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thesis entitled

Photooxidation Studies on Soybean Oil

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science

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Date 11 May 1980

O-7639

PHOTOOXIDATION STUDIES ON SOYBEAN OIL

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1980

ABSTRACT

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The photooxidation of bleached soybean oil with added chlorophylls, β-carotene, and natural and synthetic antioxidants under ultraviolet (UV), fluorescent, and incandescent radiation sources was compared by measurements of peroxide development to the photooxidation of crude, commercially refined, and bleached oil alone.

The pigments β-carotene, chlorophyll, and other color were efficiently removed from bleached oil without affecting appreciably the fatty acid composition. Although chlorophyll a and b had the same behavior in promoting photooxidation, at concentrations that can occur naturally in soybean oil, they did not appreciably accelerate the photooxidation of bleached oil. Higher concentrations of chlorophyll than those found in crude and refined oil are required to accelerate the photooxidation process. Instead of acting as a singlet oxygen ($^1O_2$) quencher, β-carotene at concentrations of 1, 5, and 10 ppm showed a noninhibiting effect in the photooxidation of bleached oil in the presence or absence of chlorophyll. The higher concentrations of β-carotene actually stimulated higher rates of oxidation. The lack of activity of chlorophyll as a sensitizer and β-carotene as a

quencher was attributed to the destruction of these pigments by oxidation in the presence of UV radiation. Chlorophyll and, to a lesser degree, β-carotene were also degraded in nonirradiated samples. Possible differences in tocopherol content were estimated by high-performance liquid chromatography. Bleached oil had markedly lower tocopherol content than did crude and refined oil, which had essentially equivalent amounts. Alpha- or γ-tocopherols failed to inhibit the oxidation of bleached oil in the presence or absence of chlorophyll under UV radiation, whereas in the absence of chlorophyll and under fluorescent and incandescent light, both gave good photooxidation protection to the bleached oil. The high energy content of UV radiation could have generated $^1O_2$, which in turn degraded the antioxidants. A combination of a vitamin E mixture and β-carotene provided the bleached oil some protection against photooxidation in the presence of chlorophyll. The four tocopherols or δ-tocopherol present in the vitamin E mixture possibly protected β-carotene from photodestruction, which then was able to act as a $^1O_2$ quencher.

TBHQ did not inhibit the photooxidation of bleached oil in the presence of chlorophyll, whereas in the absence of chlorophyll it was observed to exert a strong antioxidative effect with all radiation sources except UV. The noninhibiting effect on photooxidation by TBHQ and tocopherols in the presence of chlorophyll suggested that a different mechanism took place in conjunction with autoxidation when chlorophyll was present. A reinforcement of this concept was obtained by the lower values of the molar absorbance at 233 nm/PV ratio of

bleached oil containing chlorophyll than were found in crude, refined, and bleached oils. These results indicated the possible involvement of $^1O_2$ in the oxidation of bleached oil when chlorophyll was present.

Independent of the irradiation source, the order of resistance of the oils toward photooxidation was: crude > refined > bleached. Bleached oil developed higher peroxide values than did crude and refined oil due to the removal of the tocopherols in the bleaching process. Phosphatides were not present in commercially refined and laboratory bleached oil. This may be the reason for the higher PV developed in refined oil compared to crude oil. It appears that lecithin can act synergistically with tocopherols to inhibit photooxidation of bleached soybean oil. The effect of radiation sources on promoting photooxidation was: UV > fluorescent light > incandescent light.

To my wife, Maritza.

ACKNOWLEDGMENTS

The writer expresses his sincere appreciation to Dr. L. R. Dugan, Jr. as research chairman and major professor. The patience, understanding, and encouragement Dr. Dugan expressed throughout the graduate program leave the writer with the greatest respect and admiration.

Appreciation and thanks are extended to the members of the guidance committee, Drs. A. M. Pearson, J. R. Brunner, L. L. Bieber, and P. Markakis, for their critical review of this dissertation.

The writer is indebted to several individuals for their assistance in this research, including Dr. I. Gray for his interest and advice and S. Cuppet for her help in the analysis of tocopherols.

Special acknowledgments are also due to the Brazilian Ministry of Education and to the Universidade Federal Rural do Rio de Janeiro for providing me this opportunity to study at Michigan State University.

Last, the writer is especially grateful to his wife, for her patience and help which in a very definite way allowed me to complete my work.

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INTRODUCTION

The oxidation of unsaturated fats and oils leads to rancidity, which is the source of most of the spoilage in fats and oils. The factors that are known to accelerate oxidation are numerous. One, among them, of considerable importance is the effect of light. Exposure to light has a marked accelerating effect upon the development of rancidity and the spectral regions of light energy, which are more active in catalyzing oxidation, seem to correspond with the absorption regions of the oils. The light sources emitting short-wavelength radiant energy appear to be the most deleterious.

The majority of the research dealing with the effect of light in promoting oxidation of fats and oils was done between 1930 and 1947, when the role of singlet oxygen in photosensitized oxidation was not yet known. In recent years, there has been increasing evidence that singlet oxygen can react directly with the double bonds of unsaturated fatty acids in fats and oils to produce peroxides. This suggests that photosensitized oxidation may be important in initiating or propagating normal free radical autoxidation of unsaturated fats and oils.

Chlorophyll, which occurs naturally in soybean oil, can act as a sensitizer and, in the presence of light, generate singlet oxygen, which can initiate the oxidation process. On the other hand, carotene, which also occurs naturally in soybean oil, is suggested

to be an inhibitor for singlet oxygen just as the tocopherols are naturally occurring inhibitors of radical chain autoxidation.

Most of the recent studies carried out on fats and oils or fatty materials in the presence of photosensitizers, quenchers, or both, used conditions where the fat or fatty material was diluted with organic solvents. The photooxidation of pure fatty acids, fats, and oils in these conditions may not necessarily simulate the mechanism taking place in natural systems.

The purpose of this investigation was to study the effect of adding in concentrations that are expected to be found in soybean oil, chlorophyll a and b, β-carotene, α- and γ-tocopherols, vitamin E mixture as well as TBHQ, BHA, and lecithin on the photooxidation of bleached and refined soybean oil. Bleached oil, prepared from crude soybean oil, was used to achieve a better control of the effect of adding pigments or antioxidants. The efficiency of the bleaching process in removing pigments from soybean oil was assessed by analyzing the content of chlorophyll, β-carotene, and color before and after the bleaching process.

Ultraviolet, fluorescent, and incandescent light were chosen to study the radiant energy effects on oxidation of crude, refined, and bleached soybean oil with added pigments or antioxidants. Peroxide values, conjugated diene, and in some cases TBA absorbance were used to control the photooxidation effect in the samples.

REVIEW OF LITERATURE

Mechanism of Fat Oxidation

The reaction of oxygen with unsaturated fatty acids in lipids constitutes the major means by which lipids or lipid-containing foods deteriorate (Dugan, 1976). Among the oxidative processes, autoxidation represents the most important way by which fat or fat materials deteriorate.

The mechanism of autoxidation involves the following three steps (Boland & Ten Have, 1947):

1. Initiation

$$RH + O_2 \longrightarrow R\cdot + \cdot OOH$$

2. Propagation

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

3. Termination

$$R\cdot + R\cdot \longrightarrow RR$$

$$R\cdot + ROO\cdot \longrightarrow ROOR$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

RH refers to any unsaturated fatty acid in which H is labile by reason of being on a carbon atom adjacent to a double bend. R· refers to a free radical formed by removal of a labile hydrogen. The oxidative process becomes more complex after the development of a quantity of ROOH since the ROOH then decomposes either because of

thermal instability or through reaction with other materials to form more free radicals which in turn participate in the chain reactions (Dugan, 1961).

In food lipids, the most important problem is concerned with the initiation because, once initiated, the products of the reaction catalyze the reaction, and for this reason oxidation of fat is frequently alluded to as autoxidation.

The autoxidation of fats is affected by a number of factors. These factors are: (1) degree of unsaturation of fatty acids, (2) heat, (3) light, (4) ionizing radiation, (5) enzymes, (6) prooxidant metals and metallic compounds, (7) presence of oxygen, and (8) use of antioxidants (Lea, 1962).

In the autoxidation of fats, unsaturated fatty acids are oxidized to conjugate hydroperoxides which are called primary products of oxidation. These hydroperoxides undergo decomposition to form secondary products. Among the secondary products are a number of volatile compounds including unsaturated esters, aldehydes, ketones, alcohols, and hydrocarbons. These volatile compounds contribute to undesirable flavor in the autoxidized fats (Evans, 1961). The contribution of saturated and unsaturated aldehydes to off-flavor characteristics of the rancid fats has been reported by Hoffman (1962), Hammond and Hill (1964), and Horvat et al. (1965).

