ml/second. The viscosity of the sample was measured periodically with a Brookfield cone and plate viscometer. Antioxidants and metal chelators were initially screened at 0.01% by weight. The best antioxidants were chosen by statistical analysis and tested at higher concentrations. The best antioxidant and chelator combination was tert-butylhydroquinone at 1.28% and citric acid at 0.02%. This increased the stability of soybean oil about 5.5 fold.

Industry is now developing high-oleic soybean oil varieties that possess improved oxidative stability over normal soybean oil. However, problems arise in producing special variety oils. First, there is extra expense in seed. Secondly, poor oil yields are common. One alternative to growing special varieties to improve the oxidative stability of oil is to concentrate oleic acid. This can be accomplished by the formation of urea complexes. Urea complex formation is an old method of separating fatty acids based on length and degree of saturation. To fractionate soybean oil, 10 g of soybean oil methyl esters were combined with urea, methanol and saturated methanol in a series of

crystallizations. We were able to create three distinct fractions: oleate-rich, saturate-rich and polyunsaturated-rich fractions. The composition of the oleate-rich fraction was 0% palmitate, 0% stearate, 63.8% oleate, 22% linoleate and 3% linolenate. This fraction lowered linoleate and linolenate levels from 54% to 22% and 5.8% to 3% respectively. Also, the fraction elevated oleic acid from 26.4% to 63.8%.

# CHAPTER 1. GENERAL INTRODUCTION

## Introduction

Today, adequate petroleum is available to meet the world demands, but the costs for extracting, transporting and disposing of petroleum are rising. These factors, coupled with a rising concern for the environment, have increased the study of vegetable oils for use as lubricants (Honory and Boeckenstedt, 1998). Early in our history, when the world relied heavily on agriculture, vegetable oils and animal fats were used as mechanical lubricants. It is unlikely that vegetable oils will surpass petroleum; however, as more research is completed, new and more diverse applications, unique to vegetable oils, will emerge (Honory and Boeckenstedt, 1998).

Vegetable oil lubricants possess unique and superior features to petroleum, such as: biodegradability, higher flash point, lower toxicity, lower emissions and improved gas mileage (Honory and Boeckenstedt, 1998, Johnson, 1998). Disadvantages include: low oxidative stability, poor low-temperature properties and a bad reputation for hydraulic stability (Lal, 1995). According to Renewable Lubricants, William Garmier (1998), "The biggest obstacle for vegetable oil as a lubricant is high temperature stability, but the addition of antioxidants improves soybean oil performance."

Thus, one goal of our research was to test the efficacy of antioxidants by using viscosity increase as an indicator of oxidative stability.

DuPont has developed a high oleic soybean oil variety with improved oxidative stability that may make soybean oil more competitive in the lubricant market (Lawate and Glancey, 1998). However, there are problems in producing modified vegetable oils. First,

there is an extra expense in purchasing seeds and the segregation of seeds with this specific trait. Secondly, poor yields of special oilseed varieties are common.

One alternative to growing special varieties of soybeans to improve the oxidative stability of soybean oil is to concentrate the oleic acid in ordinary soybean oil. This can be accomplished by formation of urea complexes. Formation of urea complexes is an old, but simple method to separate fatty acids based on carbon chain length and the degree of unsaturation (Swern, 1964). Recently, Hayes et al. (1998) separated saturated fatty acids from unsaturated fatty acids in low erucic acid rapeseed oil (LEAR). Their results showed that urea separation is a viable and economical method.

Therefore, a second goal of our research was to use urea complexes to concentrate oleic acid from ordinary soybean oil for use as an engine lubricant.

## Thesis Organization

This thesis consists of two additional chapters. Chapter 2 "Using Antioxidants to Stabilize Soybean Oil for Industrial Use," includes an introduction, literature review, materials and methods, results and discussion sections. Chapter 3, "Using Urea to Isolate Oxidatively Stable Fractions from Soybean Oil," follows the same format as chapter one. References for all chapters are located at the conclusion of the thesis.

# CHAPTER 2. USING ANTIOXIDANTS TO STABILIZE SOYBEAN OIL FOR INDUSTRIAL USE

## Introduction

Currently, canola and rapeseed oils are primarily used as engine lubricants because of initiatives by Europe and Canada to become more environmentally friendly. In addition, canola and rapeseed oils are more stable to oxidation than soybean oil, so canola and rapeseed oils now are the vegetable oils of choice for lubricants (Honory and Boeckenstedt, 1998). In 1997, soybean oil production totaled 6.2 billion gallons worldwide (Honory and Boeckenstedt, 1998). Excess production of soybean oil stimulated new uses, and engine lubricants are one such use. However, due to its high percentage of linolenic acid and linoleic acid, soybean oil possesses low oxidative stability. According to Fatemi and Hammond, (1980) the relative rates of oxidation for methyl esters of oleate, linoleate and linolenate are 1:10.3:26.6, which signifies that methylene interrupted polyunsaturated fatty acids oxidize more rapidly than mono-unsaturated fatty acids. The poor oxidative stability of soybean oil is a great deterrent to its acceptance by scientists and engineers as an engine lubricant. Thus, the purpose of this project was to use antioxidants to increase the oxidative stability of soybean oil.

## Literature Review

### Fatty Acid Oxidation

The oxidation of oils with large amounts of polyunsaturated fatty acids is usually a concern to the food industry. The main concern is the effect of oxidation on flavor (Min, 1998). However, in the use of vegetable oils as engine lubricants, viscosity increase is a

common concern. Oxidation of soybean oil creates hydroperoxides, which during the final step of oxidation, may cross-link to form dimers that increase viscosity. Light, metals, temperature, fatty acid composition and antioxidants influence oxidation (Min, 1998). In addition, the rate of oxidation of methylene-interupted poly-unsaturated fatty acids is much higher than that of mono-unsaturated fatty acids (Swern, 1961). This is due to the activation of a methylene group by two adjacent double bonds, which results in greater oxidation rates than that of mono-unsaturated compounds (Swern, 1961). Soybean oil contains approximately 55% linoleic acid and 7-9% linolenic acid. Soybean oil is especially unstable to oxidation due to its linolenic acid content (Hammond, 1980).

Singlet oxygen reacts with lipids to form hydroperoxides and initiates a free-radical chain reaction. The formation of singlet oxygen by phytochemicals naturally present in the oil occurs in the presence of photosensitizers. The direct reaction of a lipid with atmospheric oxygen to produce hydroperoxides is very unlikely because the oxygen is in its triplet (stable) state. Therefore, an activation of the oxygen molecule must occur by: 1) formation of singlet oxygen by photosensitizers; 2) formation of hydroperoxides or 3) formation of active iron-oxygen complex (Min, 1998). The most common form of interaction of oxygen and fatty acids is through a free radical chain reaction. The reaction includes three steps (Min, 1998):

Initiation: $RH \rightarrow R\bullet$

Propagation: $R\bullet + O_2 \rightarrow ROO\bullet$
$ROO\bullet + RH \rightarrow ROOH + ROO\bullet$

Termination: $ROO\bullet + R\bullet \rightarrow ROOR$
$R\bullet + R\bullet \rightarrow R\text{-}R$
$2\ ROO\bullet \rightarrow ROOR + O_2$

Initiation involves the formation of a free radical. The propagation step occurs by the reaction of a free radical with triplet oxygen to form a peroxy radical. The peroxy radical can then abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide, and a new lipid radical to propagate the reaction. Termination involves the reaction of two radicals to stop the reaction (Min, 1998). Each step is discussed in detail as follows.

**Initiation**

Initiation can involve RH→ R• or the decomposition of a hydroperoxide (ROOH), which is the main initiation reaction. Metals can also catalyze the initiation of oxidation (Chan, 1987) by:

$$RH + M^{+3} \rightarrow R\bullet + H+ + M^{+2}$$

Hydroperoxides, the main initiators, can do so by two types of reactions, those that involve ROOH only, and those that involve ROOH and metals. The reaction of ROOH only can be broken into two types, unimolecular and bimolecular. Unimolecular is the cleavage of the weak O-O bond:

$$ROOH \rightarrow RO\bullet + OH^{-}$$

This reaction has an activation energy of 44 Kcal, which makes this process very unlikely. Therefore, the bimolecular reaction (Chan, 1987) is widely considered an important initiation reaction:

$$2ROOH \rightarrow RO\bullet + H_2O + RO_2\bullet$$

Although some doubt remains about the exact mechanism, the bimolecular reaction is thought to be the main culprit in initiation (Chan, 1987).

Metals affect initiation too. In the presence of trace metals, hydroperoxides can break down into alkoxyl radicals which can also initiate oxidation (Frankel, 1989).

$$ROOH + M^{n} \rightarrow RO\bullet$$

The reaction (Frankel, 1989) occurs as follows:

$$ROOH + M^{+2} \rightarrow RO\bullet + OH^{-} + M^{+3}$$

$$ROOH + M^{+3} \rightarrow ROO\bullet + H^{+} M^{+2}$$

The newly formed radical intermediates (RO•) further propagate oxidation. Metals can affect the oxidation of linolenic acid more than linoleic because the hydroperoxides of linolenic acid decompose more quickly than linoleic acid hydroperoxides (Frankel, 1989).

**Propagation**

The two propagation reactions (Min, 1987) are:

$$1)\ R\bullet + O_2 \rightarrow ROO\bullet$$
$$2)\ ROO\bullet + RH \rightarrow ROOH + R\bullet$$

The reaction of oxygen with a lipid radical (oxygenation step) is assumed to be very fast, with no activation energy needed. A consequence of this reaction is that R• concentrations are much lower than ROO•. However, this is controlled by adjusting the concentrations of R• and ROO•, and the propagation step continues very rapidly (Chan, 1987).

**Termination**

Termination of autooxidation may involve free radicals only or metal ions which react with free radicals. This reaction is thought to lead to the formation of dimers. The bimoloecular reaction of:

$$ROO\bullet + ROO\bullet \rightarrow \text{products}$$

is the most studied and well understood termination reaction (Chan, 1987). However, in an engine, where metal ions are abundant, chain termination by these metal ions may also occur. The reaction of a peroxy radical with a metal ion in its lowest valence state (Chan, 1987) forms a stable product as follows:

$$ROO\bullet + M^{+2} \rightarrow ROO\text{-}M^{+3}$$

It has been shown that high metal concentrations have an antioxidant effect with poly-unsaturated lipids by the previous reaction. Also, higher oxidation states of metals can act as chain terminators. Copper II was shown to be an efffective chain terminator (Chan, 1987).

