EFFECTS OF BETA-CAROTENE AND ALPHA-TOCOPHEROL ON STABILITY OF SOYBEAN OIL

by

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B. S. in Food Science, Rutgers University
New Brunswick, New Jersey, 1983

A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Foods and Nutrition
KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

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INTRODUCTION

Use of soybean oil as an edible oil has expanded rapidly in recent years in the United States. In the early 1900's the poor flavor of soybean products discouraged development of a domestic crop as a source of edible oil (Sanders, 1944). Over the past four decades, soybean oil has risen from an inconspicuous place in the oilseed market to the major source of edible oil in the United States. Factors which contribute to this phenomenal growth include price advantages, supply, and increased public demand for polyunsaturated fats in addition to improved processing, storage, handling, and stabilization methods (List and Erickson, 1985).

An important feature of soybean oil is its high percentage of unsaturated fatty acids. Approximately 85 percent of the fatty acids are oleic, linoleic, or linolenic and the double bonds found in them are chemically reactive sites. Lipid oxidation is thus a significant problem associated with refining, storing, and using soybean oil (Buck, 1981).

Oxidation of soybean oil can occur by either the mechanism of autoxidation or photooxidation. Autoxidation, also known as free-radical oxdation, is attributed to the presence of air, heat, light, or prooxidant metals. The reactive intermediate for this reaction is the fatty acid

free radical. Phenolic antioxidants are capable of acting as electron or hydrogen donors to effectively interrupt the free radical chain reaction (Cort, 1974). Photooxidation is the oxidation of lipids in the presence of light and is sensitized by chromophoric impurities in the oils. Chromophoric impurities absorb predominantly in the visible or near ultraviolet light (Carlsson et al., 1976). This type of deterioration does not involve free radicals but instead results from the generation of singlet oxygen by the transfer of excitation energy from excited chromophoric impurities to oxygen. Light triggered deterioration is not inhibited by conventional, free radical scavengers. Use of an efficient singlet oxygen quencher is necessary to deactivate (quench) singlet oxygen to the ground state. The obvious solution of excluding light frequently is not utilized for marketing reasons; therefore, addition of a safe, effective singlet oxygen quencher is necessary to improve the shelf-life of foods containing unsaturated oils.

Quenching of singlet oxygen ($^1O_2$) can occur by either a physical process in which the quencher (Q) undergoes no ultimate chemical change, or a chemical process where a chemical reaction takes place resulting in new products (QO_2).

Physical Quenching: $Q + {}^1O_2 \text{-----} Q + {}^3O_2$

Chemical Quenching: $Q + {}^1O_2 \text{-----} QO_2$

To be effective, a much higher rate of physical quenching than chemical quenching is necessary.

In addition to their role as inhibitors of autoxidation, tocopherols also can act as singlet oxygen quenchers. Yamauchi and Matsushita (1977) determined the quenching abilities of tocopherols on the photooxidation of methyl linoleate. They suggested alpha-tocopherol as the compound most effective toward singlet oxygen among the three tocopherols. The effect of alpha-tocopherol decreased more rapidly than that of gamma- and delta-tocopherols with reaction time because alpha-tocopherol has a higher rate of chemical reactivity with singlet oxygen than the other tocopherols. Consequently, the inhibitory effect of alpha-tocopherol on methyl linoleate photooxidation decreased rapidly, whereas the effects of gamma- and delta-tocopherols were maintained for a longer time. The physical quenching rate of alpha-tocopherol was also much higher than the other tocopherols, and alpha-tocopherol was determined as having the greatest initial singlet oxygen quenching effect. Fahrenholtz et al. (1974) estimated that alpha-tocopherol could deactivate 120 molecules of singlet oxygen before being destroyed.

Beta-carotene also has been determined as an effective quencher of singlet oxygen. Foote and Denny (1968) found that one molecule of beta-carotene could quench 250 molecules of singlet oxygen. It has been determined that the mechanism of quenching by beta-carotene involves a physical electronic energy transfer, which results in triplet state beta-carotene (Farmilo and Wilkinson, 1973). As a final result of interaction with singlet oxygen, isomerization from cis to trans beta-carotene occurs (Foote and Denny, 1970).

The inhibitory effect of delta-tocopherol in combination with beta-carotene on the photooxidation of soybean oil was investigated by Terao et al. (1980). It was determined that beta-carotene, in the presence of delta-tocopherol, was a powerful inhibitor of photooxidation. In decolorized oils, beta-carotene accelerated deterioration and it was concluded that tocopherols should coexist with beta-carotene to prevent the decomposition of beta-carotene when using this compound as an additive for preservation of oils from photooxidation.

Earlier studies with oil or model systems used excessively high levels of beta-carotene which would impart unacceptable color and flavor qualities to the oil (Frankel et al., 1979; Terao et al., 1980; Goldman et al.,

1983). Warner and Frankel (1985a) found that addition of beta-carotene at concentrations from 5 to 10 ppm did not adversely affect flavor or color quality and effectively inhibited flavor deterioration initiated by light in citrated soybean oil.

This research project is designed to investigate the effect of beta-carotene (5 ppm) and alpha-tocopherol (600 ppm) alone and in combination on the photooxidation of soybean oil. It is hypothesized that the two compounds together will provide better protection than when used separately. A study on the ability of these compounds to protect oil from autoxidation also will be conducted using an accelerated heating method. No conclusive study is available in the literature on the effect of beta-carotene and alpha-tocopherol on the heat stability of soybean oil.

REVIEW OF LITERATURE

Soybeans dominate United States and world vegetable protein and oil markets, despite competition from other oilseeds. Reasons for the success of soybeans include such factors as favorable agronomic characterics, reasonable returns to the grower and processor, high quality protein and oil products, and a plentiful supply available at a competitive price (Pryde, 1985a).

Soybeans have the approximate composition listed in Table 1 (Pryde, 1985b). Whole beans are crushed and dehulled prior to oil extraction. Crude soybean oil is generally hexane-extracted, desolventized, and filtered. Crude oil is then degummed, refined, bleached, and deodorized. Degumming removes phosphatides and is accomplished by the addition of water to warm, crude oil. Then a centrifugal force is applied to remove the gummy, hydrated phosphatides. During the refining step, degummed crude oil is heated and a solution of sodium hydroxide is added. The mixture is centrifuged to remove the sodium soaps formed, then water washed and recentrifuged to remove the final traces of soap from the oil. This process lowers free fatty acid content. Bleaching lowers color intensity and is achievedby addition of small percentages of bleaching earth to hot refined oil, followed by filtration. After this process, hydrogenation

Table 1. Composition of whole soybeans.

Component	Percentage (%)
Protein	10
Lipid	20
Cellulose and hemicellulose	17
Sugars	7
Crude fiber	5
Ash, dry weight basis	6

Source: Pryde, 1985b.

may be utilized to harden the oil. Deodorization, the final step in oil processing, removes off-flavors. This procedure uses steam to vacuum-strip volatiles (Sleeter, 1981).

Soybean oil may be visualized as a solvent in which numerous components are dissolved. Refined soybean oil contains over 99 percent triglycerides in addition to small amounts of phosphatides, unsaponifiable matter (plant sterols, tocopherols, and hydrocarbons), free fatty acids, and trace metals. Soybean oil has a high composition of unsaturated fatty acids and consists of approximately 51% linoleic, 23% oleic, 11% palmitic, and 7% linolenic acids (Brignoli et al., 1976). Other fatty acids are present in small amounts. The percentage of linolenic acid is the highest found in any of the major vegetable oils and causes a high susceptibility to oxidation.

There are few reports in the literature on the physical properties of soybean oil. This is attributed to the diversity of its composition, which depends on climate and variety, and presence of minor constituents. Also, partial hydrogenation has a great effect on the fatty acid composition and alters physical properties significantly. Some of the physical properties of soybean oil are summarized in Table 2 (Pryde, 1985c).

Table 2. Physical properties of soybean oil.

Property	Value
Specific gravity, 25°C	0.9175[a]
Refractive index, n_D25	1.4728[b]
Specific refraction, r_D26	0.3054
Viscosity, centipoises @ 25°C	50.09[a]
Solidification point, °C	-10 to 16
Specific heat, cal/g @ 19.7°C	0.458
Heat of combustion, cal/g	9,478[c]
Smoke point °F (°C)	453 (234)
Flash point °F (°C)	623 (328)
Fire point °F (°C)	685 (363)

[a] IV = 132.6

[b] IV = 130.2

[c] IV = 131.6

Source: Pryde, 1985c.