The hydroperoxides formed by autoxidized methyl oleate, methyl linoleate, and methyl linolenate were analyzed by gas chromatography-mass spectrometry (Frankel et al., 1977a, 1977b, 1977c). With methyl oleate, higher concentrations were found of the 8- and

11-hydroperoxides than of the 9- and 10-hydroperoxides. This uneven distribution of hydroperoxide isomers has an important implication to the mechanism of autoxidation and indicates that, contrary to general belief, carbons -8, -9, -10, and -11 of oleate are not equivalently subject to oxygen attack. Autoxidized methyl linoleate gave equal amounts of 9- and 13-hydroperoxides in all samples of linoleate autoxidized at different temperatures and peroxide levels. The results are consistent with the classical free radical mechanism of autoxidation involving hydrogen abstraction at carbon -11. The sites for oxygen attack at carbon -9 and carbon 13 are equivalent. No evidence was given for 11-hydroperoxide formation. Four hydroperoxides with conjugated diene systems were identified by autoxidation of methyl linolenate. The proportions of 9- and 16-hydroperoxides were significantly higher (75-81%) than those of 12- and 13-hydroperoxides (18-25%). These authors (Frankel et al., 1977c) suggested that steric factors might indeed be invoked for greater attack of O_2 on C-9 and C-16 than on C-12 and C-13 on one hand, and greater attack on C-16 than on C-9 on the other hand. The reduced yields of 12- and 13-hydroperoxides from linolenate can also be explained by their tendency to cyclize into prostaglandin-like endoperoxides.

In addition to autoxidative rancidity, many oils and fats undergo a change in flavor, known as reversion, before the onset of rancidity. The flavors that develop are quite different from rancid flavors. Soybean oil, which reverts readily, is described as developing first

a buttery or beany flavor, then grassy or hay-like, then painty, and finally fishy (Mookherjee & Chang, 1963).

Several theories for the cause of reversion flavor include: (1) oxidation of linolenic acid, (2) oxidation of isolinolenic acid of the 9,15-diene structure, (3) phosphatide reactions, (4) unsaponifiables, and (5) oxidation polymers (Smouse, 1979). Smouse and Chang (1967) isolated and identified a total of 71 volatile compounds from a reverted soybean oil. Of the 71 compounds identified, 1-decyne and 2-pentyl furan were of particular interest. At concentrations of 1-10 ppm, 2-pentyl furan imparts to an oil a characteristic beany and grassy odor and flavor reminiscent of those of a reverted soybean oil. It was concluded that 2-pentyl furan is predominantly responsible for the reversion flavor of soybean oil, particularly that which is developed when the oil has been exposed to light. Since 2-pentyl furan is postulated as originating from linoleic acid in amounts sufficient to cause reversion flavor in soybean oil but not cottonseed oil, which contains an approximately equal amount of linoleic acid, it is possible that linolenic acid in soybean oil not only catalyzes the autoxidation of linoleic acid but may also alter the decomposition pattern of its hydroperoxides.

The fatty acid that is present in soybean oil at 7%, but only in trace amounts in the nonreversion oils, is linolenic acid. It is, therefore, logical to think that the linolenate undergoes the same autoxidation reaction as linoleate to produce unsaturated pentyl furans, which then may serve as components of reversion flavor.

Ho et al. (1978) synthesized 2-(1-pentenyl) furan from triphenylbutylphosphonium bromide in the presence of n-butyl lithium and furfural. They found, through an organoleptic panel, the flavor threshold of cis-2-(1-pentenyl) furan in oils is ca. 6ppm, whereas the trans-2-(1-pentenyl) furan has a flavor threshold of only 1ppm. At a concentration of 9ppm, the cis isomer in a freshly deodorized sunflower oil had a characteristic odor and flavor slightly reminiscent of reverted soybean oil. The trans isomers at a concentration of 2ppm in oil had an odor and flavor moderately suggestive of those of reverted soybean oil.

The organoleptic results, therefore, suggested that the cis- and trans-2-(1-pentenyl) furans could contribute to the reversion flavor of soybean oil if they are formed by the autoxidation of linolenate.

Light-Induced Oxidation of Edible Oils and Fats

The action of light has long been known to cause deterioration of oils, fats, and fat-containing products during storage. Coe and LeClerc (1934a) showed that if cottonseed or corn oil was protected from light by wrapping the glass containers with metal foil, opaque black paper, or a green wrapping material whose permeability to light is limited to the interval from 490 to 580 nm, rancidity did not develop even though the peroxide value reached approximately 60 millimoles/kg oil. If the oil was not protected from light, rancidity appeared at this stage. Coe and LeClerc (1934b) also showed that when oils were irradiated using color filters, the blue light

was more conducive to the formation of peroxides and the development of rancidity than the red end of the spectrum for the same time of irradiation. The formation of peroxides and the development of rancidity were accelerated in proportion to the amount of blue light transmitted by the filter. They also showed, with respect to the formation of peroxides and development of rancidity, that lard and animal fat responded to selective light in the same way as did corn and cottonseed oils. Containers or wrappers designed for enclosing oil-bearing foods should exclude both ends of the visible spectrum, more especially the blue, in order to prevent or delay the development of rancidity. The color that affects the development of rancidity the least is green delimited by 490 to 580 nm. The use of a protective green container or wrapper alone, or in conjunction with an antioxidant, increased the period during which the oil or fat remained fresh at room temperature and, incidentally, decreased the rate of peroxide formation. Oils or fats stored at low temperature and with light excluded remained fresh longer than if treated with an antioxidant or packaged in green and exposed to light at room temperature (Coe and LeClerc, 1935).

When the intensity of light to which oil-bearing foods are exposed is the same, the ultraviolet, violet, and blue regions (below 490 nm) promote rancidity the most, whereas the regions at about 540 and 740 nm promote rancidity the least. The yellow region around 570 and the red region around 660 nm are less active than the blue region but nevertheless promote rancidity appreciably more than the green region. The regions that are more active in catalyzing

rancidity seem to correspond with the light-absorption regions of the oil (Coe, 1941b; Greenbank & Holm, 1941).

McConnel and Esselen (1947) in their studies of the effect of storage conditions and antioxidants on the keeping quality of packaged cottonseed and corn oil found that total exclusion of light was very effective in preventing the development of rancidity in oils stored in sealed containers. Many substances, when added to the oils, were slightly effective in protecting corn and cottonseed oils against the harmful effect of light, but in no case was this protection found to be as good as that provided by the total exclusion of light or its partial exclusion as by the use of amber glass bottles.

A light test to measure stability of edible oils was developed by Moser et al. (1965). The method was based on exposing samples of soybean, cottonseed, and safflower oils to fluorescent light in an easily assembled unit. They found that the flavor of soybean oil dropped significantly after 4 days' storage at 60°C; therefore, this test could be used to measure its stability. The light test can be used in the same way. A light equivalent value (LEV) of 1 was obtained for soybean oil. The LEV was described as the number of hours necessary to give a flavor score equivalent to that obtained after 4 days' storage at 60°C, and this value can be used as a test to predict stability. Because exposure to light is much more destructive than oven storage, quality testing can be performed more simply and more rapidly. The stability of soybean oil in this case can be judged in 1 hour instead of 8 by the AOM method or 4 days by the oven-storage method. Exposure of 0.5-1 hour in flourescent light

produced significant changes both in flavor and peroxide values of the oils.

Sattar et al. (1976a) used fluorescent-light energy to study the oxidizing effect in rapeseed, corn, soybean, and coconut oils, and milk fat. Among the five fats, soybean and coconut oils and milk fat were very sensitive, rapeseed oil intermediate, and corn oil was relatively stable to light-induced flavor deterioration. The susceptibility of these oils or fats to photooxidation did not depend on the degree of unsaturation alone (Sattar et al., 1976b). They also observed that the most deleterious wavelengths were those less than 455 nm. Sattar et al. (1976c) studied the oxidation effect of fluorescent light at varying time-temperature ratios on butter, butterfat, and corn, rapeseed, and soybean oils. They found that there was no increase in oxidation rate when the light was switched off. The stability of the oils did not correlate well with the ratios of C18:2 to C18:1 to C18:3 to C18:2 nor with the degree of unsaturation. Increase in temperature alone had minimal effect; however, in the presence of light the rate of oxidation increased considerably with a corresponding decrease in the content of vitamin A and β-carotene. The rate of oxidation decreased at higher wavelengths, and this effect was more pronounced in the vegetable oils than in butterfat, where the β-carotene was considered to serve as a filter for light of low wavelength.