It is during the termination step that hydroperoxides cross-link with each other to form high molecular weight products that increase the viscosity of the oil: (Gardener, 1987)

$$R\bullet + X\bullet \rightarrow RX$$

$$ROO\bullet + X\bullet \rightarrow ROOX$$

Cross-linking of lipids occurs by intermolecular addition of peroxy radicals to double bonds. Polymerization by peroxy radicals is influenced by the amount of unsaturation of a lipid, presence of oxygen and lack of antioxidants. The addition of peroxy radicals forms dimers, trimers and oligomers; however, research shows that linoleate and linolenate do not polymerize beyond dimers and trimers (Gardener, 1987).

As stated earlier, the action of polymerization and subsequent increase in viscosity are detrimental to an engine lubricant's utility, which is the main drawback of soybean oil. Therefore, antioxidants are a main defense against oxidation, and polymerization of vegetable oils.

## Antioxidants

**Production.** Rosenwald (1949) found that the alkylation of a phenol with isobutylene yielded a compound, 2-*tert*-butyl-4-methoxyphenol, (BHA) possessed excellent antioxidant properties. McConnell and Davis (1963) improved upon Rosenwald`s work by using the same mechanism to produce BHT, but with even higher yields and greater purity to allow use in foods. They produced BHT by reacting a phenol (4-methylphenol) with isobutylene gas in the presence of an acid catalyst to yield BHT (McConnell and Herman, 1963). The use of isobutylene as an alkylating agent is still common practice today for production of antioxidants.

**Mechanism of action.** Primary antioxidants inhibit autooxidation by interrupting the propagation step of the free radical mechanism. (Pokorny, 1987). Antioxidants are put into two classes. The first class (AH) are hindered phenols such as BHA, BHT and tocopherols. According to Frankel (1991), these compunds are very effective when they compete with the reaction:

$$ROO\bullet + RH \rightarrow ROOH + R\bullet$$

The second class of antioxidants include quinones, such as hydroquinone and TBHQ. These reactions compete with oxygen (Frankel, 1991) in the reaction of:

$$R\bullet + O2 \rightarrow ROO\bullet$$

The reaction of antioxidants with radicals form lipid hydroperoxides and a less active inhibitor radical. Antioxidant free radicals are not active enough to promote further oxidation. Usually, they react with other antioxidant radicals or with radicals from other oxidized lipids (Pokorny, 1987).

The effectiveness of antioxidants depends upon their concentration, rate of initiation, activity, structure and presence of metals. The critical concentration of an antioxidant is a sufficient concentration to quench all oxidation reactions formed by the initiation step. Therefore, the antioxidant concentration should be greater than this critical concentration (Pokorny, 1987). If the concentration of antioxidants is sufficient, an induction period occurs where free radical concentrations are constant. During the induction period, the antioxidant is slowly used up by the reaction with free radicals until the critical value is reached and oxidation occurs rapidly (Pokorny, 1987). The addition of antioxidants to even partially oxidized oils is useless and decreases antioxidant activity because the hydroperoxide concentration is very high (Pokorny, 1987).

The degree of unsaturation of lipids has a great effect on antioxidant effectiveness (Pokorny, 1987). Also, in the presence of trace metals, antioxidants are less effective because their reactivity toward alkoxyl radicals (RO•) is only slightly greater than the reactivity of unsaturated lipids (Frankel, 1991). Because of this, phenolic antioxidants, and others, are less effective in inhibiting oxidation in systems with a high concentration of linolenic acid. Again, this is due to linolenate hydroperoxides decomposing faster than linoleic acid hydroperoxides (Frankel, 1991).

Metal chelators, such as citric acid and EDTA, are employed because they are more effective against hydroperoxides. Chelators work by complexing with hydroperoxides preventing their decomposition into free radicals. Significant synergism and increased oxidative stability is known to occur between tocopherols and metal chelators in stabilizing soybean oil (Frankel, 1991).

The choice of antioxidants, especially in food systems is limited to just a few compounds due to toxicity of many antioxidants. In our tests though, toxicity is not of concern since our oil is for industrial use. Therefore, many antioxidants were tested, and at high concentrations.

Today most soybean oil is stabilized with TBHQ. The addition of 0.02% TBHQ to soybean oil increases its Active Oxygen Method (AOM) stability 2-fold (Pokorny, 1987).

**AOM Method**

The Active Oxygen Method (AOM) was developed in 1933, and eventually became an official method by the AOCS to test oxidative stability of fats and oils (AOCS, 1989). The AOM also became a way to test antioxidants for their effect on oxidative stability as an alternative to hydrogenation (Sherwin, 1978).

According the the AOCS Official Methods and Practices manual, Cd 12-57, The AOM method is a measure of time (in hours) to oxidize a fat or oil to a certain peroxide value. This involves heating the oil to 97.8° C , and blowing air through the oil sample at a rate of 2.33 mL/sec. in order to reach a peroxide value of 100 meq/kg.

Recently, many new methods were developed to replace the AOM method. In 1991, the AOCS officially adopted the Rancimat® method as an official alternate to the AOM (Hill, 1994). But, the AOM method is still used for comparing results from new methods. Cross (1989) compared a method for measuring oxidative stability by differential scanning calorimetry (DSC) to the AOM. A good correlation was found, but the correlation was not sufficient to replace AOM with DSC. In addition, the Rancimat® method was correlated to the AOM (Hill, 1994).

Frankel (1989) stated that high temperature tests like AOM are unreliable for PV values due to the required elevated temperatures, which affects lipid oxidation mechanisms. However, since we are not concerned with PV values, the AOM method offers a way to test antioxidants under typical industrial conditions.

**Viscosity, Oxidation and Antioxidants**

Very little research is currently being done relating viscosity of soybean oil to oxidation. Even companies heavily researching vegetable oils as engine lubricants do not use viscosity as a sole crititerion very often. Currently, companies use standard ASTM and SAE methods to study and test the functionality of vegetable oils. Lou Honory, head of Ag-Based Industrial Lubricants (ABIL), uses the ASTM D-2271 viscosity test in his research to test oxidative stability of soybean oil in hydraulic systems (Honory, 1996). The ASTM D-2271 essentially is a test that measures viscosity changes and how those changes affect performance. The results from the wear test showed viscosity changes similar to those we observed (Honory, 1996).

The Lubrizol Corporation tested high oleic vegetable oils by an industrial gear oil high temperature test (Lal, 1995). This test (ASTM D2893) measures changes in viscosity (in cSt at 100° C) for a specific time period. Results showed that soybean oil was second worst in oxidative stability. Sunflower oil was the worst, while high oleic oils had the best oxidative stability (Lal, 1995). Lubrizol also compared the viscosities of soybean oil to high oleic oils of sunflower and rapeseed oil. Soybean oil had the lowest viscosities at both 40° C and 100° C. Also, Lubrizol used a Brookfield Viscometer to compare viscosities of vegetable oils with and without pour point depressants at -25° C (Honory, 1996). Lubrizol

determined that antioxidants, detergents, viscosity modifiers and thickeners can be added to increase performance of vegetable oils and maintain 80% biodegradability (Lal, 1995).

## Materials and Methods

### Materials

Refined, bleached and deodorized oil purchased from a local grocery store was utilized for the oxidation by AOM. Colloidal copper and iron (Aldrich, St. Louis, MO) were used as trace metals to enhance oxidation. All antioxidants were obtained from Fischer (Pittsburgh, PA) and Aldrich (St. Louis, MO). Antioxidants used were:

Butylated Hydroxy Anisole (BHA)
Butylated Hydroxy Toluene (BHT)
Caffeic acid
Ethoxyquin
Ferulic acid
Oleyl-alcohol
Ceiba-Geigy L57, L65, L135
Ascorbyl palmitate
Gossipol
Hydroquinone
Quercetin
Propyl gallate
tert-Butylhydroquinone (TBHQ)
Castor oil
Lubrizol Corporation #7652

Chelators tested include:

Citric acid, Lecithin, Ethylenediaminetetraacetic Acid (EDTA)

### Methods

**Antioxidants**. Antioxidants were screened, in duplicate, using an insulated heating block operated at 105°C. The block was connected to an air manifiold, which supplied test tubes of oil with the correct air flow rate of 2.33 mL/second.. The block can be seen in figure 2.1. Antioxidants were screened in duplicate determinations at an initial level of 0.01% by weight. The antioxidants were first dissolved in absolute ethanol, then the ethanol was added to the oil. The control also had similar amounts of absolute ethanol added. Ten milligrams,

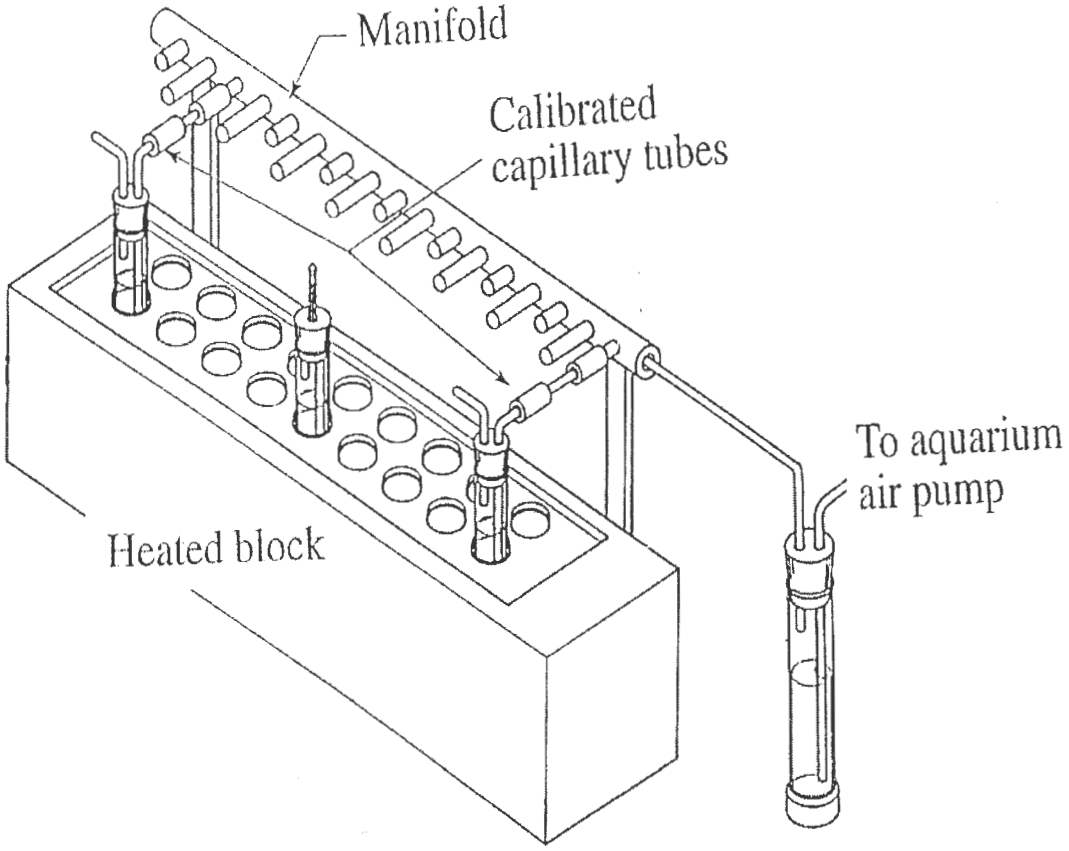



**Figure 2.1: Heating block utilized to oxidize soybean oil containing antioxidants.**

of copper and iron, each were added to 20 mL of soybean oil. The temperature of the heating block was set at 105° C, instead of the typical AOM temperature of 97.8° C. The temperature was controlled by a Barnant controller model 689 (Barrington, IL) in order to closely mimic typical engine temperatures, which do not exceed 121°C (Van Gerpen, 1999). Air, supplied by an aquarium air pump, was bubbled through the oil at 2.33 mL/second. The air pressure was controlled by a water column connected to the air pump. Periodically, 1 mL samples were taken and viscosity measured on a Brookfield LV-DV-II+ Cone and Plate Viscometer, (Middleboro, MA) operated at 40° C. Temperature was maintained by a circulating water bath. An arbitrary cutoff point selected was 150 cP. After a sample reached or closely approached this cutoff, the test was stopped.