Prior to 1950, soybean oil was considered as much an industrial oil as an edible oil. In either case, its use was considered second-rate. Today soybean oil is considered primarily an edible oil and only 6 percent is processed for non-food uses (Frankel, 1985a).

Current processing methods maintain soybean oil as the dominant edible oil of the world. Additional research and development is necessary for further improvement in the air, heat, and light stability of soybean oil (Frankel, 1985a).

This review will discuss (1) reactions which affect the quality of soybean oil, (2) methods of assessing soybean oil stability, and (3) factors which decrease the stability of stored soybean oil and methods for controlling deterioration in quality.

Deteriorative Reactions During Storage

There are basically three types of deterioration which affect soybean oil quality: hydrolysis, oxidation, and flavor reversion.

Hydrolysis

Hydrolysis of the ester bonds of triacylglycerol results in the release of free fatty acids. A high moisture content, possibly coupled with elevated temperature enhances this reaction. Hydrolysis of soybean triacylglycerol may be catalyzed by the enzymes lipase

and phopholipase (Chapman and Robertson, 1980). Flavor quality of stored soybean oil is not affected appreciably by hydrolysis since soybean oil contains high levels of non-volatile, long-chain, fatty acids which, when hydrolyzed, result in minor flavor compounds. Another reason for the minor importance of hydrolysis to overall soybean quality is that free fatty acids are removed during the refining process (Nawar, 1985) and during deodorization (Brekke, 1985). The hydrolysis reaction, which occurs principally during storage of the beans, can be minimized by storing the beans at less than 13 percent moisture (Chapman and Robertson, 1980).

Oxidation

The two major types of oxidation affecting soybean quality are autoxidation and photooxidation. Both reactions result in the formation of thermally unstable hydroperoxides and their decomposition products.

The mechanism for autoxidation involves three steps:

1. Initiation. Free radicals ($R^{\bullet}$) are formed from unsaturated fatty acids (RH) as the result of hydroperoxide decomposition, metal catalysis, or light exposure.

RH -------- free radicals ($R^{\bullet}$, $ROO^{\bullet}$)

2. Propagation. The chain reaction is propagated by abstraction of hydrogen atoms at positions alpha to double bonds. Oxygen addition occurs at these locations resulting in the production of peroxy radicals ($ROO^{\bullet}$). These in turn abstract hydrogen from alpha-

methylenic groups (RH) of other molecules yielding hydroperoxides (ROOH) and R groups. The newly generated R· groups react with oxygen and this sequence of reactions is repeated.

$$R\cdot + O_2 \text{ ------ } ROO\cdot$$

$$ROO\cdot + RH \text{ ------ } ROOH + R\cdot$$

3. Termination. Non-radical products are formed due to the reaction of free and peroxy radicals with one another.

$$R\cdot + R\cdot \text{ ------- } RR$$

$$R\cdot + ROO\cdot \text{ ------- } ROOR$$

$$ROO\cdot + ROO\cdot \text{ ------- } ROOR + O_2$$

The major pathway for oxidation of unsaturated fatty acids is the free radical mechanism previously described. The origin of the initial free radicals necessary to begin the process has been postulated as singlet oxygen (Rawls and Van Santen, 1970). Singlet state oxygen also has been implicated as the reactive intermediate in photo-oxidation (Clements et al., 1973).

An explanation concerning the theory of singlet oxygen is necessary to better understand the photooxidation process. When ground state oxygen is in the triplet state, the electrons in the antibonding $2p\pi$ orbitals have the same spin but are in different orbitals. These electrons are kept apart by Pauli exclusion andhave only a small electrostatic energy. In the singlet state, the two electrons have opposite spins. Electrostatic

repulsion is great, resulting in an excited state. Singlet oxygen is more electrophilic than triplet state oxygen and can react rapidly (approximately 1500 times faster than triplet state oxygen) with moieties of high electron density, such as carbon-carbon double bonds. This generates hydroperoxides which can cleave to initiate the conventional free radical chain reaction (Nawar, 1985).

Singlet state oxygen can be produced in a variety of ways, although most important in the deterioration of unsaturated oils is via photosensitization by natural pigments such as chromophoric impurities (Rawls and Van Santen, 1970; Clements et al., 1973). The following mechanism has been postulated (Rawls and Van Santen, 1970):

1. Light excites a photosensitizer (S) such as chlorophyll or pheophytin to the excited state.

$$^1S + hu \text{ ------ } ^1S^*$$

2. The excited singlet sensitizer is converted to its triplet state.

$$^1S^* \text{ ------ } ^3S^*$$

3. The transfer of excitation energy from the excited sensitizer to triplet state oxygen generates singlet oxygen. The singlet state sensitizer is regenerated as well.

$$^3S^* + {}^3O_2 \text{ ------ } {}^1O_2^* + {}^1S$$

4. Singlet oxygen then reacts with unsaturated fatty acids via the "ene" reaction to produce

hydroperoxides (ROOH) in the trans configuration.

$$^1O_2 + RH \text{ ------ } ROOH$$

The hydroperoxide isomers formed during photooxidation and autoxidation differ according to the mechanism of oxidation and fatty acid composition. For example, photooxidation of oleate produces the 9- and 10-hydroperoxides instead of the 8-, 9-, 10-, and 11-hydroperoxides formed by free radical oxidation. Linoleate produces 9-, 10-, 12-, and 13-hydroperoxides (instead of 9- and 13- formed from autoxidation) and linolenate produces a mixture of 9-, 10-, 12-, 13-, 15-, and 16-hydroperoxides (instead of 9-, 12-, 13-, and 16-) (Nawar, 1985).

Alkoxy radicals produced from hydroperoxides by loss of OH, undergo beta-scission leading to breakdown products which cause flavor deterioration. Olefins and alkyl radicals are the two types of aldehydes formed from this reaction and they decompose into a variety of volatile compounds (Frankel, 1985b).

Flavor Reversion

Flavor reversion is the appearance of objectionable flavors in soybean oil and other linolenate-containing oils from less oxidation (peroxide value less than 10) than is required to produce oxidative rancidity (peroxide value greater than 10). The term reversion is a misnomer

since the flavor, often described as beany or grassy in early stages and fishy or painty in more advanced stages, is not chemically related to original flavor present in the raw, unprocessed oil (Lundberg, 1962).

Causes of flavor reversion remain under investigation, although it is presumed an oxidative process. Various theories as to the problem of soybean oil reversion have been proposed which include (1) linolenic acid, (2) isolinoleic acid, (3) oxidative polymers, (4) phospholipids, (5) nonsaponifiables, and (6) light exposure.

Linolenic Acid Theory. Linolenic acid is accepted as the most important precursor in flavor reversion. Dutton et al. (1951) interesterified linolenic acid into cottonseed oil and found the oxidized product, when analyzed by a trained taste panel, produced the same flavor response as reverted soybean oil. Moser et al. (1965b) found that other vegetable oils with high linolenic acid content (crambe, mustard seed, and rapeseed) developed off-flavors similar to those in reverted soybean oil. The terminal pentene radical in linolenate is thought to be associated with flavor reversion compounds. Compounds which are associated with reverted flavor and are believed to have linolenic acid as their precursor are hexenal, 2-pentyl furans,and 2-pentenyl furans (Chang et al., 1983).

Isolinoleic Acid Theory. Isolinoleic acid is a mixture of isomeric diene fatty acids produced from linolenic acid during hydrogenation. Reversion of the isolated methyl isolinoleate was shown to produce a mixture of off-flavor products which had an odor similar to reverted soybean oil. It was concluded that isolinoleic acid may be a reversion precursor in soybean oil (Smouse, 1985).

Oxidative Polymers. Oxidized linolenic acid polymerizes rapidly into a complex mixture of oxygen-containing compounds. These high molecular weight polymers decompose easily, generating volatile aldehydes and other compounds which contribute to flavor deterioration (Frankel, 1985a).

Phosphatides. Any residual amount of phosphatides remaining in refined soybean oil can, on oxidation and decomposition, contribute to the off-flavors associated with reversion (Smouse, 1985).