Lee and Kim (1975) placed commercial edible soybean oil in plastic containers. Transparent cellophane films colorless (control), red, green, and also colorless coated with Cemedine C, Cemedine C

containing 10% pyridine, benzophenone, or P-aminobenzoic were prepared, and the percentage transmittance of each film to light in the UV and visible range was measured. The containers were covered with the films and irradiated simultaneously with direct sunlight for 4.5 hours daily. The red and green films strongly retarded peroxide value development, red showing a slightly stronger effect than green. In general, the retarding effect of films on peroxide-value development were, in decreasing order: red > green > P-aminobenzoic-acid-coated > Cemedine C-coated > control > pyridine-coated > benzophenone-coated.

The effect of light having wavelengths in the visible region appears to be primarily one of accelerating the decomposition of hydroperoxides. The hydroperoxides of pure fatty acids and esters in liquid form, however, are virtually colorless and transparent. It is not clear, therefore, to what extent the catalytic effect of visible light is attributable to absorption of light by the hydroperoxides, or by minor constituents that are present as contaminants or as secondary products of the autoxidation reactions. The effect of UV radiation is more pronounced. The autoxidation of the more common polyunsaturated fatty acids leads to the production of conjugated unsaturated systems, which absorb UV radiation strongly at certain wavelengths. In such systems, the UV radiation accelerates markedly the decomposition of peroxides, but it may also influence the course of the autoxidation reactions by other mechanisms (Lundberg, 1962).

Role of Singlet Oxygen in Fat Oxidation

Khan et al. (1954) irradiated methyl oleate and methyl linoleate with a 300-watt photoflood bulb in the presence and absence of chlorophyll. They found that appreciable amounts of hydroperoxides were formed in molecules with unconjugated double bonds, whereas in ordinary autoxidation of linoleate, all of the hydroperoxides were conjugated. It was found that α-tocopherol, which is an effective inhibitor of the ordinary autoxidation reaction, was virtually ineffective in the photochemical oxidation. These observations indicated not only a marked difference in mechanism but suggested the possibility that no chain mechanism was involved.

The presence of hydroperoxides with nonconjugated double bonds among the primary reaction products could serve to identify singlet O_2 as the reactive intermediate in photosensitized reactions and to distinguish them from free radical initiated autoxidation reactions.

After the discoveries of Foote (1976), who has shown that singlet oxygen is the intermediate in the dye-sensitized photooxidation of a number of monoenoic and dienoic compounds, Rawls and Van Santen (1970b) and Clements et al. (1973) have presented evidence for the participation of singlet O_2 in the photosensitized oxidation of fats.

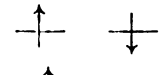
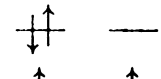
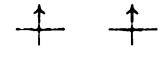
The probable roles of singlet O_2 in biological systems were described by Krinsky (1977), reactions with organic compounds by Ramby and Rabex (1978), and as activated species involved in oxidation of food constituents by Dahl and Richardson (1978).

According to molecular orbital theory, oxygen can occur in two states: triplet and singlet. In the triplet state, there are two outer electrons in two separate orbitals with their angular moment opposed but with parallel spins. The two unpaired electrons have a permanent magnetic moment if placed in a magnetic field. This may be symbolized by $^3\Sigma$, $^3O_2$, $\uparrow\uparrow$.

In the singlet state of oxygen, the outer electrons have opposed spins, which may be symbolized as $^1\Sigma$, $^1\Delta$, $^1O_2$, $\uparrow\downarrow$. The vast majority of stable organic molecules have the singlet ground state.

Singlet and triplet states of oxygen appear to have the same energy. However, because electrons of like spin tend to avoid occupying the same region of space, the repulsion energy for the triplet state is always less than for the singlet state, and thus the energy in the triplet state is always lower than in the singlet state.

Table 1: Relationship between the three states of oxygen.

State of O_2 Molecule	Symbol	Energy Above Ground State	Orbital Occupancy
Second excited	$^1\Sigma$	37 kcal	
First excited	$^1\Delta$	22 kcal	
Ground	$^3\Sigma$		

Source: N. I. Krinsky, Trends Biochem Sci 2:35 (1977).

The $^1\Sigma$ is extremely energetic and rapidly decays to the $^1\Delta$ state and is probably not of significance in chemical and biochemical reactions.

Singlet O_2 is not a free radical but is a highly electrophilic species seeking electrons to fill the vacant molecular orbital. As such, $^1O_2$ reacts readily with moities containing high densities of electrons such as the double bonds of unsaturated fatty acids.

The direct reaction of oxygen in the triplet state with a fatty acid is not possible because it requires a change in total spin, since RH and ROOH are in the singlet state while O_2 is in the triplet state. Moreover, the reaction is endothermic by about 64 Kcal/mole. Both the energy and spin barrier can be overcome, however, if instead of ordinary triplet O_2, singlet O_2 is the active species. Thus, a mechanism that can supply singlet O_2 can explain the formation of original hydroperoxides in fatty acids in an oil completely free of hydroperoxides (Rawls & Van Santen, 1970b).

Singlet O_2 can be generated in several different ways (see Figure 1). Probably the most important way by which singlet oxygen can be generated in fats and oils is through the presence of sensitizers (Sattar & deMan, 1975; Rawls & Van Santen, 1970b; Chan, 1977; Terao & Matsushita, 1977).

Chlorophyll, riboflavin, hemo- and myoglobin, and certain dyes are common photosensitizers. A sensitizer is a substance that can

transfer its excess energy to another substance, which then may react. These substances increase the quantum yield (Q):

$$Q = \frac{\text{Photon emitted}}{\text{Photon absorbed}}$$

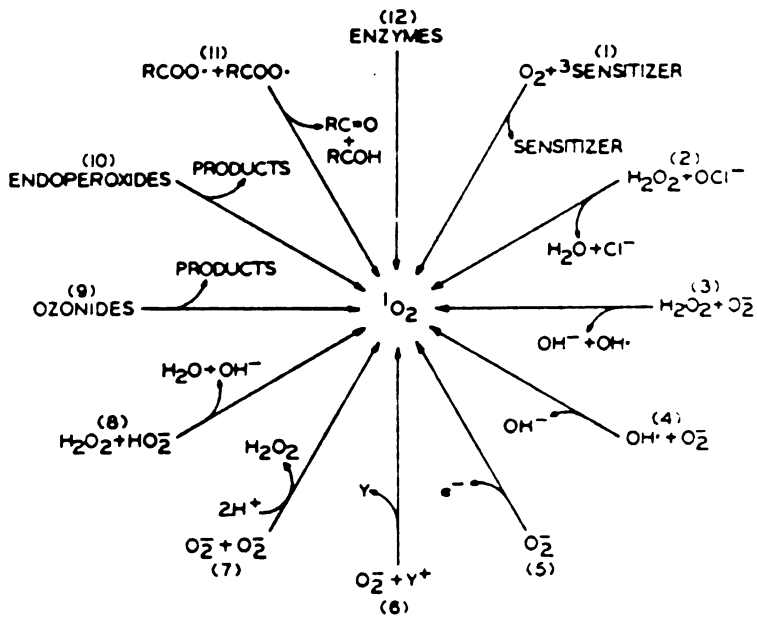


Fig. 1. Production of $^1O_2$ by photochemical, chemical, and biological systems. (From N. I. Krinsky, Trends Biochem. Sci. 2:35 [1977].)

Substances with an effect opposite to that of sensitizers are known as quenchers. These substances either decrease the quantum yield or react directly with $^1O_2$.

Two pathways for photosensitized oxidations have been identified by Foote (1976). These have been designated as Type I or Type II.

Type I

$$^1S \xrightarrow{h\nu} {}^1S^* \rightsquigarrow {}^3S^* \xrightarrow[A]{AH} \cdot SH + A\cdot \quad \text{or} \quad S^{\cdot -} + A^{\cdot +}$$

$$^3S^* + RH \xrightarrow{\text{H transfer}} \cdot SH + R\cdot \xrightarrow{\text{back reaction}} S + RH$$

$$^3S^* + R \xrightarrow{\text{electron transfer}} S^{\cdot -} + R^{\cdot +} \xrightarrow{\text{back reaction}} S + R$$

RH refers to any unsaturated fatty acid, ^1S is sensitizer in the singlet state, $^1S^*$ is sensitizer in an excited singlet state, $^3S^*$ is sensitizer in an excited triplet state, and hv refers to the energy required to produce photochemical reactions.