**Statistical Analysis.** Statistical analysis was performed by SAS (SAS Institute Inc., 1990). Analysis of Variance (ANOVA) and student T-tests were performed at the 95% confidence interval to analyze viscosity at the 0.01% level. Then, further testing of the antioxidant(s) at higher concentrations followed, along with further statistical analysis.

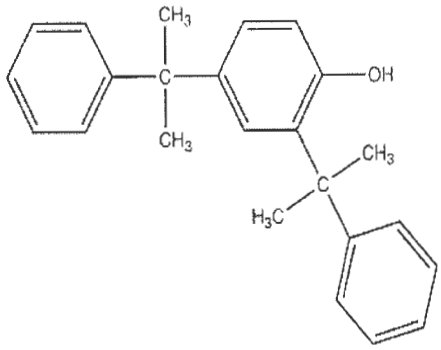
**Isolation/Identification of Lubrizol Corp. Antioxidant.** Lubrizol Corporation's antioxidant #7652 was isolated by Thin Layer Chromatography (TLC) and characterized by a Hewlett Packard (Palo Alto, CA) Gas Chromatography-Mass Spectrometer (GC-MS). A 50 microliter sample was plated on a Absorbosil Plus 1 Preparative Plate (Alltech, Deerfield, IL). The plate was placed in a TLC chamber equilibriated with 85 mL hexane, 15 mL methanol mixture. Bands were visualized by spraying the plate with 0.1% 2'7'-dichlorofluorescein (Sigma), and immediately viewed under UV light. The bands were scraped from the plate and extracted twice with 10 mL hexane.

A one microliter sample was injected into a Hewlett-Packard 5890 Series II GC and Hewlett-Packard (Palo Alto, CA) 5970 Mass Spectrometer. Oven temperature was initially 170° C, and was increased to a final temperature of 270° C. The inlet and detector temperatures were set at 270° C.

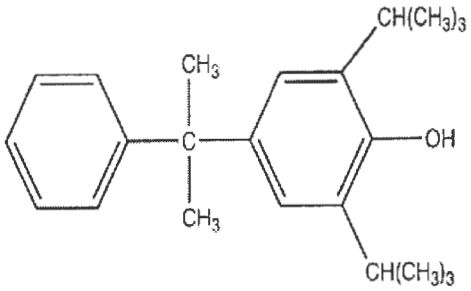
## Results and Discussion

Currently, antioxidant packages are being sold for vegetable based lubricants. The content and efficacy of these are unknown. Therefore, we initially tested an antioxidant package (#7652) by the Lubrizol Corporation. Our goal was to determine exactly what the Lubrizol Corporation was using as antioxidants in order to mimic and improve upon their formulation. To test the efficacy of their antioxidant, we used thin layer chromatography (TLC) to isolate the polar fraction, or antioxidant portion from the hydrocarbon base. Then, following standard TLC procedures, the antioxidant portion was purified, weighed and tested at 0.12% in our apparatus. Lubrizol's antioxidant did not perform well, especially when compared to TBHQ at 0.01%.

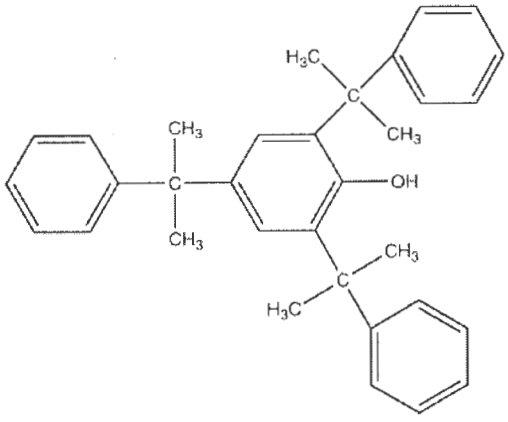
Next, we tried to characterize the antioxidant using mass spectroscopy (MS). We isolated the antioxidant portion by TLC and prepared a sample for injection into the MS. The chromatogram indicated compounds that are similar to BHA and BHT, but these compounds contained many phenolic groups attached to the toluene ring. Possible structures are located in figure 2.2.




**2,4-Bis(dimethylbenzyl) phenol**




**2,6-Bis(t-butyl)-4-(dimethylbenzyl)**




**2,4,6-Tri(dimethylbenzyl) phenol**

**Figure 2.2: Possible chemical structures of Lubrizol Corporation's antioxidant #7562.**

These compounds probably were created by a mechanism similar used to produce BHT. Production of BHT involves isobutylene gas as the alkylating agent, which adds to a phenol. Possibly, Lubrizol started with an alkylating agent other than isobutylene, namely 2-phenylpropylene. It is thought that Lubrizol reacted 2-phenylpropylene with 4-methylphenol to produce an alkylated polyphenol. This reaction can add more than one 2-phenylpropylene to create a highly phenolated compound, which may account for the chromatogram produced from Lubrizol's antioxidant package. The 2-phenylpropylenes added to the 4-methylphenol are thought to prevent volatilization, and loss of the antioxidant during use.

Because Lubrizol Corporation`s antioxidant did not work well, we started with readily available antioxidants to test in soybean oil. Tests were run on a number of antioxidants including: ferulic acid, caffeic acid, castor oil, hydroquinone, oleyl alcohol and ascorbyl-palmitate (figure 2.3 and 2.4). These antioxidants were initially tested at 0.01% by weight and compared to TBHQ at 0.01%. Statistically, TBHQ was significantly superior to these antioxidants. The statistical analysis is found in tables 2.1and 2.2 Therefore, TBHQ was considered the best antioxidant.

Quercetin, gossipol and ethoxyquin are known to be effective antioxidants; however, they did not perform very well in our tests, as figure 2.5 indicates. The test oil had its normal level of tocopherols, and it is not surprising that addition of primary chain terminating antioxidants did not significantly improve stability. Propyl gallate and the gallate family have good activity in lard, and oils, but are sensitive to heat (21), which makes figure 2.5 unusual because the oxidation is carrried out at a high temperature.

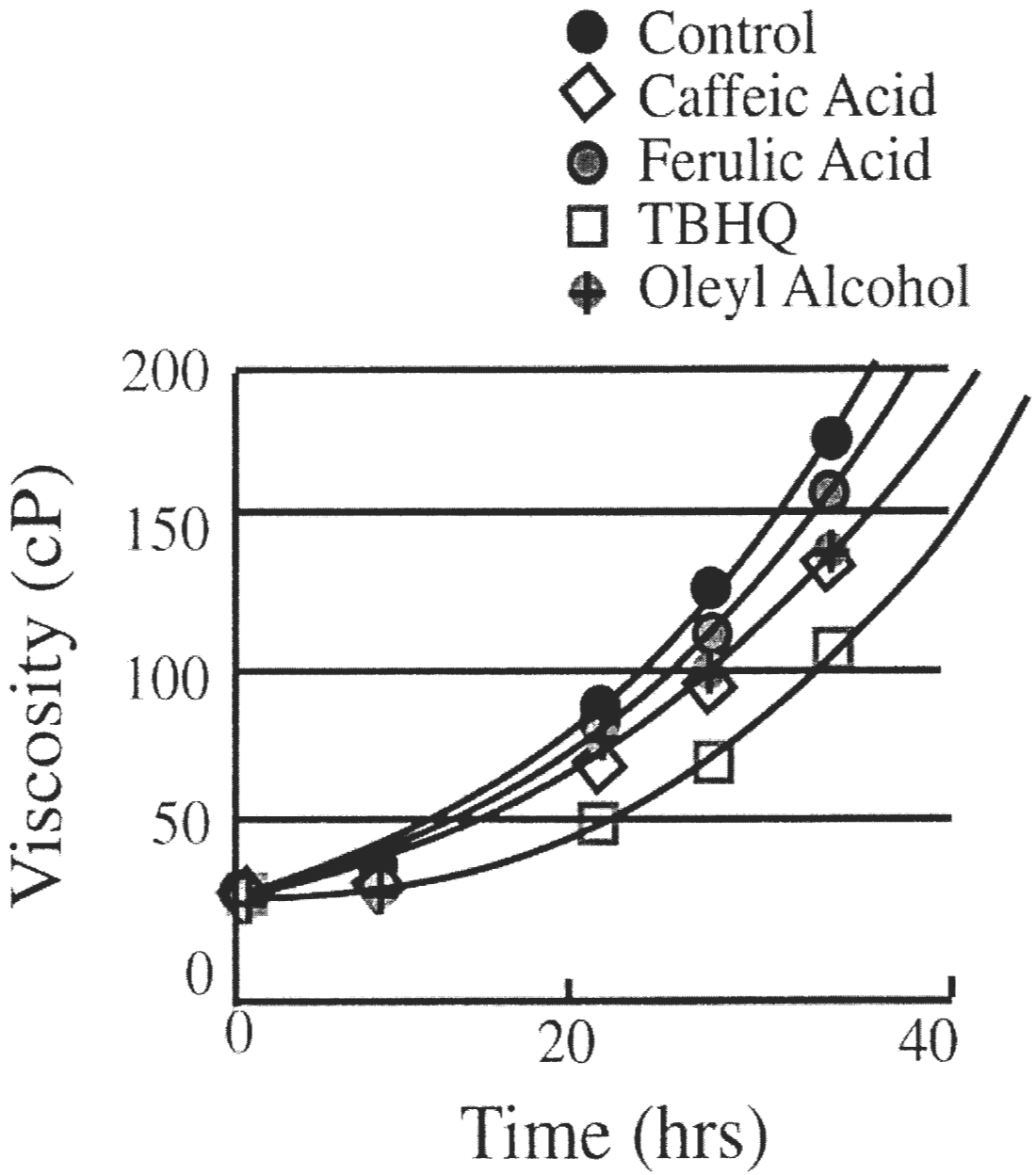



**Figure 2.3 Time vs Viscosity: Soybean oil with caffeic acid, ferulic acid, oleyl alcohol, and TBHQ at 0.01% by weight.**