Nonsaponifiables. Researchers have found that the addition of nonsaponifiable material from hydrogenated soybean oil produced reversion flavor when added to cottonseed oil and concluded that the nonsaponifiable matter was responsible for flavor reversion. Later work contradicted these findings and currently it is believed that the role of unsaponifiable matter in the development of reversion flavor in soybean oil is of minor

significance (Smouse, 1985).

Light Exposure. Light has a much more adverse effect on soybean oil than dark storage and will reduce its flavor stability within a few hours. Effect of light upon soybean oil proceeds by two mechanisms: direct photochemical oxidation or photosensitized oxidation. Direct photochemical oxidation is not of primary concern since ordinary glass will filter out the UV light responsible for this type of oxidation (Smouse, 1985). Photosensitized oxidation, previously described, proceeds via singlet oxygen. Sufficient amounts of photosensitizers are left in commercially refined, bleached, and deodorized soybean oil to contribute to its light instability (Clements et al.,1973).

Methods for Assessment of Soybean Oil Quality

Quality of a fat or oil depends on the extent to which deteriorative reactions have occurred. Lipid oxidation can be measured by objective or subjective methods. The spectrum of tests ranges from simple sensory evaluation to chemical and physical techniques. A variety of objective methods have been reported and the ultimate criterion for the suitability of any test is its agreement with sensory perception of rancid flavors and odors (Gray, 1978).

Sensory Evaluation

The ultimate measure of quality of a fat or fat-containing food is the flavor of the product (Warner, 1985). For taste assessment on a commercial level, a panel of experience testers rate the flavor according to an established intensity system (Williams and Applewhite, 1977). An appropriate score sheet assists the panelist in making evaluations. The score sheet developed at the Northern Regional Research Center (NRRC, United States Department of Agriculture, Peoria, IL) requires the panelist to rate the oil on a 10-unit scale, with 10 indicating bland and 1 extreme (Mounts and Warner, 1985).

Objective Measurements

Objective techniques can be classified into static methods which measure the degree of oxidation at a certain moment in time using chemical or physical analysis, and dynamic methods, which subject the fat or oil to an accelerated aging process.

Peroxide Value. Peroxide determination is the most widely accepted method for oil flavor quality determination (Min and Kim, 1985). Peroxides are the major initial products of lipid oxidation and are measured by techniques based on their ability to liberate iodine from potassium iodide or to oxidize ferrous to ferric iron (Nawar, 1985). Their content usually is expressed as

milliequivalents of oxygen per kilogram of fat. Peroxide values of 0.5 meq/kg or less generally are necessary for a high flavor score (Evans et al., 1973). Because of the transitory nature or instability of peroxides, the level of peroxides may not serve as a true indicator of the actual state of oxidative rancidity of the fat or oil. During the course of oxidation, peroxide values reach a peak, then decline (Nawar, 1985). The American Oil Chemists' Society official iodometric method Cd 8-53 (AOAC, 1984), which is a modification of the procedure developed by Wheeler (1932), commonly is used for peroxide determination of fats and oils. This procedure is empirical and any variation in method may produce a variation in results.

Thiobarbituric Acid Test (TBA Test). This colorimetric test can be used to detect rancidity of fats or fatty foods. A red color, produced from the condensation of two molecules of TBA with one molecule of malonaldehyde, is measured spectrophotometrically. The red color is produced on the oxidation of the more unsaturated fatty acids such as linolenic acid. Oxidized linoleic acid gives only a slight reaction (Lundberg, 1962). The TBA test is not generally useful in predicting oil flavor stability (Gray, 1985). A modified TBA test developed by Pokorny et al. (1985)

was found useful for evaluation of soybean oil in the initial stages of rancidity.

Kreis Test. This test was one of the first procedures to be used commercially to evaluate lipid oxidation. The reaction of epihydrin aldehyde (an isomer of malonaldehyde) or other oxidation products with phloroglucinol produces a red color which is then measured with a spectrophotometer. Although the Kreis test may be useful to indicate slight changes in oxidation under some circumstances, it does not provide a satisfactory index of rancidity (Nawar,1985).

Total and Volatile Compounds. An alternative approach to measuring the extent of lipid oxidation is to measure the carbonyl compounds formed by degradation of hydroperoxides. Advantages of methods which measure carbonyl compounds are that they attempt to measure products which contribute to off-flavor development and are not limited, as is peroxide determination, to early stages of oxidation. Methods for determining total carbonyl compounds are based on the reaction of oxidation products (aldehydes and ketones) with 2,4-dinitrophenylhydrazine. Absorbance of colored hydrazones formed is measured. A problem associated with these tests is that carbonyl compounds may be generated by decomposition of hydroperoxides, thus interfering with quantitative

results. Hydroperoxide interference can be minimized by reduction of hydroperoxides to non-carbonyl compounds prior to determination of carbonyls or by conducting the reaction at low temperatures (Gray, 1985). A further weakness of carbonyl determination is that the major portion of carbonyl compounds in oxidized systems are of high molecular weight and make little or no contribution to flavor. Measurement of the volatile carbonyl compounds in oxidized systems is necessary to assess off-flavors of oxidized fats or oils. This can be achieved using gas chromatography, a physical method.

Gas Chromatography. Objective instrumental methods which reflect the sensory qualities constantly have been sought to replace or minimize sensory panel work. Gas chromatographic (GC) methods are simpler, more reproducible, less time-consuming, less expensive, and less subjective than sensory tests (Min and Kim, 1985). Also, GC analyses provide information concerning the cause of flavor quality resulting from refinery processes.

Some of the earliest GC work was accomplished by Scholz and Ptak (1966). These researchers used direct injection to measure the degree of rancidity in samples of cottonseed oil. Pentane, a decomposition product of hydroperoxides, was chosen as the basis of the analysis. A direct correlation was indicated between ppm pentane

obtained by gas-liquid chromatography and flavor scores, based on ranking, but this was not an incremental response to the concentration of pentane.

Using direct injection gas-solid chromatography (GSC), Evans et al. (1969) found high correlation of pentane concentration with peroxide values and flavor scores of fresh and aged soybean and cottonseed oils. Jarvi et al. (1971) modified the GSC method of Evans et al. (1969) to analyze rancidity of soybean oil. The GC peaks were treated as one group and an oxidation value (OV) was computed by means of an internal standard (n-octanol). The OV's correlated well with peroxide values ($r = 0.99$) and with flavor scores ($r = 0.82$).

Although these early GC analyses were simple and correlated well with sensory testing, direct injection of oil into a gas chromatograph can ruin columns in a short period of time and reproducibility would be difficult to obtain due to decomposition of residual oil in the GC column (Min and Kim, 1985). To increase detection and reproducibility, methods which isolate and concentrate volatile compounds prior to injection were developed.

Hartman et al. (1971) isolated the volatile compounds in an oil sample by bubbling helium gas through a measured quantity of heated oil. Volatile compounds were collected on activated charcoal and extracted from the

charcoal with carbon disulfide. The concentrated compounds were injected into a gas chromatograph. A 400-fold concentration of volatile compounds was achieved. Although good reproducibility was indicated, this method has been criticized as tedious, time-consuming, and complicated (Min and Kim, 1985).

Dupuy et al. (1976) also developed a method to isolate and separate volatile compounds without contaminating the column. For this analysis, the oil sample was placed onto glass wool contained in a GC injection port liner. The liner was inserted into a heated injection port and the volatile compounds were eluted rapidly by heat and carrier gas onto the column. Temperature programming enabled attainment of volatile profiles of samples. Flavor scores of soybean oil correlated well with individual volatile components ($r \geq 0.72$) and with total volatiles ($r = 0.79$). Modification of this GC method (Dupuy, 1977) increased sensitivity and applicability. A second inlet liner was used which served to double the quantity of oil samples and volatiles eluted. Sensitivity was increased substantially and mass spectral analysis was made possible. Trans 2-trans 4-decadienal was the volatile component which correlated best with flavor scores ($r = -0.97$).

Williams and Applewhite (1977) analyzed 23 soybean

oils stored up to 5 wks in the light using Dupuy's method (1976) and sensory analysis. High correlation was found between the volatile profile data and flavor scores ($r = 0.87$).