In a Type I mechanism, the sensitizer initiates a radical reaction through either electron or hydrogen atom abstraction, and this can proceed in the absence of molecular O_2. The types of molecular structure that favor a Type I reaction are those that are readily oxidized (phenols, amines, etc.) or those that are readily reduced (quinones). Substances that are not readily oxidized or reduced by the sensitizer (olefins, dienes, and aromatic compounds) tend to favor a Type II mechanism in which O_2 participates in the reaction.

Type II

$$^1S \xrightarrow{h\nu} {}^1S^* \rightsquigarrow {}^3S^*$$

$$^3S^* + {}^3O_2 \longrightarrow {}^1O_2^* + {}^1S$$

$$^1O_2^* + RH \longrightarrow ROOH$$

$$ROOH \longrightarrow \text{free radical products}$$

Most natural pigments such as chlorophyll and hematoporphyrins tend to favor the singlet oxygen mechanism.

A direct assay for $^1O_2$ involves measuring the chemiluminescence resulting from the energy liberated when $^1O_2$ decays to the ground triplet state. This energy is liberated as a photon according to the following reaction:

$$^1O_2 \longrightarrow {}^3O_2 + h\nu$$

In this reaction, the light emitted is in the infrared region at 1269 nm, equivalent to the energy difference between the first excited state and the ground-state oxygen. In addition, $^1O_2$ can undergo an energy-pooling reaction, which results in "dimol" emission according to the following equation:

$$2\ {}^1O_2 \longrightarrow 2\ {}^3O_2 + h\nu$$

In this case, the emitted light occurs primarily at 634 nm and can be readily detected from chemical reactions that generate $^1O_2$, such as the H_2O_2/OCl^- reaction.

Another method of detecting $^1O_2$ is through the use of specific quenchers of $^1O_2$ reactions. These compounds can inhibit oxidation initiated by $^1O_2$ either by physically or chemically reacting with $^1O_2$ to render it ineffective.

In the case of photosensitized oxidation of unsaturated fatty acid methyl esters, the participation of singlet oxygen has been evidenced indirectly either by kinetic studies or by the kinds of hydroperoxides formed. It was found that singlet-state oxygen reacted

10^3 to 10^4 times faster than normal oxygen with methyl linoleate (Rawls & Van Santen, 1970). Gunstone and Hilditch (1945) found that the rates of autoxidation of methyl oleate, methyl linoleate, and methyl linolenate are: 1:12:25, respectively. Terao and Matsushita (1977) found the relative reactivities of these esters with $^1O_2$ to be 1.0:1.7:2.3.

It was demonstrated by autoxidation studies that oleate gave the 8-, 9-, 10-, and 11-isomers; linoleate, the 9- and 13-isomers; and linolenate, the 9-, 12-, 13-, and 16-isomers (Frankel et al., 1977a, 1977b, 1977c). The distribution of hydroperoxide isomers of photosensitized oxidation is different from that of autoxidation. Methyl oleate gave 9- and 10-isomers; methyl linoleate, the 9-, 10-, 12-, and 13-isomers; and methyl linolenate, the 9-, 10-, 12-, 13-, 15-, and 16-isomers, respectively (Terao & Matsushita, 1977). The position of the hydroperoxide group in photosensitized oxidation is the carbon atom located at each side of the double bond. Therefore, the number of isomers produced is two times that of the double bonds in each unsaturated fatty acid methyl ester. The double bonds shift to the adjacent position, and hence both conjugated and non-conjugated hydroperoxide isomers are formed.

The role of singlet oxygen in oxidation was studied by analyzing hydroperoxide isomers in unsaturated fats and esters by gas chromatography/mass spectrometry (Frankel et al., 1979). On oxidation photosensitized with methylene blue at 0°C, methyl oleate produced a 50-50% mixture of 9- and 10-hydroperoxides, linoleate a mixture of 66% conjugated (9 + 13) and 34% unconjugated (10 + 12) hydroperoxides,

and linolenate a mixture of 75% conjugated (9 + 12 + 13 + 16) and 25% unconjugated (10 + 15) hydroperoxides. Several lines of evidence supported the conclusion that $^1O_2$ may contribute to the unique hydroperoxide composition of vegetable oil esters at low levels of oxidation.

In the presence of photosensitizers such as methylene blue and chlorophyll, the unique hydroperoxide composition (high levels of 10- and 12-hydroperoxides) obtained in soybean esters was similar to that produced by oxidation at low peroxide values.

A common mode of attachment of an oxygen molecule ($^3O_2$) to the hydrocarbon chain in the autoxidation of unsaturated fatty acid is the reaction of molecular oxygen with an already formed conjugated free radical (Dugan, 1976). In contrast to this, the reaction between singlet oxygen and olefinic bonds does not involve a free radical but proceeds via a spin-allowed addition reaction. This is a concerted reaction between $^1O_2$ and a carbon-carbon double bond in which the oxygen molecule is inserted at either carbon atom of the C=C bond, which is shifted to yield an allylic hydroperoxide (Chang, 1977).

The autoxidation of methyl linoleate at low temperature yields predominantly cis, trans conjugated hydroperoxides, whereas oxidation catalyzed by visible light or UV radiation leads to the formation primarily of trans, trans hydroperoxides (Khan et al., 1954).

Role of Chlorophyll in Oxidation of Fats

Crude soybean oil contains 1.0 to 1.5 mg/liter of chlorophyll. Ordinary caustic-refining reduces the chlorophyll by about 25%.

Bleaching with earth adsorbents is one of the most effective means of removing chlorophyll from soybean oil (Prichett et al., 1947). Hinners et al. (1946) showed in their evaluation of bleaching earths that activated materials adsorb chlorophyll very efficiently and that the adsorptive capacity of such a material for this pigment is directly proportional to the acidity of the earth.

The use of copper chromite as hydrogenation catalyst has been shown to be particularly effective in reduction of the green color due to chlorophyll (Beal et al., 1974).

Coe (1938) pointed out that small quantities of chlorophyll derivatives, which are sometimes present in refined oils, can act as sensitizers for fat and fatty acid oxidation.

Coe (1941a), in his study of the chlorophyll value in relation to autoxidation, showed that the lower the chlorophyll value for a given oil normally treated, that is, without excessive heat, the longer is the induction period of that oil. The chlorophyll value was believed to be indicative of the keeping quality of a normal oil.

Taufel et al. (1959) observed that chlorophyll in the presence of light acts as a prooxidant for methyl oleate. This pigment, however, had no prooxidant effect in the dark; on the contrary, it acted synergistically with phenolic antioxidants. In order to have oxidation in the presence of chlorophyll in the dark, Tollin and Green (1960) showed that very strong prooxidants are necessary. Lundberg (1949) studied the oxidation of methyl linoleate in the presence of chlorophyll and light at 37°C and found that this reaction proceeded

extremely rapidly in comparison with autoxidation at the same temperature.

Khan et al. (1954a) used the concentration of 2.0 mg/ml of substrate of crude chlorophyll to study the photochemical oxidation of methyl oleate and methyl linoleate. The visible light source was a 300-watt photoflood bulb. Autoxidation of methyl linoleate at low temperature yielded predominantly cis-trans conjugated hydroperoxides. The products obtained by autoxidation at -10°C, in the dark, with copper catalyst, with visible light irradiation, or with UV in methyl linoleate were qualitatively similar. The two major dienoic reduction products exhibited cis-trans and trans-trans conjugation. With chlorophyll-sensitized photooxidation, four major reduction products were found. One was found to exhibit no conjugation and to have hydroxyl groups and isolated trans double bonds (Khan et al., 1954b).

Clements et al. (1973) used sodium chlorophillin to study the participation of singlet oxygen in photosensitized oxidation of 1,4 dienoic systems and soybean oil. Photooxidized refined soybean oil (8.7 g oil in 500 ml propanol) in the presence of light at 330 nm and 0.44 mg/l of sodium chlorophillin gave a peroxide value of 86 after 6 hours of irradiation. In the same period of time and under conditions using light with wavelength greater than 500 nm, the peroxide value was only 14. It was also indicated that chlorophyll-like sensitizers are probably unimportant in well-refined soybean oil.

Satry and Lakshminarayana (1971) photooxidized methyl laureate and methyl stearate at 30-40°C under intermittent exposure to light from a 500-watt tungsten bulb in the presence of 1% of chlorophyll extracted from spinach leaves. Gas liquid chromatography, TLC, IR spectrophotometry, NMR, and mass spectrometry of the hydroxy esters showed that the oxygen attack was exclusively on the α-methylenic carbon atom.