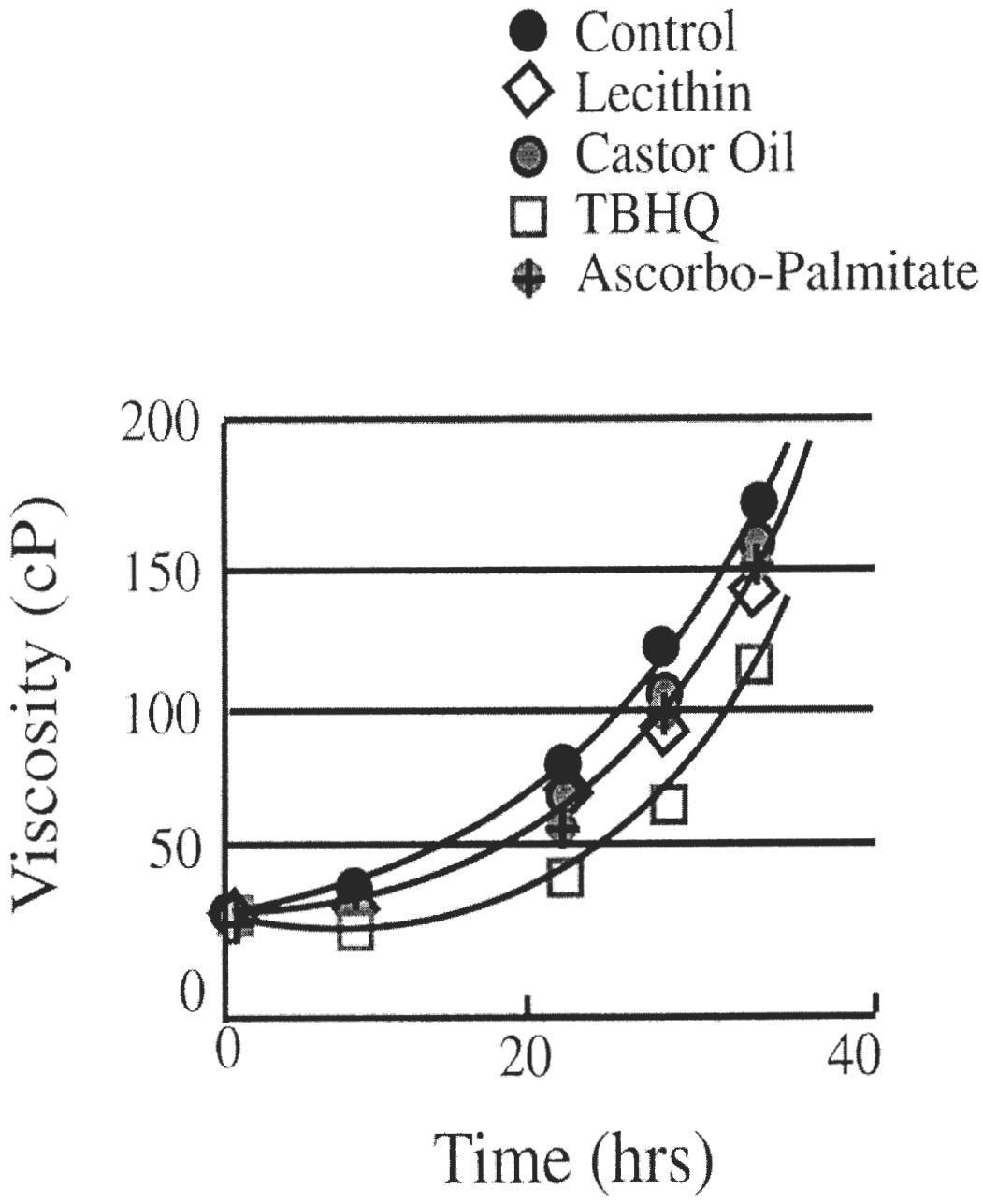



**Figure 2.4 Time vs. Viscosity: SBO with TBHQ, lecithin, castor oil, ascorbyl-palmitate at 0.01%.**

**Table 2.1 Statistical analysis of mean viscosity (cP) of antioxidants using T-test for Least Significant Difference (LSD) at 95% confidence interval.**

| Antiox. | Means | Antiox. | Means | Antiox. | Means | Antiox. | Means | Antiox. | Mean |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | $70.8^{A,B}$ | **Control** | $76.1^{A}$ | **Control** | $90.0^{B,C}$ | **Control** | $60.6^{A}$ | **Control** | $59.8^{A}$ |
| **Castor Oil** | $72.9^{A}$ | **Ferulic Acid** | $73.7^{A}$ | **BHA** | $100.5^{A,B}$ | **A-P[a]** | $48.4^{A}$ | **Lubrizol:** | |
| **Lecithin** | $66.7^{B,C}$ | **PG[b]** | 70.7A | **BHT** | $103.2^{A}$ | **TBHQ** | $36.9^{B}$ | **# 7652** | $66.7^{A}$ |
| **HQ[c]** | $66.0^{C}$ | **Oleyl Alcohol** | $72.3^{A}$ | **BHA/BHT** | $84.7^{C}$ | | | **TBHQ** | $44.4^{B}$ |
| **TBHQ** | $56.8^{D}$ | **Caffeic Acid** | $68.5^{A}$ | **TBHQ** | $60.5^{D}$ | | | | |
| | | **TBHQ** | $56.1^{B}$ | | | | | | |
| **LSD** | 4.2 | | 8.3 | | 11.3 | | 4.0 | | 9.6 |

$^{A-D}$Means with different letters within a column are significantly different.
[a]A-P= Ascorbyl Palmitate
[b]PG= Propyl Gallate
[c]HQ= Hydroquinone

**Table 2.2: Statistical analysis of mean viscosity (cP) of antioxidants using T-test for Least Significant Difference (LSD) at 95% confidence interval.**

| Antiox. | Means | Antiox. | Means | Antiox. | Means | Antiox. | Means |
|---|---|---|---|---|---|---|---|
| **Control** | $77.1^{B}$ | **Control** | $100.2^{A}$ | **Control** | $90.0^{B,C}$ | **TBHQ** | |
| **Gossipol** | $84.4^{A}$ | Ceiba-Geigy: | | Citric Acid: | | 0.64% | $67.5^{A}$ |
| **Quercetin** | $76.8^{B}$ | L57 | $91.5^{B}$ | 0.01% | $64.4^{A}$ | 1.28% | $29.6^{B}$ |
| **Ethoxyquin** | $76.5^{B}$ | L135 | $73.9^{C}$ | 0.02% | $56.8^{C}$ | 2.56% | $31.0^{B}$ |
| **Propyl Gallate** | $66.3^{C}$ | L67 | $85.7^{B}$ | 0.04% | $59.7^{B,C}$ | | |
| **TBHQ** | $45.1^{D}$ | TBHQ | $27.8^{D}$ | 0.08% | $61.9^{A,B}$ | | |
| | | | | 0.16% | $62.7^{A}$ | | |
| | | | | | $63.5^{A}$ | | |
| LSD | 4.0 | | 8.1 | | 2.9 | | 3.7 |

$^{A-D}$Means with different letters within a column are significantly different.

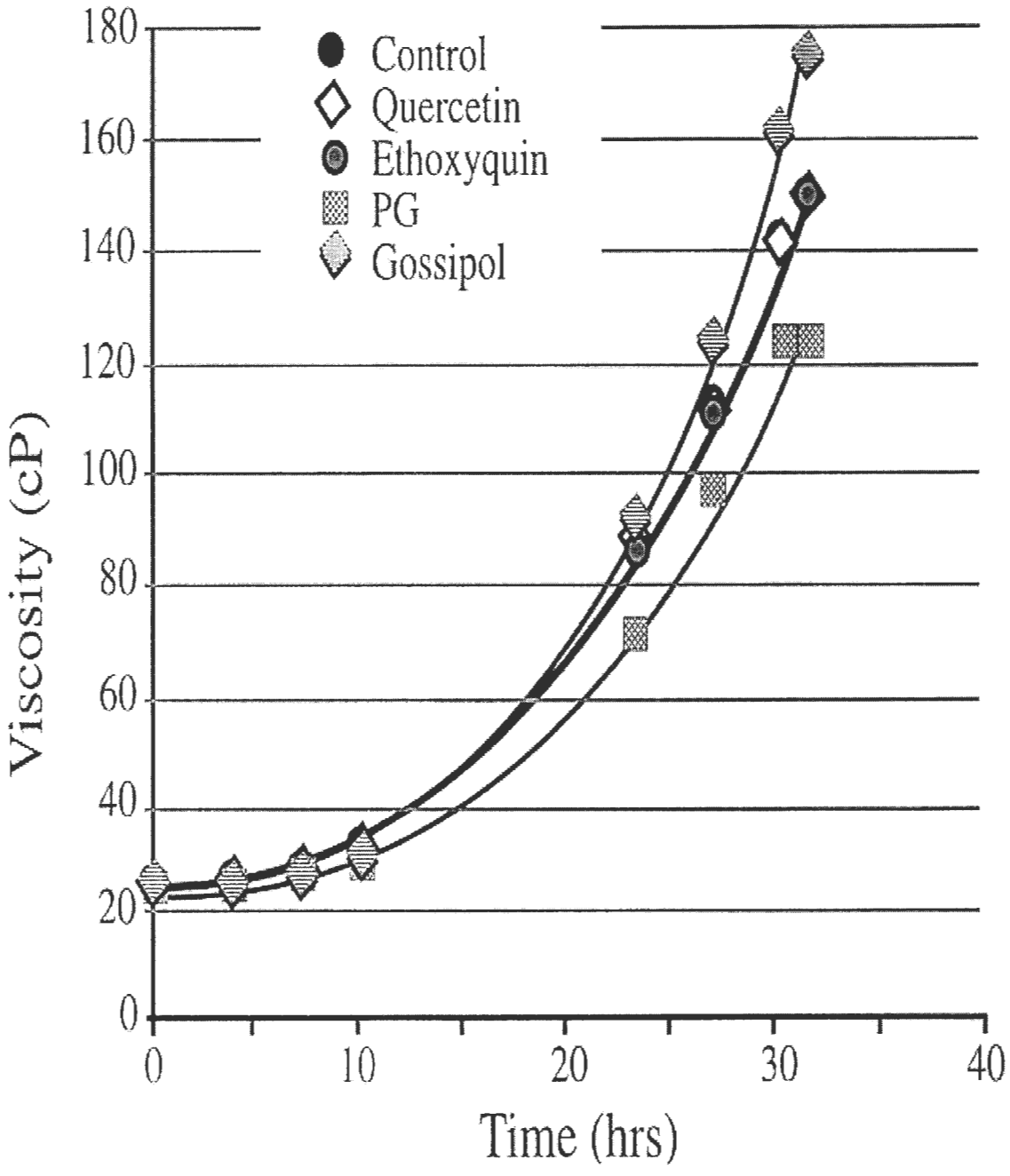



**Figure 2.5 Time vs Viscosity: Soybean oil with propyl gallate, ethoxyquin, gossipol and quercetin at 0.01% by weight.**