Warner et al. (1978) used direct GC (Dupuy, 1976) to quantify pentanal and hexanal contents in vegetable oils. High correlation was found with the pentanal or hexanal content and flavor scores.

A later study by Warner and Frankel (1985b) attempted to use Dupuy's method (1976) to predict flavor stability of soybean oil by measuring induction periods based on the time required for rapid formation of volatile compounds. High correlation coefficients ($r = 0.96$) were obtained between flavor scores and total volatiles.

Waltking and Zmachinski (1977) developed a procedure which utilized direct injection of an oil sample containing an internal standard into a packed precolumn. Differences in the volatile profile generally were reflected in flavor panel results, but there was some variability.

Min (1981) reported a method which did not require a sample inlet but instead collected volatiles external to the GC in a heated U-tube apparatus. The flavor compounds isolated by this method were collected on the GC column. The column was disconnected from the U-tube, connected to

the GC, and flavor compounds were separated. Excellent correlation between this instrumental analysis and sensory analysis ($r = -0.99$) was found with soybean oil. Using this method, Min (1983) analyzed the flavor quality of soy and corn oil exposed to fluorescent light for different time periods. Sensory evaluation was conducted by a panel of 94 members from 8 different laboratories. Excellent correlation coefficients ($r \geq 0.95$) between sensory and instrumental analysis were reported.

A gas chromatographic headspace technique was used by Warner et al. (1974) to study rancidity of vegetable oils and potato chips by determining the concentration of pentane. Significant correlation ($r = 0.79$) between rancid descriptions and ppm pentane was indicated. Headspace analysis was found to be a simple, direct procedure requiring small sample size and minimal sample preparation.

Use of capillary columns for flavor analysis is currently under investigation. Capillary analyses generally result in superior peak resolution and shorter analysis times. Marsili (1984) developed a capillary headspace technique to analyze light induced volatiles in soybean oil. Research by Snyder et al. (1985) resulted in a reproducible capillary GC method to analyze headspace volatiles of different vegetable oils. Dupuy et al.

(1985) improved the direct GC method (Dupuy, 1976, 1977) for the determination of volatiles in vegetable oils by using a capillary column coupled with an external inlet device. This method gave increased sensitivity and better resolution than packed column methods.

Other Physical Methods. In addition to gas chromatography, the use of other physical methods to measure rancidity also have been examined. Some of the techniques investigated include: conjugated diene methods, fluorescence, infrared spectroscopy, refractometry, and polarography (Gray, 1978). These techniques are of minor importance in the analysis of soybean oil deterioration because they do not correlate as well with sensory scores.

A novel method to analyze the oxidative deterioration of oils and foods is measurement of chemiluminescence (Usuki et al., 1979; Timms and Roupas, 1982). Although an increase in emission intensity was found to correlate with oxidative deterioration in oils, more research is necessary before this method can be adopted as an analytical method to evaluate oil quality.

Dynamic Methods

Shelf-storage tests, which expose the sample to conditions simulating those encountered in distribution, provide the most realistic estimate of actual storage life

of a fat or oil (Gray, 1985). Researchers at NRRC undertook a 12-month storage investigation of soybean and cottonseed oils (Evans et al., 1973). This is the only long term storage study that was available in the literature.

Due to the considerable amount of time required for shelf-storage tests, accelerated methods of aging have been developed to predict the stability of oil by subjecting it to conditions designed to simulate storage and light exposure (Mounts and Warner, 1985). Extent of oxidation then is assessed using an objective or sensory method, as previously discussed.

There are basically three types of accelerated storage tests: Schaal or oven test, light test, and oxygen absorption methods (Gray, 1978).

Schaal or Oven Test. This is, perhaps, the simplest of the accelerated storage methods for determining stability of fats or fat products (Joyner and McIntyre, 1938). The sample is placed in an oven maintained at elevated temperatures (40 - 70°C) until rancidity is detected by sensory evaluation or peroxide value. An oven test specifically for soybean oil was developed by Moser et al. (1950). Bottles two-thirds full of soybean oil, capped loosely with cellophane covered corks were stored for four days at 60°C. This accelerated test is

equivalent to approximately three months storage under ambient conditions (Evans et al., 1973).

Light Test. An accelerated test to evaluate light stability of soybean oil also was developed by Moser et al. (1965). For this procedure, 150 ml of an oil sample to be tested was placed into a 250-ml (8-oz) clear glass bottle, loosely covered, and placed into an apparatus consisting of six 38.1 cm (15 in), 15-watt daylight fluorescent tubes mounted inside of a 44.5 cm (17.5 in) diameter stainless steel cylinder 44.5 cm (17.5 in) high. The top was open so that air could circulate freely. A 4-hr exposure test was determined to give sufficient deterioration to ascertain stability differences in soybean oils. The light test is especially useful in testing soybean oil which may develop grassy or green flavor described as "light struck" when exposed to light.

Oxygen Absorption Methods. These methods involve exposure of the oil sample to oxygen, usually at elevated temperatures. The Activated Oxygen Method (AOM) is widely used. This procedure involves maintenance of the sample at 97.8° while air is bubbled continuously through it at a constant rate. The time to reach a specific peroxide value is determined (Nawar, 1985). Other methods which sometimes are used include the oxygen absorption procedure, Barcroft-Warburg method, oxygen bomb method,

Eckey procedure, and weight-gain procedures (Gray, 1985).

Factors Affecting Quality Deterioration and Methods of Control

Stability of soybean oil is affected by conditions which accelerate deteriorative reactions. Factors affecting soybean oil stability include: heat, light, oxygen, and prooxidant metals. Measures to control the rate of deterioration will be discussed. These include (1) antioxidants, (2) metal inactivators, (3) packaging, and (4) hydrogenation.

Antioxidants

In the early 1930's, a distinct relationship between tocopherol content of soybean oil and its resistance to oxidative rancidity was discovered. Lecithin also was shown to have antioxidant properties (Evans, 1935). However, significant quantities of these natural antioxidants are removed from soybean oil during processing and refining. Soybean oil processors and users began to look for other methods to increase the stability of soybean oil and products containing it (Buck, 1981).

Primary antioxidants are those substances which function by inhibiting or interrupting the free radical mechanism of autoxidation. The primary antioxidant or phenolic substance functions as a free radical acceptor,

and terminates oxidation at the initiation step. The phenolic structure of these compounds allows them to donate a proton to a fatty free radical. This regenerates the glyceride molecule and interrupts the free radical mechanism. As a result of this reaction, the phenolic compound becomes a free radical. However, these free radicals can stabilize themselves through hybridization and do not promote or propagate further oxidation (Sherwin, 1976; Buck, 1981).

Primary or phenolic antioxidants which currently are approved in the United States include: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary butylhydroquinone (TBHQ), and tocopherols (Sherwin, 1976; Fung et al., 1985).

Mounts et al. (1978) studied the effect of added citric acid and citric acid in combination with BHA and BHT on the flavor stability of hydrogenated and unhydrogenated soybean oil. Addition of the phenolic antioxidants improved the oxidative stability of citrated oils, but did not impart flavor stability during an accelerated heat test. Flavor stability was improved in hydrogenated oils exposed to fluorescent light by the addition of BHA and BHT.

A later study by these researchers (Mounts et al., 1981) evaluated and compared the oxidative stability of

citrated soybean oil treated with TBHQ to oils treated with citric acid alone and with citric acid plus BHA. Oxidative stability of oil treated with TBHQ was much greater than the other oils.

Moulton (1985) produced soybean oils of various levels of linolenate content and studied the effect of TBHQ on these oils. Results indicated that TBHQ significantly improved the oxidative stability, but not the flavor stability.

Effects of various antioxidants (BHA, PG, and TBHQ) on stability of crude oil also has been investigated (Sherwin and Luckadoo, 1970). These researchers found PG and TBHQ effective in inhibiting oxidative deterioration of crude oil. Min and Wen (1983) found TBHQ more effective than PG, BHA, or BHT using an analysis that measured the rate of dissolved oxygen disappearance in soybean oil during storage. Pinkowski (1986) used sensory and GC analysis to evaluate effectiveness of various antioxidants (TBHQ, BHA, BHT, PG, and dl alpha-tocopherol). This study also found TBHQ the most effective antioxidant.