Frankel et al. (1979) used a mixture of chlorophyll a and b prepared chromatographically from soybean leaves to study the role of singlet oxygen in the oxidation of unsaturated fats and esters. In the presence of photosensitizers such as methylene blue and chlorophyll, the unique hydroperoxide composition (high levels of 10- and 12-hydroperoxide) obtained in soybean oil esters was similar to that produced by oxidation at low peroxide values.

Rawls and Van Santen (1970b), in their studies of a possible role of singlet oxygen in the initiation of fatty acid autoxidation, photooxidized methyl linoleate with a 100-watt tungsten projection lamp in the presence of chlorophyll a and pheophytin a. Chlorophyll a and pheophytin a were purified from spinach leaf extracts by chromatographing the extracts on silica TLC plates in the dark. When larger quantities were needed, the chlorophyll and pheophytin were used as mixtures that had been roughly separated from the carotenoids in the spinach leaf extracts. Chlorophyll a, pheophytin a, and roughly purified spinach leaf extract all gave the same pattern of TLC spots. Although singlet oxygen formation can account for approximately 80% of the observed chlorophyll photooxidation, at least one

other mechanism must be involved. It was postulated that proton abstraction by the photoactivated carbonyl group of chlorophyll could account for the remaining 20% of the observed photooxidation.

The probable mechanism by which chlorophyll or other pigments can be photosensitized to generate singlet oxygen is explained in the section "Role of Singlet Oxygen in Fat Oxidation" of this dissertation.

Role of β-carotene in Oxidation of Fats

The carotenoids are widely distributed in crude animal and vegetable fats although their concentration is low. Crude unbleached palm oil has one of the highest contents of carotenoids (0.05-0.2%). Carotenoids are not separated from fats by alkali refining, but selective hydrogenation of a fat will reduce the unsaturation of the carotenoid pigments sufficiently to effect a significant reduction in color. These pigments are also unstable to heat to some degree; hence oils are bleached by high-temperature treatment, such as steam deodorization, which succeeds in removing objectionable volatiles from the oil and partially eliminating the color from carotenoids. Carotenoids are readily adsorbed by Fuller's earth or activated carbon. The carotenes are largely removed from the oil during the bleaching step in the refining operation (Sonntag, 1979).

Sherman (1940) reported the total pigment content of five varieties of soybeans to vary from 0.92 to 1.74 ug/g, of which 2.5 to 11.8% was α-carotene, 80.0 to 88.8% was β-carotene, and 6.5 to 9.8% was unidentified residue. The β-carotene content in mature soybeans varied from 0.2 to 2.4 ug/g (Smith & Circle, 1972).

Carotenoids undergo coupled oxidation in the presence of lipids at rates dependent on the system. Carotenoids can act as antioxidants or prooxidants, depending on the system (Clydesdale & Francis, 1976).

The effect of antioxidant properties of carrot oil in methyl esters obtained from olive oil was studied by Heftmann (1947). Under the conditions of the experiment, pure carotene inhibited the autoxidation of the substrate in concentrations below 3 mg%, but accelerated the initial rate of peroxidation in higher concentrations. Carrot oil lost most of its antioxidant activity on hydrogenation. Studies in which spectral changes in carotene-ethyl linoleate mixtures were related to oxygen uptake indicated that carotenes were almost completely destroyed before 10% of the ester was oxidized.

The products of autoxidation of carotene and of coupled oxidation of carotene are qualitatively similar (Holman, 1949). As in the case of carotene, vitamin A acetate was virtually destroyed before 10% of the linoleate carrier was oxidized, emphasizing the importance of protection of vitamin A bearing oils from even traces of oxidation. The course of oxidation of linoleate was unaffected by the presence of vitamin A acetate (Holman, 1950).

Thompson and Steenback (1944) found β-carotene to be an active prooxidant. It shortened the induction period and accelerated the rate of oxidation after the end of the induction period for both plant and animal fats from which the antioxidants had been removed chromatographically. The prooxidant effect of carotene was greater with plant fats (cottonseed and soybean oil) than with animal fats.

Similarly, it was greater with ethyl linoleate than with ethyl oleate.

McConnell and Esselen (1947) used carotene (90% β, 10% α) to study the effect of keeping quality of cottonseed and corn oil packed in sealed glass containers and stored under different conditions. As judged organoleptically, carotene at concentrations of 0.0013% and less, accelerated the development of rancidity in sealed oils exposed to light. However, at higher concentrations, the carotene was found actually to retard the development of rancidity. Therefore, the protective action may be due to the carotene absorbing much of the energy of the light falling upon the oil.

Du and Armstrong (1970) irradiated milk fat in hexane with four types of fluorescent lamps. After milk fat had been treated with activated charcoal to remove carotene, the depigmented product showed a pattern of oxidation different from that of the untreated samples. Addition of β-carotene did not alter the pattern, except that oxidation in the near-ultraviolet region was intensified.

Foote and Denny (1968) found that β-carotene deactivated singlet oxygen at a rate much higher than the rate at which it reacted with singlet oxygen. With this evidence, they postulated a protective role for β-carotene in photosynthesis.

Seely and Meyer (1971) reported that β-carotene itself is photooxidized to products absorbing in the violet and near-ultraviolet regions of the spectrum.

Beta-carotene quenches triplet chlorophyll a by an energy-transfer mechanism at a very high rate. However, since oxygen also

quenches triplet sensitizer at a similar rate, quenching of chlorophyll by carotenes cannot be responsible for the protective effect unless the local concentration of carotene greatly exceeds that of oxygen (Foote, 1976).

Koka and Song (1978) reported the inhibition of the photooxidation of chlorophyll a in ethanol and ethanol-benzene. The quenching of $^1O_2$ by β-carotene occurs by a collisional quenching mechanism with a diffusion-controlled rate of $1.7 \times 10^{11} M^{-1} S^{-1}$. The carotenoid thus effectively protects chlorophyll a from photodynamic damage, providing a direct proof for the protective role of carotenoids in the photosynthetic pigment complex.

Rawls and Van Santen (1970b), in the chlorophyll photooxidation studies, found β-carotene to act as an inhibitor of oxidation of methyl linoleate.

Frankel et al. (1979) found a normal isomeric hydroperoxide composition when β-carotene was added to quench singlet oxygen in photosensitized oxidation of unsaturated fats and esters. These researchers pointed out that although β-carotene is known as a quencher of singlet oxygen, the oxidation products of β-carotene would be expected to catalyze free radical oxidation.

Matsushita (1979) reported that addition of β-carotene enhanced the peroxide value of decolorized soybean oil. Decolorization of soybean oil increased stability against photoirradiation by eliminating chromophoric impurities. However, it appears likely that decolorized oil is more susceptible to autoxidation, because tocopherols are eliminated.

Mechanism of Antioxidant Action in Autoxidation

Numerous studies have indicated that more than one type of action may occur, depending upon the conditions of the reaction, the type of system being studied, and the antioxidant used. Dugan (1963) noted that antioxidants might be considered to function in two ways, either as inhibitors of free radical formation or as peroxide decomposers.

Antioxidants that function as free radical inhibitors react with free radicals to form inert products as in a termination step in the chain-reaction mechanism. Studies by Bolland and Ten Have (1947) led to proposal of a simple mechanism in which the antioxidants acted as hydrogen donors or free radical acceptors. From the kinetics of the reaction, it appeared that the antioxidant reacted primarily with $RO_2^{\cdot}$ and not with $R\cdot$, as follows:

$$RO_2^{\cdot} + AH_2 \longrightarrow ROOH + AH\cdot$$

$$AH\cdot + AH\cdot \longrightarrow A + AH_2$$

Boozer et al. (1955) proposed a different mechanism, involving complex formation, as follows:

$$RO_2^{\cdot} + AH_2 \longrightarrow (RO_2AH_2)\cdot$$

$$(RO_2AH_2)\cdot + RO_2^{\cdot} \longrightarrow \text{stable product}$$

As peroxide decomposers, antioxidants act as catalysts in the decomposition of the peroxides that are initially present or are formed during the oxidation. The decomposition function of

antioxidants appears when they are used in high concentration exceeding 0.02% (Hill et al., 1969). Dugan (1961) noted that the decomposition process results in the formation of products that are not free radicals.

Synergism is a phenomenon that occurs when two or more compounds used together give a more pronounced antioxidant effect than the sum of their individual effects. The original description of antioxidants and synergists (Olcott & Matill, 1936) differentiated between substances that were effective alone in relatively low concentrations (tocopherols, phenols) and those that had little activity by themselves but were effective in combination with the phenolic inhibitors. They were called synergists (citric and ascorbic acids, phospholipids, etc.).