Since propyl gallate and TBHQ were the two best antioxidants found so far in our laboratory testing, they were compared directly. As figure 2.6 illustrates, TBHQ was superior to propyl gallate, again, which possibly is caused by the heat sensitivity of propyl gallate. Next, TBHQ was tested at even higher concentrations ranging from 0.01% to 2.56%. As figure 2.7 shows, 1.28% TBHQ was the optimum concentration. Increasing TBHQ to 2.56% showed no improvement of oxidative stability and is not shown in figure 2.7. Although 1.28% TBHQ worked well, loss of antioxidant by volitilization was noticed. This was observed by the formation of purple crystals along the air inlet and outlet tubes. The exact loss of TBHQ is not known, but significant improvement in the oxidative stability of soybean oil was still observed.

Chelators were tested under the same conditions as antioxidants. Figure 2.8 shows the comparison of EDTA and citric acid at 0.01%. Citric acid was slightly better as a chelating agent. Also, EDTA has poor solubility in absolute ethanol, and was very difficult to add directly to the soybean oil. Therefore, there may be some error in the amount of EDTA added; however, this error resulted in slightly more EDTA added than calculated. In addition, lecithin was tested and did not perform well. Citric acid was then tested at higher concentrations, and a concentration of 0.02% was determined to be the optimum for citric acid.

Finally, having determined the best antioxidant and its concentration, and the best chelator concentration, the two were combined. One test was run with TBHQ at 1.28% along with another test with 1.28% TBHQ combined with 0.02% citric acid. Figure 2.9 shows that the combination of TBHQ and citric acid prolonged the life of soybean oil by 25 to 30 hours