Current studies focus on the application of natural antioxidants to prolong the shelf-life of soybean oil. Compounds under investigation include tocopherols, carotenoids, phospholipids, and chlorophylls.

Tocopherols have been recognized as antioxidants for over 50 years. Research indicated that alpha-, beta-, and gamma-tocopherols are effective antioxidants at concentrations of 0.02 - 0.10 percent in lipid systems (Houlihan and Ho, 1985). Antioxidant effectiveness increases in the order alpha-, delta-, gamma-tocopherol (Sherwin, 1976; Hudson and Ghavami, 1984). This order of effectiveness may be influenced significantly by temperature and light conditions (Parkhurst et. al, 1969). The antioxidant potential of tocopherols is related directly to their stability. Cillard and Cillard (1980) found alpha-tocopherol as most easily oxidized by air. Tocopherol oxidation results in the formation of free radicals which act as prooxidants.

Tocopherols have their greatest effect in protection of animal fats, carotenoids, and vitamin A (Cort, 1974). Bickoff (1951) found alpha-tocopherol an effective antioxidant for carotene in mineral oil.

Carotenoids and tocopherols may act as singlet oxygen quenchers to inhibit photooxidation of oils. Yamauchi and Matsushita (1977)determinedalpha-tocopherol asthemost efficient quencher of singlet oxygen among the tocopherols. A later study by Yamauchi et al. (1981) found alpha-tocopherol and ascorbic acid acted synergistically on the photooxidation of methyl linoleate

and soybean oil. Sattar et al. (1976) noted that beta-carotene provided strong protective properties against the photooxidation of milk fat. Terao et al. (1980) observed that beta-carotene efficiently inhibited the photooxidative deterioration of soybean oil and this effect was elongated in the presence of delta-tocopherol. Frankel et al. (1979) found alpha-tocopherol (0.15%) much more effective than beta-carotene (0.10%) in inhibiting the photooxidation of soybean esters. Warner and Frankel (1985a) showed that beta-carotene, at levels ranging from 5 to 10 ppm, was useful in the protection of soybean oil from light deterioration without affecting its color acceptability. Peroxide values, GLC headspace analysis, and flavor evaluation were used for the analysis of the beta-carotene treated oils.

Hildebrand et al. (1984) conducted a study to determine the effects and interaction of phospholipids and tocopherols on soybean oil stability. Phosphatidylinositol (PI) and phosphatidylethanolamine (PE) appeared to increase the stability of soybean oil, whereas phosphatidylcholine (PC) had little effect and phosphatidic acid (PA) had no effect. The mechanism of the effectiveness of the phospholipids was not attributed to prooxidant metal inactivation, but rather an ability of these compounds to extend the effectiveness of tocopherols

in free radical termination. Dziedzic and Hudson (1984) showed that PE had little synergistic activity at lower temperatures, but at temperatures above 80°C the synergistic effect increased. Yoon and Min (1986) studied the effect of phospholipids on the flavor stability of purified soybean oil. They reported that PE and PA were more effective antioxidants than PC. The mechanism of phospholipid action was elucidated. They hypothesized that phospholipids act as prooxidants by increasing oxygen solubility when no ferrous iron is present, and prevent oxidation by metal chelation in oils containing 1 ppm ferrous iron.

Using oven tests, Endo et al. (1985) studied the effects of chlorophyll and pheophytin on the autoxidation of oils stored in the dark. Their results indicated that chlorophyll increased the oxidative stability of soybean oil. Pheophytin exhibited antioxidant activity in methyl linoleate, but not in soybean oil.

Addition of phenolic compounds generally offers little improvement in flavor stability but some increase in oxidative stability can be incurred. Crude soybean oil, which contains natural antioxidants such as tocopherols, carotenoids, phospholipids, and chlorophylls has greater stability than partially or fully refined soybean oil (Kwon et. al., 1984). Use of natural antioxidants,

therefore, should be subjected to additional research.

Metal Inactivators

Certain metals exhibit a prooxidant effect in soybean oil. Copper, the strongest prooxidant, exerts an oxidative effect at concentrations as low as 0.005 ppm. Other metals implicated as prooxidants include iron, cobalt, manganese, and chromium (Flider and Orthoefer, 1981). Although these metals naturally are present in soybean oil in trace amounts, they also can be introduced during processing.

The effect of prooxidant metals can be diminished by use of compounds known as chelating agents, metal scavengers, or metal inactivators. Dutton et al. (1948) found that addition of polycarboxylic acids and polyhydric alcohols to soybean oil containing prooxidant metals increased the oxidative and flavor stability of the oil. These researchers concluded that the necessary structural groups for chelating agents are the carboxyl and hydroxy groups. Currently, citric acid or phosphoric acid is added during processing. Use of lecithin and phytic acid also has been investigated.

Citric Acid. A later study by Dutton et al. (1949) found that the addition of 0.01 percent citric acid improved the oxidative and flavor stability of oil processed in a commercial operation. These researchers

recommended addition of citric acid during the cooling stage of deodorization since citric acid was found to decompose at deodorization temperatures. It has been proposed that heating of oils prior to addition of citric acid is necessary because heating releases metals from a complex formed with hydroperoxides in the oil. Application of heat is believed to break the metallic complex so that metals can be chelated by later addition of metal inactivators (List and Erickson, 1985).

Phosphoric Acid. Phosphoric acid also may be added to deactivate trace amount of prooxidant metals. The use of excess phosphoric acid as a metal scavenger may lead to the development of cucumbery, melony, or fruity off-flavors (List and Erickson, 1985).

Lecithin. The use of lecithin as a metal inactivator also has been studied (Dutton et al., 1949). It was found that levels of lecithin which improve oxidative stability also cause darkening of the oil and melony bitter, or cucumbery flavors.

Phytic Acid. A study by Evans et al. (1953) determined phytic acid highly effective as a metal inactivator in edible oils. Winters et al. (1984) used peroxide values to test the effect of added phytate on the oxidative stability of oil exposed to accelerated test conditions. Results of this research contradicts the

findings of Evans et al. (1953) since phytate did not effect oil stability.

Packaging

Flavor and oxidative stability of soybean oil can be preserved through proper packaging techniques which exclude light and/or minimize contact with air (List and Erickson, 1985). Few studies have been conducted on packaging techniques.

Moser et al. (1965a) showed that oil packaged in brown glass bottles maintained flavor and oxidative stability after 2 hr exposure to fluorescent light. The same oil packaged in clear glass or translucent plastic and exposed to the same conditions, deteriorated to a point such that its quality was no longer acceptable.

Warner and Mounts (1984) used accelerated light and heat tests to determine the effect of packaging on the flavor and oxidative stability of hydrogenated and unhydrogenated soybean oils. A comparison was made between oils packaged in polyvinylchloride (PVC), acrylonitrile (AN), clear glass, and amber glass. No significant difference in flavor and oxidative stability between plastic and clear glass containers was found. The use of amber glass bottles significantly improved oil stability during the light exposure tests. These researchers concluded that packaging in PVC or AN bottles

was an acceptable alternative to glass.

Use of nitrogen as a headspace gas is important to flavor and oxidative stability. List and Erickson (1985) recommended the use of nitrogen blanketing of oils in bulk storage tanks. Evans et al. (1973) conducted a long term storage study on oils packaged with nitrogen in the headspace and oils packaged with air as the headspace gas. After 26 wks of storage at 100 °F,the flavor of partially hydrogenated oils packaged under nitrogen showed minimal loss of flavor and little, if any, reduction in oxidative stability. Soybean oil demonstrated progressive flavor when it was not protected with nitrogen.

Hydrogenation

Hydrogenation is the reaction of hydrogen gas with fats and oils in the presence of a catalyst. Partial hydrogenation is performed to increase the oxidative stability by the selective reduction of linolenic acid. Commercially, a dual purpose salad/cooking oil is produced by hydrogenation of soybean oil with nickel catalysts under selective conditions such as 5 - 14 psi, 0.05% catalyst at 177°C (Mounts and Warner, 1985).

Moulton et al. (1985) found that hydrogenated oils were more stable than unhydrogenated oils to oxidative deterioration. However, flavor stability of hydrogenated soybean oil from a continuous process was not improved

significantly.