It has been well demonstrated that the chelation of metals is one of the principal mechanisms involved. Kraybill et al. (1949) showed that BHA exhibited synergism with certain acids, including citric, as well as with hydroquinone, methionine, lecithin, and thiodipropionic acid. Citric acid was more effective against iron (Morris et al., 1950). Cowan et al. (1962), with soybean oil and lard, showed that both sorbitol and citric acid were acting as a metal inactivator and did not have a true synergistic effect. Citric acid readily forms stable complex salts with many metallic ions (Lockwood & Irwin, 1963); thus it is able to retard the increase in rate of free radical formation.

Smith and Dunkley (1962) showed ferrous ion was more effective than ferric ion in the peroxidation of linoleate. It was proposed that a perhydroxyl radical was produced by the reduced metal ion. Strouse et al. (1977) reported that triionized citrate formed a tridentate chelate with Fe (II) in which the protonated hydroxyl group, the central carboxyl group, and one terminal carboxyl group are coordinated to a single Fe (II) ion. Both oxygen atoms of the other terminal carboxyl group were coordinated to two other symmetric-related Fe (II) ions. Hexaquoiron (II) was the counter ion.

Cort et al. (1975) stated that it was not the metals, per se, but their oxidation state that was important. Ascorbate converted Fe^{3+} to Fe^{2+} and Cu^{2+} to the lower oxidation state. It was the higher oxidation state that reacted with tocopherol, Trolox C, and ascorbic acid. Berger (1975) reported that ascorbic acid had some antioxidant activity, and possibly it acted as a somewhat inefficient free radical scavenger. On the other hand, citric acid was particularly valuable as a metal chelating agent.

Synergism has been observed also in fat by a combination of two antioxidants (Mahon & Chapman, 1953; Dugan et al., 1954). There are several theories regarding synergism. It may act as metal scavenger, peroxide decomposer, and sparing agent, as in the interaction of phenolic antioxidants or the interaction of other agents with phenolic antioxidants (Dugan, 1963). Ikeda and Fukuzumi (1977) with methyl linoleate showed that nucleic acid acted as a synergist with tocopherol through H-bonding, which protected tocopherol from direct air oxidation.

Tocopherols as Natural Antioxidants in Soybean Oil

The tocopherols, which are the best-known and most universally distributed antioxidants, include fat-soluble vitamin E as a member of the class. There are four commonly occurring tocopherols designated α- (alpha), β- (beta), γ- (gamma), and δ- (delta) tocopherols. The relative effectiveness of these four tocopherols as antioxidants is δ > γ > β > α (Daubert, 1950). Since approximately 30% of the total tocopherol of soybean oil is δ-tocopherol, it might be assumed that this oil should have a relatively high resistance to oxidative rancidity compared to cottonseed and peanut oils, which contain only small amounts of this tocopherol (Stern et al., 1947). Carpenter (1979) found by high-performance liquid chromatography (HPLC) analysis the following values for tocopherols in soybean oil: α- 0.07 ug/mg, trace amounts of β, γ- 0.924 ug/mg, and δ- 0.37 ug/mg. The relative amount of δ- among the four tocopherols in this analysis is 27.1%, which is in agreement with the value found by Stern et al. (1947).

Mag (1973) indicated a reduction of tocopherols in soybean oil from 0.148% to 0.138% during caustic-refining and a further reduction to 0.126% during bleaching. Clay-heat refining reduces tocopherols to 0.136%, about the same loss as in either refining or in bleaching.

Cort (1974) indicated that α- and γ-tocopherols at concentrations of 0.02% had low antioxidant activity in stripped soybean oil. On the other hand, α- and γ-tocopherols showed good antioxidant activity in chicken, pork, and beef fats. Neither one had increasing antioxidant activity above the level of 0.02%. Gamma-tocopherol had

more activity than α, and activity increased as the concentration increased.

The tocopherols as carry-through antioxidants were studied by Dugan and Kraybill (1956) in crackers, pastry, and potato chips prepared with stabilized lards. They found that α-tocopherol had little carry-through effect but that γ-tocopherol had a marked effect. The carry-through activity of tocopherols was much less than that provided by BHA or BHT.

Sherwin (1976) reported that the order of antioxidant activity of tocopherols is found to be: $\delta > \gamma > \alpha$, but this order of antioxidant potency in vegetable oils may be influenced significantly by temperature and light conditions. He also pointed out that not only are tocopherols widely distributed in vegetable matter from which edible oils of commerce are extracted, but it is very important to recognize that high proportions of these tocopherols survive oil-processing steps and end up in finished vegetable oils. If the residual tocopherols are stripped completely from vegetable oil by distillation or some other efficient method, it will be observed that the oxidative stability of the oil is reduced to an extremely low level. If tocopherols are added back to the stripped oil at levels normally present in refined vegetable oils, the oxidative stability of the oil will be restored to a level typical of normally processed vegetable oil. Lea and Ward (1959) reported that the antioxidant activity of δ-tocopherol exceeded that of γ-tocopherol in various substrates. They also pointed out that the tocopherols were much less effective antioxidants in light than in dark. Parkhurst et al.

(1968) reported the antioxidant effectiveness of the tocopherol in lard to increase in order α, δ, γ. The antioxidant efficiency decreases with increasing concentration of tocopherols such that addition of any single tocopherol above a concentration of 250 ug/g has little effect on oxidative stability.

Chow and Draper (1975) investigated the oxidative stability of the natural tocopherols in corn and soybean oil heated at 70°C and aerated at a rate of 100 ml/minute. Vitamin E oxidation and peroxide formation occurred more rapidly in corn oil than in soybean oil. In soybean oil, γ-tocopherol was destroyed faster than δ-tocopherol.

Like other antioxidants, the tocopherols are themselves readily oxidizable. Mild oxidation of a tocopherol opens the heterocyclic ring to form tocoquinone, which is not an antioxidant (Sonntag, 1979).

Yoon and Kim (1974), in their studies on relative effectiveness of some antioxidants in dark and sunlight-irradiated conditions, pointed out that α-tocopherols in soybean oil showed some retarding effect on oxidation, but the effect decreased rapidly as storage time increased.

Frankel et al. (1979) pointed out in studies of photooxidation of soybean esters that although β-carotene and α-tocopherol are known to be quenchers of $^1O_2$, α-tocopherol was much more effective in lowering oxidation rates than was β-carotene. Matsushita (1979), in his study of the effect of tocopherol and β-carotene on the photo-oxidation of methyl linoleate and soybean oil initiated by $^1O_2$, found that γ- and δ-tocopherols inhibited autoxidation mainly and had little

effect on $^1O_2$ oxidation. Alpha-tocopherol had weak inhibitory effects on both $^1O_2$ oxidation and autoxidation as compared with δ-tocopherol. Coexistence of α- and δ-tocopherol had no appreciable effect on the inhibition by δ-tocopherol alone.

TBHQ and BHA as Antioxidants in Soybean Oil

Synthetic antioxidants have played an important part for more than 30 years in preserving the quality of edible fats, oils, and food products (Lundberg, 1962). The most widely used, food-approved stabilizers include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG). These antioxidants are used frequently in mixture with each other and with a synergist or a metal deactivator such as citric acid.

Desirable features of an antioxidant include the following: it must be effective at low concentrations, nontoxic, conveniently and safely handled, and low in cost, and it must not impart undesirable characteristics to the system in which it is used (Dugan, 1963).

All antioxidants are structurally similar in that they contain unsaturated benzene rings plus either hydroxy or amino groups. Most natural and synthetic food-grade antioxidants belong to the phenolic class of compounds. Although the presence of hydroxy or amino groups on the aromatic ring is necessary for antioxidant activity, the potency of a given compound can be greatly enhanced by the introduction of certain substituents into the proper position on the aromatic nucleus. Morawetz (1949) and Thompson and Symon (1956) showed, after evaluating many phenolic compounds, that alkyl substitution in the

ortho and para positions greatly enhance the potency of a given compound. The addition of the tertiary butyl group in the ortho position seems to be particularly effective in this respect.