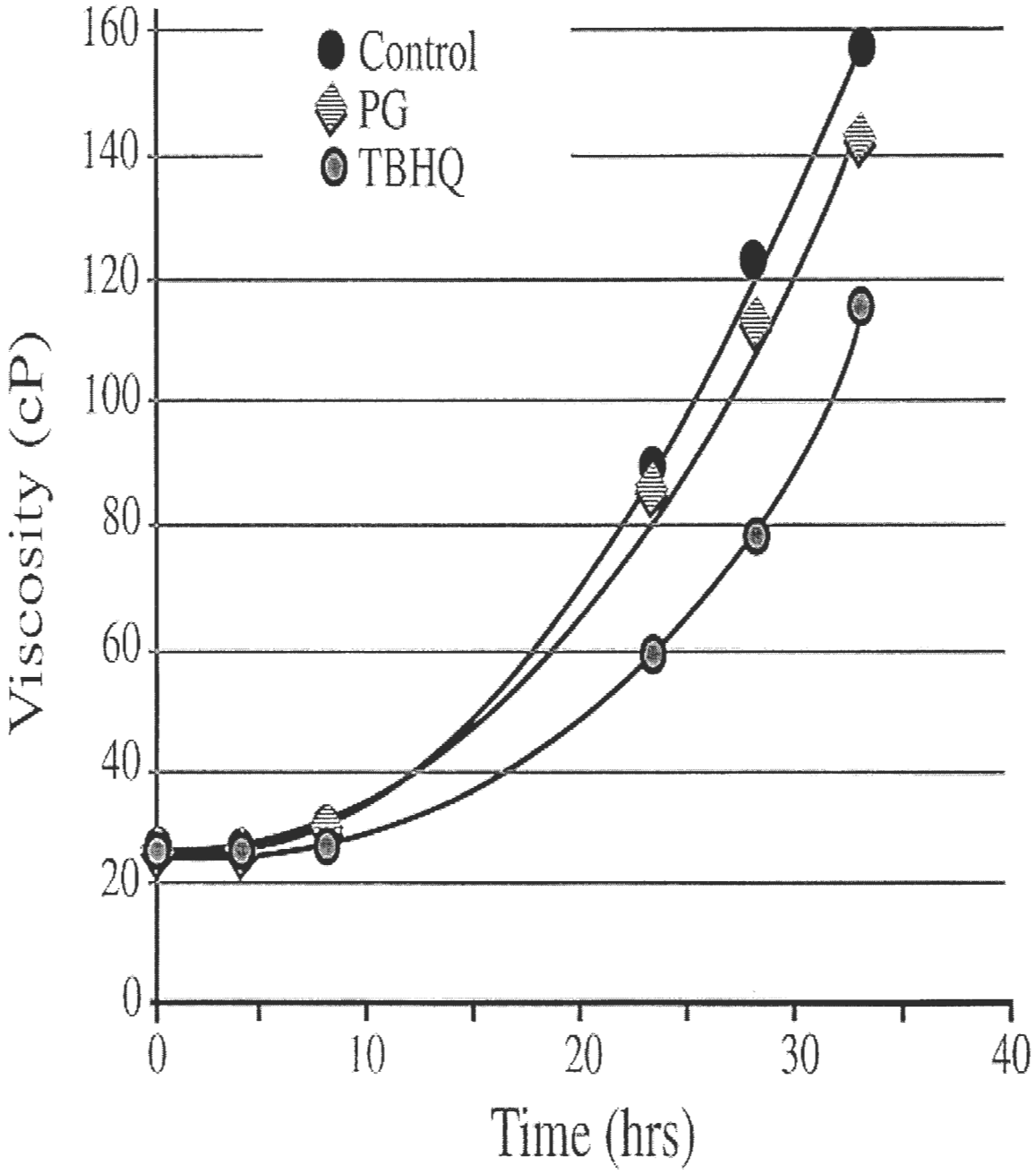



**Figure 2.6 Time vs. Viscosity: Soybean oil with TBHQ, and propyl gallate at 0.01% by weight.**

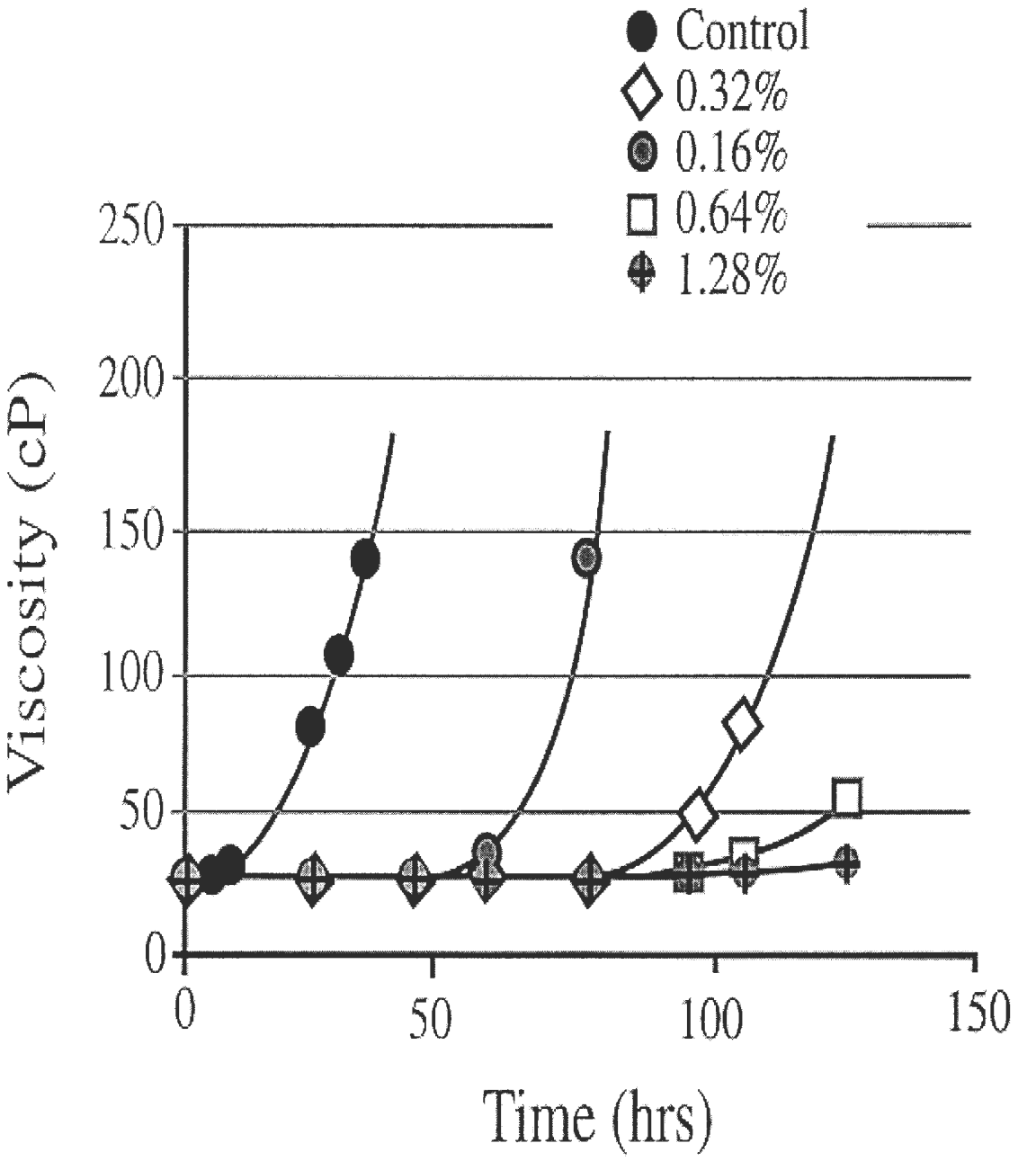



**Figure 2.7 Time vs. Viscosity: Soybean oil with TBHQ at increased concentrations.**

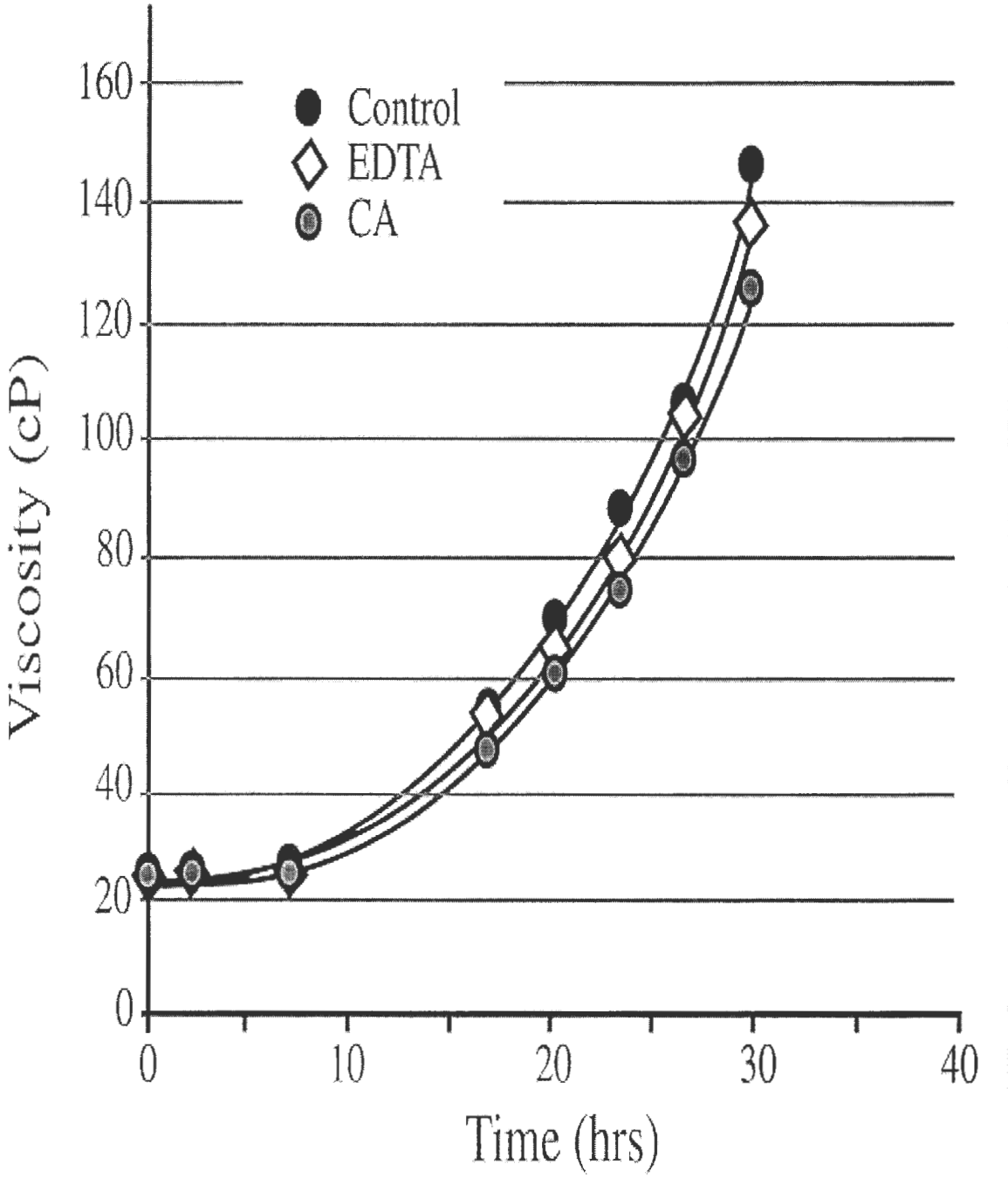



**Figure 2.8 Time vs. Viscosity: Soybean oil with citric acid and EDTA at 0.01% by weight.**

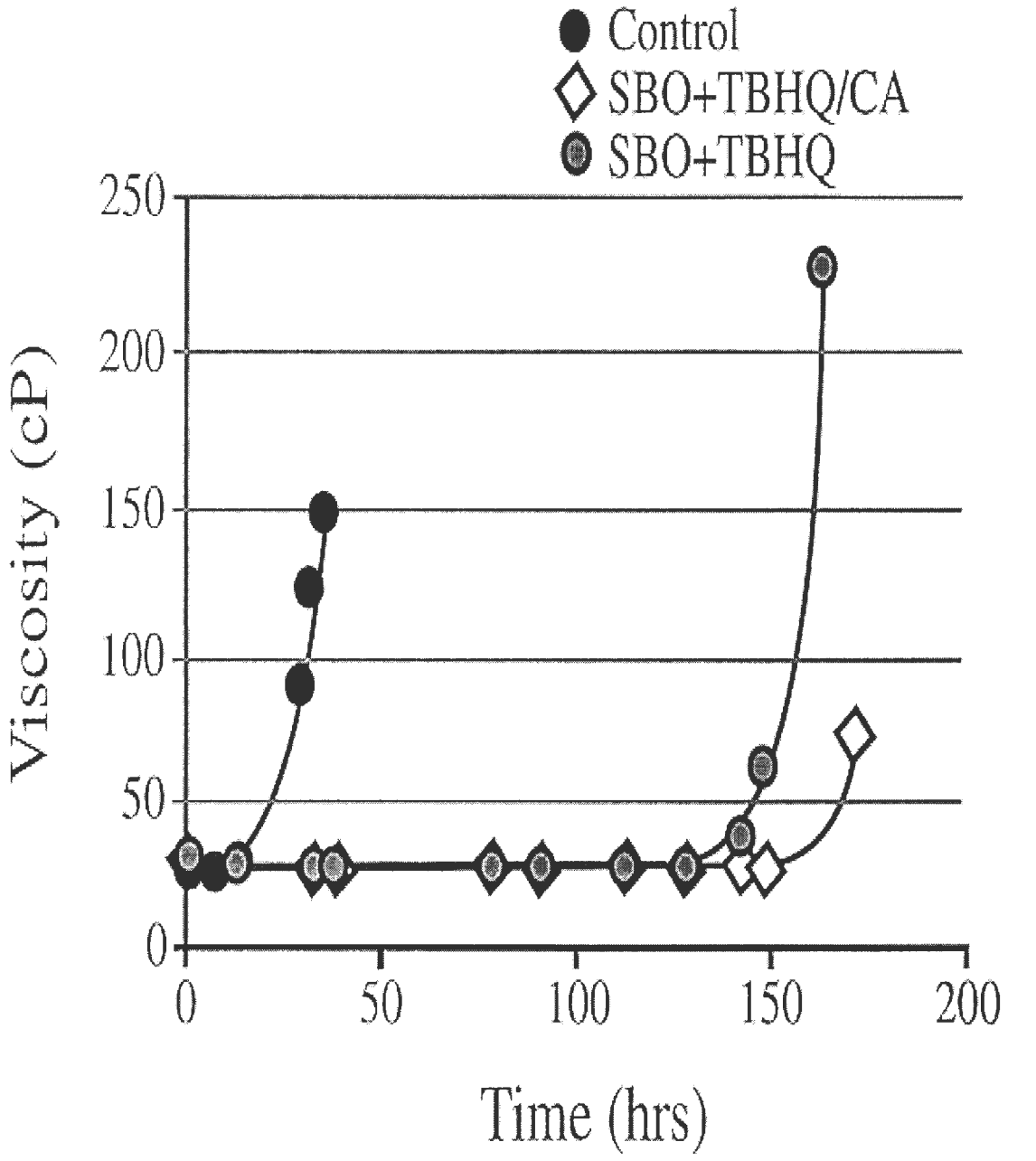



**Figure 2.9 Time vs. Viscosity: Soybean oil plus 1.28% TBHQ with, and without 0.02% citric acid.**

over the soybean oil with just 1.28% TBHQ. Again, loss of TBHQ was noted by volitilization as seen by crystal formation throughout the air tubes.

Subsequently, we found that the combination of 1.28% TBHQ and 0.02% citric acid prolonged the useful life of refined soybean oil about 5.5 fold. The AOM method is a good method to mimic industrial conditions; however, it is limited by loss of antioxidant at high concentrations by volatilization. Also, the method requires significant reaction time as seen in figure 2.9.

Since TBHQ is thought to work by competing with oxygen during the propagation step, TBHQ should be even more effective in an engine, where there is substantially less oxygen present compared to our conditions. Also, oleyl alcohol and castor oil were tried because they are used in some oil formulations. According to Yanishlieva and Kortenska (1995), these oils may have a prooxidant effect due to hydrogen bonding with hydroperoxides, and may affect bimolecular decomposition. This may explain their relatively poor antioxidant effect on soybean oil. One drawback to TBHQ is its cost. The cost of TBHQ is approximately $13.00/lb. This can increase the cost of soybean oil by $0.18/lb. However, if TBHQ extends the life of the soybean oil 5.5 fold, its antioxidant ability may outweigh the cost.

# CHAPTER 3. USING UREA TO ISOLATE OXIDATIVELY STABLE FRACTIONS FROM SOYBEAN OIL

## Introduction

The formation of urea adducts is an old, but effective method for separating fatty acids. This method's simplicity, feasibility and use of less toxic solvents are bringing it back to the forefront in fatty acid separation. The use of free fatty acids from vegetable oils for industrial and commercial applications is growing. Recently, Hayes et. al (1998) separated saturated fatty acids from unsaturated fatty acids in low erucic acid rapeseed (LEAR) oil. These unsaturated fatty acids have commercial use in paints, foods and pharmaceuticals (Hayes et. all, 1998). High oleic soybean oil, like rapeseed and canola, possesses greater oxidative stability than normal soybean oil. Subsequently, upon separation of the oleic acid, the other fatty acids, as demonstrated by Hayes et. al, (1998) will have great commercial value. The primary goal of this research is to increase the oxidative stability of soybean oil by concentrating oleic acid by urea complexes for use as an engine lubricant and hydraulic oil.

## Literature Review

Urea adducts were first discovered by Bengen in 1940 (Swern, 1964). Bengen created adducts that contained straight-chained, but not branched compounds, that formed a well-defined crystal (Swern, 1964). Future studies showed normal compounds could be separated from branched compounds (Schlenk and Holman, 1950). Under certain conditions, urea complexes will form between urea and fatty acids. Fatty acids act as a template in

which urea molecules spiral around the fatty acid (Swern, 1964). If one were to look down the unit cell of a urea complex, it would look like a doughnut with the included compound (fatty acid) occupying the doughnut hole. These two molecules are held together by secondary forces. Hydrogen bonds, London forces and electrostatic forces all keep the molecules attached (Swern, 1964).