Mounts et al. (1978) showed that hydrogenation of soybean oil to linolenic acid contents of 3.3 and 0.4 percent greatly improved the oxidative stability, but did not increase flavor stability during an accelerated heat storage test. Improvement of oxidative stability of soybean oil after partial hydrogenation has been attributed to the conversion of linolenic acid to isolinoleic acid and monoenoic acids, which are more difficult to oxidize. Some of the compounds formed during hydrogenation have double bonds between C-14 and C-16 and may produce flavor compounds similar to those of linolenic acid upon oxidation. This may account for the lack of impact on flavor (Frankel, 1985b).

A problem associated with hydrogenation of soybean oil is the development of hydrogenation flavor during storage. Yasuda et al. (1976) identified volatile compounds associated with hydrogenated soybean oil, which could contribute to total hydrogenation flavor. Compounds isolated include 2-trans 6-trans octadienal.

Hydrogenation lowers the linolenic acid content and thus increases oxidative stability of soybean oil asan edible oil. But this is only a partial solution to the problem and oxidative deterioration will eventually produce objectionable odors and flavors.

MATERIALS AND METHODS

This research project consisted of two separate investigations to study effect of heat and effect of light on four different treatments for soybean oil. The four treatments were: control (no additive), 5 ppm beta-carotene, 600 ppm alpha-tocopherol, and a mixture of 5 ppm beta-carotene plus 600 ppm alpha-tocopherol.

Various additive combinations at different levels were added to soybean oils in preliminary studies. Additives exhibiting greatest stabilizing potential were found to be alpha-tocopherol, beta-carotene, and a combination of the two. Levels of additives to be used in the project were determined as the lowest concentration that exerted an inhibition of photooxidation.

Treatment of Soybean Oil

Six different samples of partially hydrogenated soybean oil were obtained from Cargill, Inc. (Wichita, KS). Oil specifications are listed in Table 3.

Soybean oils were taken off-line at the Cargill facility in Wichita, packaged in one gallon aluminum cans, and shipped via United Parcel Service on the same day as processed. A time range of 24 to 48 hours elapsed before the oils were received. Immediately upon arrival, the additives were placed in the oils.

Additives used in this study were D-alpha-tocopherol

Table 3. Specifications of partially hydrogenated soybean oil.

Sample	Iodine Value	Free Fatty Acid Value	Lovibond Color[a]	Flavor Score[b]
1	105.9	0.012	2.0/0.2	7.5
2	105.7	0.015	3.0/0.3	7.5
3	105.4	0.022	3.0/0.4	7.5
4	106.5	0.015	3.0/0.3	7.5
5	104.9	0.016	3.0/0.3	7.5
6	102.6	0.017	4.0/0.4	8.0

[a] yellow/red

[b] Based on a 10-unit scale with 10 as bland, and 1 extreme.

(Fisher Scientific Company, Fairlawn, NJ) and 80 - 90% beta - isomer, 10 - 20% alpha - isomer beta-carotene (Sigma Chemical Company, St. Louis, MO).

The alpha-tocopherol (100 mg) was dissolved in a small amount (0.05 ml) of reagent grade ethanol. This solution was added to 166.7 g oil producing a final concentration of 600 ppm alpha-tocopherol. The additive was mixed thoroughly into the oil with a magnetic stirrer. The solvent was removed in vacuo during stirring with the application of low heat for approximately 25 min.

Beta-carotene (1.75 mg) was added directly to the soybean oil (350.0 g) giving a final concentration of 5 ppm beta-carotene. A magnetic stirrer was used to mix the additive thoroughly into the oil for approximately 10 min under vacuum. After mixing, a portion of the beta-carotene treated oil (166.7 g) was removed and alpha-tocopherol was introduced as described previously. The remaining beta-carotene treated oil was subjected to the same mixing conditions as the alpha-tocopherol treated oils.

A control sample (no additive) also was subjected to the mixing conditions previously described for the alpha-tocopherol treated oils.

After preparation, all samples were refrigerated (4 - 7°C) immediately after stirring and were held at

refrigeration temperature until needed.

Light Exposure

The light exposure test was conducted using an apparatus constructed with six 41 cm (16 in), 15-watt daylight fluorescent tubes mounted inside a 44.5 cm (17.5 in) diameter stainless steel drum, 52.7 cm (21.0 in) high. The interior of the drum was painted white to reflect lightfrom the back side of the fluorescent tubes. Circular perforations were cut into the bottom 6.5 cm of the drum and since the top was open, air was able to circulate freely. This apparatus was constructed in the Department of Physics, Kansas State University, based on specifications used by Moser et al.(1965a) in similar light exposure tests.

Light intensity was measured as 7535 lux (700 ft candles). Randomized samples were exposed to light for a period of 0 to 8 hours. To ensure maximum exposure, only one sample was exposed to the light source at a time.

Prior to the light exposure test, a sample was allowed to reach room temperature (approximately 20 min). Clear 250-ml glass bottles were filled approximately two-thirds full with 150 ml of the sample oil. A bottle was placed in the center of the light apparatus on top of an inverted 1000-ml glass beaker. The beaker was used to elevate the sample and further enabled maximum light exposure.

Analysis for Oxidation

Peroxide values were conducted in duplicate at the following time intervals: 0, 1, 2, 3, 4, 6, and 8 hours. At these time intervals, a 25-ml sample was removed from the glass bottle. Approximately 15 ml of this subsample was used in the peroxide analysis. The remainder was frozen for later gas chromatographic analysis.

Gas Chromatography

As a further measure of light deterioration, total volatile compounds were determined by a Hewlett Packard Gas Chromatograph (GC) model 5880A (Avondale, PA) in samples exposed to light for 0 and 8 hr. The technique used for this study was based on the procedure used by Marsili (1984) with the following modifications: a packed column was used instead of a capillary column, samples were incubated for 30 min instead of 40, and temperature programming was slightly altered to ensure maximum peak separation. The chromatograph was equipped with a flame ionization detector and a glass column (2.5 m x 2.0 mm i.d.) packed with 10% Carbowax 20M on 100/120 mesh Supelcoport (Supelco Co., Bellefonte, PA). The carrier gas was nitrogen (20 ml/min) while hydrogen (28 ml/min) and air (400 ml/min) were used in the detector. Temperature at the injection port was maintained at

220°C, and detector temperature was 230°C. For the analysis, 1.00 ± 0.01 g of the sample oil was weighed into a 5-ml reaction vessel (Supelco Co., Bellefonte, PA). A vial was capped with teflon closure lined with rubber septa and sealed using a crimper (closure, septa, and crimper obtained from Supelco Co., Bellefonte, PA). Prior to GC analysis, a sample was placed into a laboratory oven (Precision Scientific Co., Chicago, IL) maintained at 150 ± 1.0°C for 30 min. After incubation, the vessel was allowed to cool for 5 min at room temperature. A sample of headspace gas (1.0 ml) was withdrawn from the vessel using a 1.0-ml gas syringe (Precision Scientific Corp., Baton Rouge, LA). The sample was injected immediately and temperature programming was initiated. The column was maintained at 50°C for the initial 5 min, then elevated to 170°C at 15°C/min. The column was maintained at 170°C for 15 min to allow elution of remaining peaks. The value of total volatiles was obtained using GC integrator counts.

Heat Exposure

The heat exposure test was conducted in a 220 volt, 5.5 amp incubator (Precision Scientific Co., Chicago, IL.). Temperature was maintained at 60± 0.5°C, in accordance with other accelerated heat tests (Moser, 1965b; Evans, 1973).

For the heat exposure test, samples were allowed to equilibrate to room temperature and poured into 250-ml clear glass bottles similar to those used in the light study. The bottles were filled to two-thirds full with 150 ml oil and then covered loosely with clean plastic wrap. A small portion of each sample was refrigerated as a control. Four samples were placed randomly into the incubator for a period of four days. After heat exposure, peroxide values were conducted, in duplicate, on the control and heat-treated samples.

Peroxide values were obtained using the American Oil Chemists' Society Method Cd8-53 (AOAC, 1984; Appendix). Reagents used for the analysis were chloroform, glacial acetic acid, sodium thiosulfate solution N/10 (0.1002 - 0.00998 N) and soluble starch (Fischer Scientific Co., Fairlawn, NJ); and potassium iodide (Mallinckrodt Inc., St. Louis, MO.)