Butylated hydroxyanisole (BHA), commercially available as a mixture of 2- and 3-isomers of tertiarybutyl-4-methoxy phenol, was first approved in 1948 for food use in the United States and has since found widespread use in food fats and oils in many countries. Because of the tertiarybutyl group ortho- or meta- to the hydroxyl group, BHA is referred to as a hindered phenol. This steric hindrance is believed responsible for the relative ineffectiveness of BHA in vegetable oils because the tertiarybutyl group interferes with the antioxidant activity of the phenolic structure. On the other hand, this same steric hindrance also serves to protect the active hydroxyl group under some conditions and is probably responsible for the carry-through effect of BHA in fats and oils used in baked or fried foods. Although it is not a particularly effective antioxidant in vegetable oil, BHA is commonly used in combination with other primary antioxidants in order to take advantage of the synergistic effects observed when certain phenolic antioxidants are used together and also to benefit from the carry-through protection that BHA may afford (Sherwin, 1976). Despite the widespread use of BHA, BHT, and PG, these antioxidants have been relatively ineffective in some of the more highly unsaturated fats and oils. Thompson and Sherwin (1966) screened approximately 500 compounds to determine their relative antioxidant potencies in polyunsaturated oils. They reported mono-tertiary-butyl hydroquinone (TBHQ) was a compound that could

possibly fill the increasing need for an antioxidant with greater potency in more highly unsaturated types of fats and oils. Their work culminated in the issuance in 1972 of regulations by the Food and Drug Administration of the United States that permit the use of TBHQ or its combination with other antioxidants up to 0.02% based on the weight of fat, oil, or fatty containing of a food product.

Sherwin and Thompson (1967) showed by active oxygen and oven-storage tests that TBHQ provided much better keeping quality in soybean oil than BHA, BHT, and PG. Sherwin and Luckadoo (1970), in their studies on antioxidant treatments of crude vegetable oils, showed that TBHQ was effective in inhibiting oxidative degradation of crude oil subjected to long-term storage.

Yoon and Kim (1974) found that the retarding effect of BHA on the peroxide value of soybean oil was in general more pronounced in the case of oils stored in the dark than in the case of irradiated oils.

Toxicological and biochemical studies with BHA and TBHQ were reviewed by Branen (1975) and Astill et al. (1975), respectively.

Measurement of Lipid Oxidation

The off-flavors developed as a result of the reaction between oxygen and an unsaturated fatty acid are identified as oxidative rancidity. One way to define rancidity is that it is the development of an off flavor that makes the food or food product unacceptable on a consumer-market level (Labuza, 1971).

Many methods have been developed for measuring lipid oxidation. The most commonly used methods are: organoleptic evaluation, peroxide value, thiobarbituric acid (TBA) test, total and volatile carbonyl compounds, and conjugated diene methods. A review of measurement of lipid oxidation was presented by Gray (1978).

Peroxide Value

Traditionally, the practical aspects of detecting and monitoring rancidity development in unsaturated fats and oils has been through measurement of the peroxide content. The iodometric methods of Lea (1931) and Wheeler (1932) are widely used, and these are based on the measurements of the iodine liberated from potassium iodide by the peroxides present in the oil. The peroxide number of a fat is a measure of its content of reactive oxygen in terms of mmoles of peroxides or milliequivalents of oxygen per 1000 g of fat.

Conjugated Diene Absorption Method

Oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption due to the formation of conjugated diene and triene hydroperoxides. Fatty acids with conjugated unsaturation absorb strongly in the region 230 to 375 nm, diene unsaturation at 233 nm, and triene unsaturation at 268 nm. The magnitude of change is not related to the degree of oxidation because the effects upon the various unsaturated fatty acids vary in quality and magnitude. However, the changes in the ultraviolet spectrum of a given substance can be used as a relative measurement of oxidation (Gray, 1978).

Oils containing linoleate or more highly unsaturated fatty acids are oxidized to conjugated diene systems that can be measured by ultraviolet absorption at 233 nm. Farmer and Sutton (1943) indicated that this absorption increased proportionally to the uptake of oxygen and to the formation of peroxides in the early stages of oxidation.

Chan (1977) studied the photosensitized oxidation of methyl oleate and methyl linolenate using erythrosine and riboflavin as sensitizers. The molar absorptivity was calculated at 234 nm. The molar ratio absorption/peroxide content of the sample was calculated by assuming a molar absorbance at 234 nm of 26,000 for conjugated diene hydroperoxides. The molar ratio of UV absorbance/peroxide from oxidation of methyl linolenate was 1.01, 0.69, and 0.97, respectively, for autoxidation and for erythrosine and riboflavin sensitized oxidation. Golumbic et al. (1946) showed absorption curves of refined and deodorized soybean oil after exposure to visible radiation in air and in nitrogen. Samples of oil exposed to visible radiation in an atmosphere of nitrogen did not develop the maximum at 234 nm characteristics of the samples exposed to air.

Angelo et al. (1975) studied the autoxidation of peanut butter by measuring the peroxide value and the increase in absorption at 234 nm due to diene conjugation. They concluded that the conjugated diene hydroperoxide (CDHP) method can be used as an index of progressive staling in place of, or in addition to, the peroxide value. The CDHP method is faster than the peroxide value method, is simpler, requires no chemical reagents, and can be used on smaller samples.

This method is applicable for analysis of peroxides in vegetable oils containing polyunsaturated fatty acids.

Thiobarbituric Acid (TBA) Test

The 2-thiobarbituric acid (TBA) test has been used widely for measuring oxidative changes in food containing unsaturated fatty acids. Early investigation by Sinnhuber et al. (1958) helped to clarify the nature of the colorimetric reaction that occurs during the TBA test. They proposed that the chromogen was formed through the condensation of two molecules of TBA with one molecule of malonaldehyde. However, no evidence was presented that malonaldehyde could be found in all oxidizing systems.

Dahle et al. (1962) postulated a mechanism for the formation of malonaldehyde as a secondary product in the oxidation of polyunsaturated fatty acids. This mechanism was based on investigations which showed that no color developed for linoleate even at peroxide values of 2,000 or greater and that, with fatty acids containing three or more double bonds, the molar yield of TBA color increased with the degree of unsaturation. These results indicated that only peroxides which possessed unsaturation β, γ to the peroxide group were capable of undergoing cyclization and scission with the ultimate formation of malonaldehyde. It therefore becomes imperative to know the fatty acid profile of the sample to be tested. This work also indicated that meaningful results from the TBA test can only be obtained by comparison of samples of a single material at different stages of oxidation.

A mechanism was proposed by Pryor et al. (1976) in which the malonaldehyde arises at least in part from the acid catalyzed, or thermal decomposition of endoperoxides (2,3-dioxanorbornane compounds). They applied Dahle et al.'s (1962) theory to explain the formation of the thiobarbituric acid-reactive material in a diene system and demonstrated that endoperoxides can be produced in a diene system but in a lower ratio than in a triene system. The TBA test has been criticized on several points.

Tarladgis and his co-workers (1962) considered the effect of acid, heat, and oxidizing agents on the TBA reagent. They suggested steam distillation of the product to remove the volatile constituents that were assumed to be responsible for sensorial rancidity. These workers concluded that the structure of TBA was altered by acid and heat treatment as well as by the presence of peroxides and recommended that blank determinations be carried out in conjunction with the test. In another investigation of the effect of reaction conditions, Yu and Sinnhuber (1964) suggested the following corrections to the conclusion of Tarladgis et al. (1962): (1) the color interference was the result of impurities in the acid, (2) hydrogen peroxide reacted with TBA only if the acid concentration was high, and (3) since colored complexes formed when the TBA reagent was passed through a cellulose column, the column chromatography results were suspect. This investigation stressed the importance of using purified reagents.

Color development during the TBA test is usually assessed by measuring the absorbance of the red pigment at 532 nm. However,

other pigments have been observed to form, notably a yellow pigment with maximum absorbance at 450 nm. Using a modified one-phase system, Jacobson et al. (1964) observed that dienals showed an absorption peak at 432 nm, whereas TBA reaction products of the saturated aldehyde showed a peak at 452 nm. The location of the double bond in monoenes was found to influence the spectral behavior of the TBA reaction products.

Pohle et al. (1964) evaluated the peroxide value and TBA test on stored fats and shortenings. They found that either test can be used to measure flavor development but that the flavor score cannot be so estimated. Significantly, within any one product, development of off-flavor is related to the peroxide value and TBA intensities, but the relative levels vary from product to product.

Evidence that TBA can react with compounds other than those found in oxidizing systems to produce the characteristic red pigment has been presented in the literature. Dugan (1955) reported that sucrose and some compounds in woodsmoke react with TBA to give a red color so that cured and smoked meats require corrections for the sugar and for the smoke in the outer layers. Baumgartner et al. (1975) also found that a mixture of acetaldehyde and sucrose when subjected to the TBA test produced a 532 nm absorbing pigment identical to that produced by malonaldehyde and TBA.