Various techniques were developed to prepare urea complexes. Consideration of a technique depends on the type of lipid to be separated, the yield and purity required, or which type of separation is needed (Swern, 1964). Urea complexes are formed by adding free fatty acids (FFA's) to a solution of urea and a polar alcohol. Methanol is the most common alcohol (Hayes et al, 1998 and, Swern, 1964). The solution is heated to the boiling point, cooled under constant agitation to room temperature or lower, and crystals will form. Urea complexes form rapidly and consistently at room temperature (Hayes et al, 1998), and found that cooling to 35° C instead of 24-25° C formed fewer crystals.

In separations based on chain length, urea preferentially complexes with longer chained molecules. If the urea added is limited, longer-chained molecules will bind with urea first, excluding shorter-chained molecules (Swern, 1964). Usually, the best separations occur when the carbon length differs by 4-6 carbons. Unsaturated compounds also can be separated. If the chain length is the same, fully saturated fatty acids will bind first, then monounsaturated fatty acids followed by polyunsaturated fatty acids (Swern, 1964).

One great advantage of urea complexes is their ability to protect unsaturated fatty acids from autooxidation. The basis for this phenomenon is that autooxidation is a free-radical mechanism. In urea complexes, the contact between free radicals and unsaturated

fatty acids is limited; subsequently, the reaction cannot proceed. In addition, urea may act as a barrier to atmospheric oxygen (Swern, 1964).

## Materials and Methods

### Materials

Crude, undistilled, soybean oil methyl esters (West Central CO-OP, Ralston, IA) were used for separation. Methyl ester content was determined to be 95.6% by gas chromatography. Urea (Fischer, Pittsburgh, PA) and methanol (Fischer Pittsburgh, PA) were used to create urea complexes. Filtration was carried out using a Buchner funnel with a #4 Whatman filter paper.

### Methods

**Separation.** Initially, the amount of urea that would preferentially bind with saturated and unsaturated fatty acids was determined. For saturates, urea weighing 6, 7, 8 and 9g were added to 50 mL methanol and 10g of soybean oil methyl esters. The solution was heated to dissolve the urea, and then cooled to room temperature. Upon separation, the adduct (crystals), and filtrate, were analyzed by GC to determine fatty acid percentages in each.

The same procedure was utilized for unsaturated fatty acids. The data showed that 17g worked well, but the selectivity needed improvement. Therefore, an additional 50 mL of saturated urea (1g urea/6 mL methanol) was added. This improved selectivity greatly.

**Separation scheme.** Ten grams of soybean oil methyl esters were combined with 15g urea and 100 mL methanol. The resulting mixture was separated, by vacuum filtration,

with a Buchner funnel into the adduct (A1) and filtrate (F1). Next, the adduct (A1) was recrystallized by adding 50 mL methanol, heated, cooled to room temperature, and filtered. Then, 17g of urea and 100 mL-saturated methanol were added to the filtrate (F1) from the first step, heated and cooled to room temperature. Upon separation, the resulting adduct (A2) was recrystallized with 75 mL methanol, heated and cooled to room temperature. In addition, the funnel and filter paper from all separations were washed with 50 mL of methanol to recover missed methyl esters.

**Sample preparation (Adducts).** One-gram samples from adducts were treated with 5 mL of hot water (70°C), and 3 drops of concentrated hydrochloric acid to neutralize any trace of ammonium soaps. The samples were centrifuged for 5 minutes at 3500 rpm and appropriate amounts of standard and hexane were added. Standard was prepared by dissolving 249mg heptadecanoic acid methyl esters in 100 mL hexane.

**Sample preparation (Filtrates).** For filtrates, 1 mL samples were evaporated under nitrogen, leaving only urea and fatty acid methyl esters. The methyl esters were extracted from the residue as before using 5 mL of hot water, hydrochloric acid, and addition of hexane and standard.

**Sample preparation (Filter wash).** Samples from the filter and funnel wash were treated exactly as the filtrate samples.

**GC analysis.** One microliter samples were run in duplicate determinations on a Hewlett-Packard 5890 Series II Gas Chromatograph with a polar column. The injector and flame ionization detector were set at 250° C. The oven temperature was set at 170° C. The amount of fatty acids (mg) was determined from the internal standard. The area of each fatty

acid was divided by the area of the standard. These quotients were summed and multiplied, by the standard, to give the number of milligrams in the sample. Then, the weight or volume of each fraction was multiplied by the milligrams calculated for the sample to determine the total amount of soybean oil methyl esters recovered.

## Results and Discussion

To determine the optimum conditions for selectively fractionating oleic acid from soybean oil, the optimum amounts of urea were calculated. Samples were run in duplicate by gas chromatography. Table 3.1 shows the preferential binding of urea to saturated and unsaturated fatty acids.

From the results, we determined that seven grams of urea would be ideal to preferentially bind to saturated fatty acids. The results from the addition of seven grams of urea show a higher percentage of palmitic and stearic acids than did six, and eight grams. Conversely, less palmitic and stearic acid were found in the filtrate. Nine grams of urea had similar results; however, since the same results could be achieved with less urea, seven grams was considered ideal.

These initial results were very good; however, we needed to improve the selectivity of the urea-methanol solution. We accomplished this by adding 50 mL-saturated methanol (1g urea/6mL methanol) to the original 50 mL of methanol. The addition of 50 mL more methanol along with enough urea to keep it saturated at the filtration temperature of 25° C increased the selectivity as indicated in table 3.2.

**Table 3.1: Effect of various amounts of urea on fatty acid methyl ester fractionation using 10g soybean oil methyl esters and 50 mL methanol.**

| Grams of Urea | %Fatty Acid in Filtrate | | | | |
|---|---|---|---|---|---|
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| 6 | 8.7 | 2.5 | 23.2 | 56.8 | 8.8 |
| 7 | 5.7 | 1.0 | 25.1 | 29.3 | 8.9 |
| 8 | 3.3 | 0.1 | 24.7 | 29.9 | 9.2 |
| 9 | 3.2 | 0.09 | 24.8 | 62.8 | 9.1 |
| 15 | 0.03 | 0.01 | 12.6 | 76.3 | 11.1 |
| 17 | 0.0 | 0.0 | 10.9 | 77.3 | 11.7 |
| 19 | 0.0 | 0.0 | 9.1 | 78.7 | 12.1 |
| **Grams of Urea** | **%Fatty Acid in Adduct** | | | | |
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| 6 | 20.4 | 11.0 | 20.8 | 40.9 | 6.8 |
| 7 | 23.1 | 13.2 | 19.4 | 37.7 | 6.6 |
| 8 | 20.0 | 10.0 | 21.0 | 42.2 | 6.8 |
| 9 | 25.0 | 12.6 | 20.9 | 35.6 | 5.8 |
| 15 | 16.8 | 6.5 | 24.3 | 45.5 | 6.9 |
| 17 | 10.9 | 4.3 | 19.9 | 56.4 | 8.4 |
| 19 | 8.8 | 3.6 | 21.8 | 57.2 | 8.7 |

**Table 3.2: Effect of various amounts of urea on saturated fatty acid methyl ester fractionation using 10g soybean oil methyl esters, 8 extra grams urea and 100 mL methanol.**

| Grams of Urea | %Fatty Acid in Filtrate | | | | |
|---|---|---|---|---|---|
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| 14 | 1.3 | 0.0 | 25.0 | 64.5 | 9.1 |
| 15 | 1.1 | 0.0 | 24.6 | 64.6 | 9.6 |
| 16 | 0.2 | 0.0 | 23.2 | 66.9 | 9.7 |
| 17 | 0.0 | 0.0 | 21.3 | 68.4 | 10.0 |
| **Total grams of Urea** | **%Fatty Acid in Adduct** | | | | |
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| 14 | 36.6 | 17.2 | 19.1 | 23.7 | 3.3 |
| 15 | 36.3 | 17.4 | 20.1 | 23.0 | 3.2 |
| 16 | 30.3 | 13.4 | 27.2 | 25.4 | 3.6 |
| 17 | 39.4 | 13.7 | 27.9 | 16.8 | 2.2 |

From those results, we determined 15g of urea to be ideal. The adduct possessed more stearic acid. Also, the filtrate contained more linoleic and linolenic acid. These results showed that the combination of 7g of urea with 50 mL saturated methanol (8 extra grams urea) preferentially binds saturated fatty acids.

The initial results to determine the optimum amount of urea to bind unsaturated fatty acids were inconclusive. Therefore, we needed to increase the selectivity. This was accomplished by using 100 mL-saturated methanol, which contained enough urea (15 grams) to keep the methanol saturated at our filtration temperature of 25° C. The selectivity was greatly increased as indicated in table 3.3.

**Table 3.3: Effect of various amounts of urea on unsaturated fatty acid methyl ester fractionation using 10g soybean oil, 100 mL methanol and 15 extra grams urea.**

| Total Grams of Urea | %Fatty Acid in Filtrate | | | | |
|---|---|---|---|---|---|
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| 30 | 0.0 | 0.0 | 3.9 | 83.1 | 12.9 |
| 32 | 0.3 | 0.0 | 3.4 | 83.7 | 12.5 |
| 34 | 0.0 | 0.0 | 1.0 | 85.2 | 13.5 |
| **Total Grams of Urea** | **%Fatty Acid in Adduct** | | | | |
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| 30 | 19.1 | 8.7 | 39.1 | 29.5 | 3.6 |
| 32 | 17.5 | 8.9 | 35.9 | 33.6 | 4.0 |
| 34 | 16.3 | 17.1 | 25.2 | 36.6 | 4.8 |

The results indicated that 32g urea with 100 mL methanol was ideal. The adduct possessed less palmitic acid than before. Also, the adduct contained more oleic acid than the sample with 34g of urea. Conversely, the filtrate contained less oleic acid and more linoleic and similar amounts of linolenic acid. These results indicate that the combination of 17g

urea with 100 mL-saturated methanol (15 extra grams urea) preferentially binds oleic acid. The addition of more than 100 mL-saturated methanol did not improve selectivity.

Our goal now was to combine the data from tables 3.2 and 3.3 to fractionate soybean oil to achieve an oleate-rich fraction. A schematic diagram (figure 3.1) shows how we reached that goal. Table 3.4 shows the fatty acid composition of each fraction, and table 3.5 shows the amount of each fatty acid in each fraction.

The results show that we were successful in fractionating soybean oil. The most important fraction being A4, which contains elevated amounts of oleic acid, and reduced levels of linolenic acid. This fraction is most useful as an engine lubricant because of its lowered linolenic and linoleic concentrations, which will increase its oxidative stability. In addition, the oleic acid concentration is increased to simulate the fatty acid composition of canola oil, which is the current choice for engine lubricants (Honory and Boeckenstedt, 1998).