Data were collected using a split-plot statistical design and analysis of variance between and within the samples was computed. F - values were calculated and when significance was determined, t-tests were performed.

RESULTS AND DISCUSSION

The overall objective of this research was to determine the effect of additives (alpha-tocopherol, beta-carotene, and a combination of alpha-tocopherol and beta-carotene) on the shelf-life of soybean oil. The investigation was divided into two parts: the effect of additives on the light stability of soybean oil and the effect of additives on the heat stability of soybean oil.

Effect of Additives on Light Stability of Soybean Oil

The mechanism of light deterioration of soybean oil proceeds via the reactive intermediate singlet state oxygen (Clements et al., 1973). Singlet oxygen reacts directly with unsaturated fatty acids generating hydroperoxides which subsequently decompose, forming a variety of volatile products. To inhibit the deleterious effect of singlet oxygen, efficient singlet oxygen quenchers are necessary to deactivate this reactive molecule to the ground state.

The effect of the additives used in this study is summarized in Table 4. Significant differences were found between the control oil (no additive) and the oil treated with beta-carotene alone and the oil treated with beta-carotene in combination with alpha-tocopherol. No significant difference was detected between the control and the alpha-tocopherol treated oils.

Table 4. Effect of additives on peroxide values[a] (meq/kg) in soybean oil exposed to fluorescent light.

Additive	Light exposure (hr) 1	2	3	4	6	8
Control	0.7 (±0.1)	1.1 (±0.1)	1.4 (±0.0)	1.7 (±0.0)	2.2 (±0.1)	2.7 (±0.2)
Alpha-tocopherol	0.7 (±0.1)	1.0 (±0.1)	1.4 (±0.1)	1.8 (±0.1)	2.2 (±0.1)	2.7 (±0.2)
Beta-carotene	0.3* (±0.1)	0.4* (±0.0)	0.6* (±0.1)	0.8* (±0.0)	1.2* (±0.1)	1.5* (±0.1)
Alpha-tocopherol + beta-carotene	0.2* (±0.1)	0.4* (±0.1)	0.6* (±0.0)	0.8* (±0.1)	1.1* (±0.1)	1.4* (±0.1)

[a] Initial PV < 0.1 in all samples.

[b] Each mean represents 2 determinations for four replications.

* Significant at the 1 % level.

Alpha-tocopherol

Although Frankel et al. (1979) found alpha-tocopherol more effective than beta-carotene at slowing the rate of photooxidation, Terao et al. (1980) concluded that inhibition of photooxidative deterioration could not be achieved by tocopherols. The results of the current study support that of Terao et al. (1980). A possible explanation for the poor inhibitory effect of alpha-tocopherol on singlet oxygen initiated oxidation is the high chemical reactivity of alpha-tocopherol toward singlet oxygen.

Beta-carotene

Addition of 5 ppm beta-carotene was effective in preventing significant light deterioration when compared to the control for all time exposures tested ($p < 0.01$). This finding is in agreement with previous work which used much higher concentrations of beta-carotene (Frankel, 1979; Terao et al., 1980), and recent work which used < 20 ppm beta-carotene (Warner and Frankel, 1986). The effectiveness of beta-carotene can be attributed to its ability to physically quench singlet oxygen.

Alpha-tocopherol in Combination with Beta-carotene

This treatment also offered significant improvement ($p < 0.01$) in deterioration as compared to the control for all light exposure periods tested. However,

combination of alpha-tocopherol with beta-carotene was not significantly different than the beta-carotene treated oils ($p < 0.05$) Terao et. al. (1980) found that when soybean oil was chromatographed through Florisil, beta-carotene acted as a prooxidant because tocopherols were removed; but in the presence of delta-tocopherol, beta carotene acted to synergistically prevent autoxidation. These researchers concluded that tocopherols protect beta-carotene from free radical autoxidation. Warner and Frankel (1986) attributed the ability of beta-carotene (<20 ppm) to provide light stability to the protective effect of natural tocopherols present in soybean oil. Thus, photooxidation of soybean oil is inhibited by beta-carotene only when oxidation of this quencher is prevented by tocopherols. Since no significant increase in light stability was found between soybean oil treated with tocopherol and beta-carotene as compared to soybean oil treated with beta-carotene alone, the oils used in this study must have contained sufficient amounts of tocopherols to prevent the autoxidation of beta-carotene. Addition of alpha-tocopherol was unable to increase the light stabilizing effect of beta-carotene.

Analysis of Light Induced Volatiles

The values of total volatiles as measured by gas chromatograph integrator counts are summarized in Table 5.

Table 5. Effect of additives on total volatiles as measured by gas chromatography integrator counts[a] in soybean oil exposed to fluorescent light.

Additive	Light exposure (hr) 0	8
Control	2800	4800
Alpha-tocopherol	1800	4000
Beta-carotene	1500	2000*
Alpha-tocopherol + beta-carotene	1300	2600

[a] Each value is a mean for four replications.

* Significant at the 5 % level.

Results of this analysis indicate that beta-carotene alone provided significant protection against photooxidation ($p < 0.05$). Oils treated with tocopherol alone and tocopherol in combination with beta-carotene did not inhibit photooxidation ($p < 0.05$). It is possible that the heat treatment used to generate volatiles affected the outcome of this study by adversely affecting the tocopherol causing it to decompose and act as a prooxidant. This may account for the insignificant differences betweenthe oils containing the tocopherol-beta-carotene combination and the untreated oils. The two major peaks isolated by this GC method, based on retention time, are pentane and hexanal. Work using similar column conditions also identified these two compounds,and concentration of pentane correlated well with sensory analysis (Scholz and Ptak, 1966). Warner and Frankel (1986) identified pentane and hexanal in addition to 2-heptenal, 2,4-heptadienal, and 2,4-decadienal in oils exposed to light (8 and 24 hr) using capillary gas chromatography and mass spectral analysis. Their work determined significantly lower amounts of these volatiles in oils treated with 20 ppm beta-carotene in comparison to untreated oils.

Effect of Additives on Heat Stability of Soybean Oil

The mechanism of autoxidation proceeds via the free

radical intermediate. To effectively inhibit this reaction, phenolic antioxidants or free radical scavengers are necessary. The additives used in this study were unable to decrease the heat-catalyzed autoxidation reaction and, in fact, a significant prooxidant effect was noted in samples treated with beta-carotene in combination with alpha-tocopherol. Table 6 summarizes the effect of additives on peroxide values in soybean oil samples exposed to storage at 60°C for a period of 4 days.

Alpha-tocopherol

Alpha-tocopherol is known for its free radical scavenging ability, but did not exert a positive effect on the soybean oils used in this study. Similar results were reported by Werman and Neeman (1986). These researchers found alpha-tocopherol (250 ppm) an ineffective antioxidant in avocado oil exposed to 60° C for a time period of to 6 weeks.

Beta-carotene

This additive offered no improvement in stability of oils exposed to the heating conditions used in this study This result is in agreement with work by Warnerand Frankel (1986). These researchers found peroxide values of oils treated with beta-carotene (< 20 ppm) were not significantly different than control oils when stored at 60°C for a period of 4 days. It was noted that after 8

Table 6. Effect of additives on peroxide values [a] (meq/kg) in soybean oil exposed to heat treatment.[b]

Additive	Peroxide Value[c]
Control	1.5 ± 0.3
Alpha-tocopherol	2.6 ± 1.0
Beta-carotene	2.2 ± 1.2
Alpha-tocopherol + beta-carotene	3.5 ± 1.4*

[a] Initial PV < 0.1 in all samples.

[b] Each value represents 2 determinations for four replications.

[c] Determined after four days storage at 60°C.

* Significant at the 1 % level.

days of storage at 60°C oils containing beta-carotene had significantly higher peroxide values than the control. These researchers suggested that free radical oxidation was promoted by beta-carotene in the dark.

Alpha-tocopherol in Combination with Beta-carotene

This combination had a significantly higher peroxide value ($p < 0.05$) as compared to the control. It is possible that the heat caused the beta-carotene and/or the alpha-tocopherol to decompose and act as a prooxidant.