Another goal involved with this project was to determine economic feasibility of fractionating soybean oil. This involved calculating the yield of methyl esters after fractionating, which presented some additional problems.

Theoretically, the first two fractions, A1 and F1 should amount to 10g of soybean oil methyl esters. However, in early trials we recovered 7 to 7.8g of methyl esters. The samples showed a clear hexane layer on top, but a milky white layer on the bottom. Our technique included one-gram samples from adducts and 1 mL samples from filtrates.

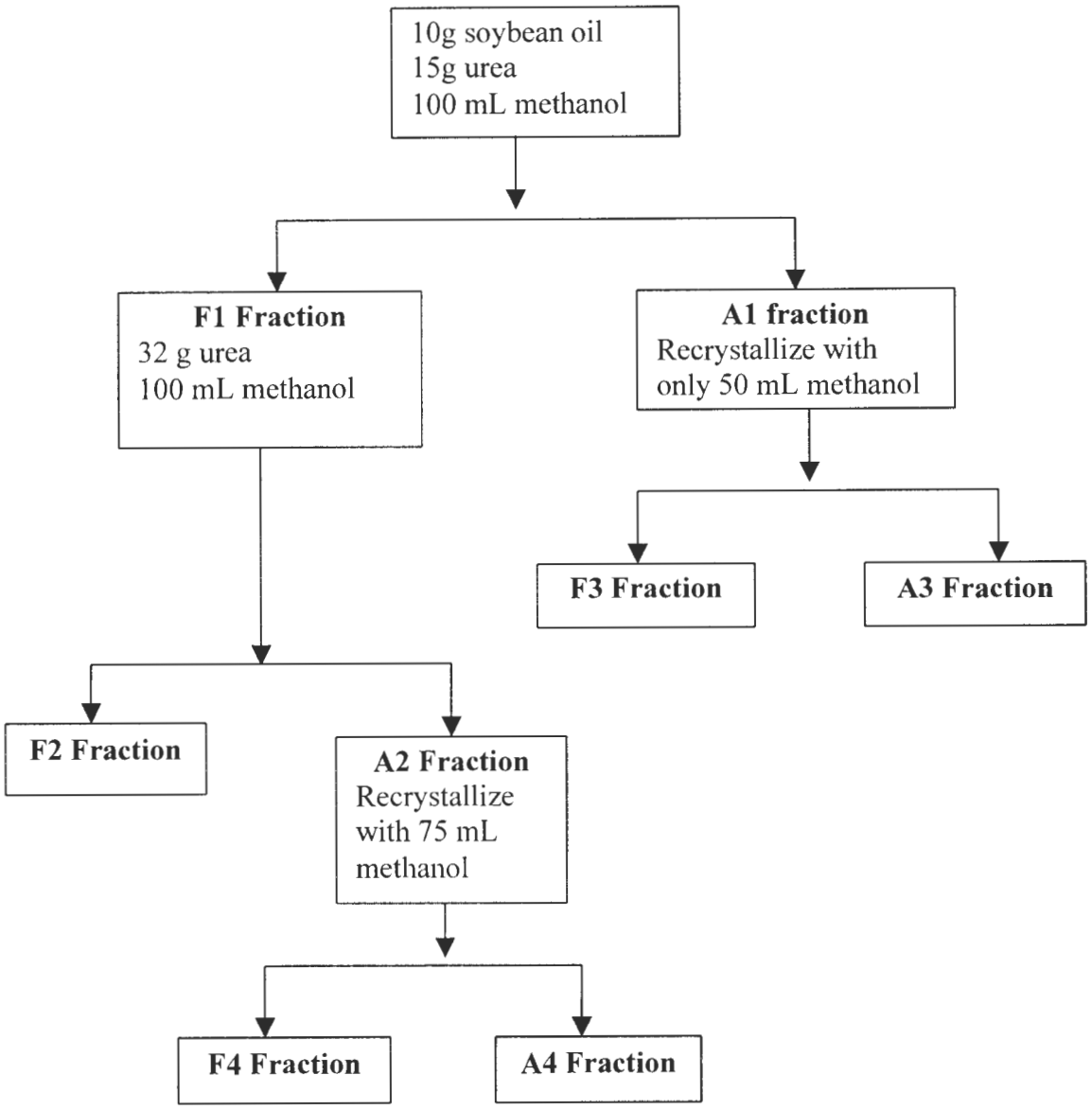



**Figure 3.1: Schematic of soybean oil fractionation.**

**Table 3.4: Fatty acid composition of soybean oil methyl ester fractions achieved using the sequence of steps shown in figure 3.1.**

| Fraction | %Fatty Acid in Filtrate | | | | |
|---|---|---|---|---|---|
| | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| Original Comp. | 10.0 | 3.0 | 25.0 | 55.0 | 8.0 |
| A1 | 38.0 | 17.9 | 23.6 | 19.9 | 2.6 |
| A2 | 1.6 | 0.0 | 30.0 | 56.9 | 7.1 |
| A3 | 46.9 | 42.5 | 6.3 | 3.8 | 0.4 |
| A4 | 3.8 | 0.0 | 63.9 | 29.6 | 2.6 |
| F1 | 1.2 | 0.0 | 24.3 | 64.3 | 10.0 |
| F2 | 0.0 | 0.0 | 0.3 | 80.2 | 19.4 |
| F3 | 35.0 | 9.4 | 26.6 | 25.5 | 3.4 |
| F4 | 0.1 | 0.0 | 7.0 | 82.6 | 10.5 |

**Table 3.5: Grams of fatty acid methyl ester in each fraction calculated from data in table 3.4.**

| Fatty Acid | Grams of Fatty Acid in Each Fraction | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | A4 | F1 | F2 | F3 | F4 |
| Palmitic | 0.88 | 0.09 | 0.25 | 0.09 | 0.08 | 0.00 | 0.51 | 0.00 |
| Stearic | 0.41 | 0.00 | 0.22 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 |
| Oleic | 0.55 | 1.62 | 0.03 | 1.46 | 1.64 | 0.00 | 0.39 | 0.17 |
| Linoleic | 0.44 | 3.07 | 0.02 | 0.68 | 4.34 | 1.11 | 0.37 | 2.05 |
| Linolenic | 0.06 | 0.38 | 0.00 | 0.06 | 0.67 | 0.27 | 0.05 | 0.26 |
| **Total (g)** | **2.34** | **5.16** | **0.52** | **2.29** | **6.73** | **1.38** | **1.46** | **2.48** |

Three milliliters of water were added to each to break up the urea complex and 3 mL of both C17 standard and hexane were added. Then, an additional 7 mL of water was added to the adducts and the filtrates with similar results. Upon the addition of 5 mL standard to the adduct fractions, the amount of methyl esters found in the adducts became very consistent; however, the amount found in the filtrate fractions were very inconsistent. Next, the filtrate samples were put under a nitrogen stream to evaporate the methanol. Then, 5 mL of water was added to break up the urea complex and standard and hexane was added.

After addition of water and hexane, the sample was vortexed for approximately one minute. This improved recovery in the filtrate fraction, but not to sufficient levels. Further literature review found that the addition of acid breaks up urea complexes. Therefore, three drops of HCL (0.4 mL) were added to the adduct and filtrate samples. After the addition of acid, the samples were centrifuged for 5 minutes at 3500 rpm. This improved our yield for F1 and A1 to 95%. We believe the low yields resulted from an emulsion of the urea complexes in the form of ammonium soaps, which were responsible for the milky white layer we observed. The addition of HCL and centrifugation broke the emulsion and allowed for a more complete extraction of the methyl esters.

**Cost analysis**

A cost analysis was completed to determine if fractionating soybean oil is more economical than growing special varieties (Table 3.6). Cost factors included, soybean oil methyl esters, heat for distillation, urea and methanol. The estimate that follows was calculated for the fractionation of 1000kg of soybean oil methyl esters.

**Table 3.6: Some of the costs associated with soybean oil fractionation by urea complexes.**

| Factors | Cost Analysis ($/MT[1]) | |
|---|---|---|
| | **Cost ($/MT)** | **Loss/Cycle (#/MT)** |
| **Heat to Distill Methanol** | | 254.00 |
| **Heat to Distill Water** | | 48.00 |
| **Urea** | 936.00 | 9.36 |
| **Methanol** | 2543.00 | 25.43 |
| **Total Dollars** | 3479.00 | |

[1]MT is metric ton = 1000 kg

Since we evaporate the methanol from the filtrates, the methanol can be recycled and used for further fractionation cycles. We assumed that 1% methanol would be lost per cycle. Coal costs were estimated based on calories to heat and distill methanol and water. In addition, the cost of distillation may be lowered by using mechanical vapor recompression (MVR) to preheat incoming methanol and water.

The urea also could be recycled if the water was evaporated, but it still may be cheaper to sell the urea solution as fertilizer or as a feed additive. In addition, this cost analysis is a minimum estimate. It does not include the cost for labor, buildings, taxes and other cost of running and maintaining this operation.

In our process, certain fractions may possess definite market possibilities. The A4 fraction, rich in oleate, would have increased oxidative stability that is valued in many industrial uses. Also, the linoleate and linolenate fractions (F2, F3, F4) have excellent potential for use in inks and paints. These fractions may well offset the cost of fractionation.

The saturate rich fraction, A3, has limited value because lard and tallow are cheap, competitive sources of saturates. Therefore, it is difficult to compare the costs of fractionating soybean oil to growing special variety plants.

The cost of producing special variety oils today is speculative. Currently, low yields of high oleic soybean oil varieties are common, but this may change in the future as technology and knowledge increase. Also, special varieties carry a premium of approximately $0.40/bushel, which raises the price of soybean oil about $0.04/lb. The current cost of making methyl esters is about $0.24/lb, but this value would come down if there was effective competition and increased scale. Therefore, it is difficult to establish an exact comparison, but soybean oil fractionation works well, provides an alternative to biotechnology, and may create new markets and uses for soybean oil.

**Concerns**

Recently, Hayes et al. (1998) fractionated LEAR oil by urea complexes. In their research, they used ethanol as the polar alcohol. Yurawecz and Conas (1999) submitted a letter to the editor in the *Journal of the American Oil Chemists' Society*, citing several sources that the reaction of ethanol with urea may form urethane (ethyl carbamate), which is a known carcinogen in humans. Urea can also react with methanol to form methyl carbamate; however, according to Mirvish (1968), methyl carbamate is either noncarcinogenic or questionably carcinogenic. In addition, a review of ethyl carbamic acids by Adams and Baron (1965) state, "of all carbamates, only ethyl carbamate has been found to have significant carcinogenic effect on mammals." Therefore, we believe that our process is

safe for humans and the environment, and provides an excellent alternative for improving the oxidative stability of soybean oil.

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