CONCLUSIONS

Based on the conditions of this study, the following conclusions can be made:

1. Addition of beta-carotene to soybean oil provided significant protection against photooxidation. Beta-carotene was determined an efficient quencher of singlet oxygen.
2. Beta-carotene was ineffective in preventing autoxidation of soybean oil. It is possible that this compound is oxidized in the presence of heat. Further research is necessary to verify this theory.
3. Addition of alpha-tocopherol was found ineffective at increasing the stabilizing effect of beta-carotene. Soybean oil, when processed under suitable conditions, apparently retains sufficient amounts of tocopherol to protect beta-carotene from autoxidation.

RECOMMENDATIONS

1. Use of beta-carotene at a concentration of 5 ppm in soybean oil or foods containing soybean oil (such as popcorn oil or salad dressing) may have potential to extend shelf-life.
2. Interactions of natural additives in simple and food systems containing soybean oil warrants further investigation.

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ACKNOWLEDGMENTS

I would like to express sincere thankfulness and appreciation to my major professor, Dr. Martha B. Stone, Associate Professor, Foods and Nutrition Department, for her support and guidance throughout this research project.

Appreciation is also extended to Dr. Daniel Y. C. Fung, Professor and Chairman, Food Science Graduate Program, Dr. Gerald Reeck, Professor, Department of Biochemistry, and Dr. Jon Faubion, Assistant Professor, Department of Grain Science and Industry, for serving on my supervisory committee; to Dr. Paul Nelson, Associate Professor, Department of Statistics, for assistance with the experimental design and data analysis; and to Dr. William Klopfenstein, Professor, Department of Biochemistry, for technical assistance.

My specialthanks to my husband, Paul, for his support, understanding, and patience throughout my graduate studies. I am grateful to the Department of Foods and Nutrition and the Kansas Agricultural Experiment Station for financial support.

Finally, I wish to express my gratitude to my parents, Mr. and Mrs. Joseph Bauer, my husband's parents, Mr.and Mrs. Paul Kramer, and the staff in the Department of Foods and Nutrition for their support and encouragement throughout the study.

APPENDIX

Table A - 1. Analysis of variance table of effect of additives on peroxide values in soybean oil exposed to fluorescent light.

Source of variance	SS	d.f.	MS	F
Between subjects	15.22	15		
Treatment (A)	14.99	3	4.99	262.6*
Subjects within a group	.23	12	0.19	
Within subjects	49.24	96		
Time periods (T)	44.73	6	7.46	2572.4*
A x T	4.30	18	0.24	82.7*
T x subj. within a group	0.21	72	0.0029	
Total	64.46	111		

* Significant at the 1 % level.

Table A - 2. Analysis of variance of effect of additives on total volatiles as measured by gas chromatography integrator counts in soybean oil exposed to fluorescent light.

Source	SS	df	MS	F
Between subjects (subj.)	5.8063×10^7	15		
Treatment (A)	2.1067×10^7	3	7.0224×10^6	2.28
Subj. within group	3.6996×10^7	12	3.0830×10^6	
Within subj.	3.9121×10^7	16		
Time periods (T)	1.8101×10^7	1	1.8101×10^7	12.63*
A x T	3.8220×10^6	3	1.2740×10^6	0.89
T x subj. within group	1.7199×10^7	12	1.4332×10^6	
Total	9.7184×10^7	31		

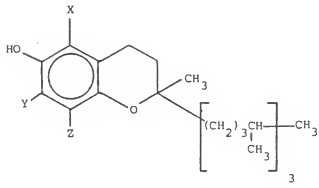
* Significant at the 1 % level.

Table A - 3. Analysis of variance of the of additives on peroxide values in soybean oil exposed to heat treatment.

Source	SS	df	MS	F
Between subjects (subj.)	11.18	15		
Treatment (A)	4.27	3	1.42	2.45
Subj. within group	6.91	12	0.58	
Within subj.	55.52	16		
Time periods (T)	44.42	1	44.42	77.92*
A x T	4.26	3	1.42	2.49
T x subj. within group	6.84	12	0.57	
Total	66.70	31		

* Significant at the 1 % level.

Figure A-1. Structures of various tocopherols and beta-carotene.



α-Tocopherol, X = Y = Z = CH_3

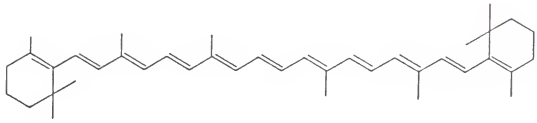
β-Tocopherol, X = Z = CH_3; Y = H

γ-Tocopherol, Y = Z = CH_3; X = H

δ-Tocopherol, X = Y = H; Z = CH_3

Structures of the various tocopherols.

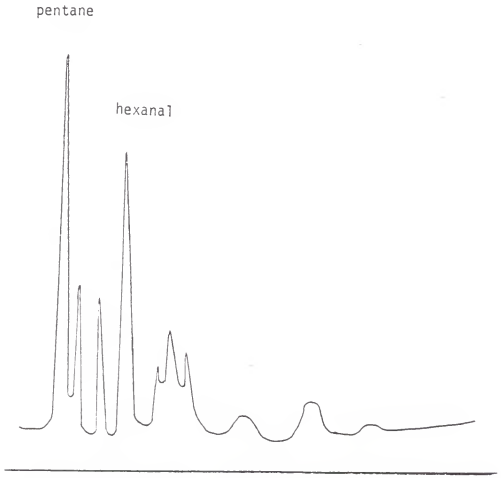
Source: Pryde, 1985c.



Structure of beta-carotene.

Source: Zapsalis and Beck, 1985.

Figure A-2. Typical gas chromatogram of headspace volatiles.



Peroxide Value of Oils and Fats

Titration Method

Final Action

American Oil Chemists' Society Method

Weigh 5.00±0.05 g sample into a 250 ml glass erlenmeyer. Add 30 ml HOAc-$CHCl_3$ (3:2) and swirl to dissolve. Add 0.5 ml saturated KI solution from Mohr pipet, let stand with occasional shaking for 1 min, and add 30 ml H_2O, Slowly titrate with 0.1N $Na_2S_2O_3$ with vigorous shaking until yellow is almost gone. Add approximately 0.5 ml 1% starch solution and continue titration, shaking vigorously to release all I from $CHCl_3$ layer, until blue just disappears. If <0.5 ml 0.1N $Na_2S_2O_3$ is used, repeat determination with 0.01N $Na_2S_2O_3$.

Conduct blankdetermination daily (must be ≤0.1 ml 0.1N $Na_2S_2O_3$). Subtract from sample titration.

Peroxide value (meq peroxide/kg sample) = S x N x 1000/g sample, where s = ml $Na_2S_2O_3$) blank corrected and N = normality $Na_2S_2O_3$ solution.

Source: AOAC, 1984.

EFFECTS OF BETA-CAROTENE AND ALPHA-TOCOPHEROL ON STABILITY OF SOYBEAN OIL

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE
in
FOOD SCIENCE
Department of Foods and Nutrition
KANSAS STATE UNIVERSITY
Manhattan, Kansas
1986

ABSTRACT

Soybean oil was treated with beta-carotene (5ppm) and alpha-tocopherol (600 ppm) alone or in combination. Effect of these additives on the stability of soybean oil was studied using the following accelerated aging conditions: fluorescent light exposure for predetermined time periods ranging from 0 to 8 hr and a storage test conducted at 60°C for a period of 4 days. Beta-carotene treated oils exhibited significant increased light stability over all time intervals monitored based on peroxide values. Addition of alpha-tocopherol did not extend this effect. Soybean oils used in this study may have contained sufficient amounts of tocopherol to prevent autoxidation of beta-carotene. Gas chromatography of light induced volatiles confirmed these results. Oils treated with beta-carotene alone had significantly lower amounts of total volatiles. Results of the storage test conducted at 60°C indicated that beta-carotene was unable to inhibit autoxidation of soybean oil since no significant difference in peroxide values was found between oils treated with beta-carotene and those untreated. Oils containing alpha-tocopherol in combination with beta-carotene exhibited significantly increased peroxide values. Heat caused beta-carotene and/or alpha-tocopherol to decompose and act as a prooxidant. Beta-carotene can

be classified as an efficient singlet oxygen quencher because it reduced the rate of photooxidation and was unable to inhibit autoxidation.