The TBA test may be performed in two ways, either directly on a food product followed by extraction of the colored pigment, or on a portion of a steam distillate of the food. Both methods have in common the use of acid and heat. Dekoning and Silk (1963) reported

that they were unable to successfully apply the TBA test in either of its forms to determine rancidity in fish oils. Poor results were attributed to the two-phase system of the direct method and to inefficient extraction of the malonaldehyde by the distillation method.

MATERIAL AND METHODS

Soybean Oil

Five gallons each of crude and refined oils were obtained from Central Soya Co., Decatur, Indiana. These oils had no added antioxidants.

Chlorophylls and Beta-carotene

Chlorophyll a and chlorophyll b from Sigma Chemical Company. Oil-soluble chlorophyll (30% chl) from ICN Pharmaceutical Corporation. Beta-carotene (100% beta) from Eastman Kodak Company.

Antioxidants

Tocopherol-d-alpha; d-gamma-tocopherol. Vitamin E4-50; lecithin; butylated hydroxyanisole (BHA); 2-tert-butyl hydroquinone (TBHQ). All of these antioxidants were acquired from Eastman Kodak Company.

Bleaching Agents and Chemicals

Activated carbon Darco S-51 grade (ICI United States Corporation). Silicic acid 100 mesh (Mallinckrodt Corporation). MN Kieselgel G (Macheney, Nagel Company). Adsorptive Magnesia Sea Sorb 43 (Fisher Scientific Company). All the reagents used during analyses were analytical grade.

Light Sources

Ultraviolet (UV) lamp model XX-15, 120V, 15W, 45.72cm of length, wavelength 366 nm (Arthur H. Thomas Company). Fluorescent lamp, GE, 120V, 15W, 45.72 of length. Floodlight, GE, 120V, 150W.

Preparation of Samples for Irradiation

Twenty-five grams of crude, refined, and laboratory bleached oils were added separately into 100 ml beakers with 18.63 cm^2 of surface area. Where beta-carotene, chlorophyll a, chlorophyll b, alpha and gamma tocopherols, TBHQ, BHA, and lecithin were used, they were dissolved in a mixture of 10 ml hexane and 1 ml acetone and mixed into the oil. These sensitizers, quenchers, or antioxidants were added only to the laboratory bleached oil.

Irradiation System

The 100 ml capacity beakers containing each sample of oil were irradiated in a stainless steel 45X30X20 cm water bath (Blue M.). The temperature of the bath was kept at 17±2°C either when UV light or fluorescent light was used. With the floodlight, the temperature was kept at 20±2°C. The temperature was maintained by a controlled flow of tap water in the bath. In either case, when UV radiation or fluorescent light was used as the irradiation source, two bulbs of 15W each were adapted into a Black-Ray Lamp apparatus Model XX-15 (Arthur H. Thomas Company). The floodlight bulb was placed into a Smith-Victor adaptor (Smith-Victor Corporation). All irradiation sources were placed separately at the upper part of the water bath. The remaining open part of the top was covered to avoid the light

coming from the laboratory environment. The distance between the light source and the surface of the oil in the beaker was 18 cm. The amount of irradiation was measured with a quantum flux meter (Mod. Li-185A) with quantum sensor (Mod.s.r. N^{o} 02382-7710) "Li-Co." These values were 0.08 W/m^2, 11.4 W/m^2, and 117 W/m^2 for UV radiation, fluorescent light, and floodlight, respectively.

In all experiments the radiation source was turned off at fixed intervals of time for the control of oxidation of the samples. The interruption of irradiation existed for time required for weighing the samples and, as soon as this procedure was terminated, the beakers containing the samples were placed back into the bath and the switch turned on.

The position of the beakers containing the samples was changed inside the bath at fixed time intervals to allow the samples to occupy all the positions equally. Beakers containing controls were shielded with aluminum foil and placed in the corner of the bath.

Bleaching the Soybean Oil

A glass column 4.5 cm internal diameter and 50 cm long was packed in the following way: 2 cm of glass wool at the bottom, followed by a 2 cm layer of Kieselgur G and then 100 g of a prepared mixture containing 50% activated carbon, 35% MN Kieselgel G, and 15% florisil was placed in the column. Finally, layers of silicic acid (1cm) and anhydrous sodium sulfate (1cm) were added to complete the packing. The column was packed under reduced pressure produced by a water aspirator and pressed with a glass rod. About 100 ml of hexane

was percolated through the packing material before adding the oil. After all hexane percolated thoroughly, 100 g of crude oil was mixed with 150 ml hexane, and this mixture was added to the column. The column was washed with 150 ml of hexane to elute off the remainder of the oil.

The eluate containing bleached oil and hexane was collected in a 500 ml filtering flask. The overall time spent in this operation was about 10 hours. The eluate was transferred to a 500 ml round glass flask and the solvent evaporated in a rotary evaporator under vacuum at a temperature of 40°C. The yield from this procedure was 90%. The bleached oil obtained by this technique was kept under a nitrogen atmosphere in a freezer at 0°C for later use.

Analytical Techniques

Preparation of Methyl Esters

Methyl esters were prepared by the rapid procedure of Metcalfe et al. (1966). A total of 4 ml of 0.5N methanolic NaOH was added to approximately 150 mg of oil in a 50 ml test tube with screw cap. This mixture was heated in a boiling water bath for 5 minutes. Five ml of a 14% BF_3-methanol solution (Supelco Corporation) was added to the test tube, and the mixture was boiled for 2 minutes. Enough saturated NaCl colution was added to the test tube to float the methyl esters; then the entire mixture was transferred into a separatory funnel. About 20 ml of petroleum ether (b.p. 30-60°C) was added to the separatory funnel, and the layers were allowed to separate. The aqueous layer was drained off and discarded. The petroleum ether

layer was drained through filter paper into a 50 ml beaker. The solvent was then evaporated on a 60°C water bath or removed by a gentle stream of air at room temperature. The esters were then ready for GLC analysis.

Fatty Acid Composition of Soybean Oils

The methyl esters obtained as mentioned above were injected into a Hewlett Packard Gas Chromatograph 5830A equipped with a hydrogen flame detector. A coiled stainless column 180 cm long and 2 mm i.d. packed with 15% (W/W) DEGS on 80/100 mesh chromosorb-W was used for methyl ester separation. The column oven temperature was 200°C, the injection temperature was maintained at 210°C, and the detector at 300°C. The nitrogen carrier gas was adjusted to 26 ml/minute. The flow rate of hydrogen was 26 ml/minute and the compressed air 180 ml/minute. The emerging components were identified by comparing the retention time for each to those of standard mixtures of known fatty acid methyl esters (Supelco Corporation). Peak areas were calculated by an electronic integrator 18850A HP.

Determination of Chlorophyll a and b

One mg of both pure chlorophyll a and b were dissolved separately in 100 ml volumetric flasks with diethyl ether. From each of these solutions, 0.25, 0.50, 2.5, 5.0, 7.5, and 10.0 ml were taken and added separately to 50 ml volumetric flasks to prepare a standard curve. Each one of these solutions contained 0.05, 0.10, 0.50, 1.00, 1.50, and 2.00 mg Chl/l. A special holder to fit 5 cm cuvettes was fitted into a DU spectrophotometer to read the percentage transmittance

of the chlorophyll solutions. A spectrum from 645 to 675 nm was run to determine the wave-length at which chlorophyll a had the maximum absorption. This was determined to be at 660 nm. For chlorophyll b, a spectrum was run from 630 to 650 nm and the maximum absorption was at 642 nm. These values for absorption of chlorophyll a and b are in agreement with those reported by Comar and Zscheile (1942). The standard curve for chlorophyll a as for chlorophyll b was determined by reading the percentage of transmittance at 660 and 642 nm. The regression line for standard curve of chlorophyll a was $Y = 0.482x + 0.0001$; $r^2 = 1.00$ at 660 nm and $Y = 0.07x + 0.0074$; $r^2 = 0.995$ at 642 nm. For chlorophyll b the regression lines were $Y = 0.29x + 1.75 \times 10^{-5}$; $r^2 = 1.00$ and $Y = 0.04x - 0.0029$; $r^2 = 0.998$ at 642 and 660 nm, respectively. To get a general equation to determine chlorophyll a and b in the oils, the regression lines obtained for each curve were combined in the following way:

Absorbance at 660 = 0.482 mg/l Chl a + 0.0001 + 0.04 mg/l Chl b - 0.0029

Absorbance at 642 = 0.07 mg/l Chl a + 0.0074 + 0.29 mg/l Chl b + 1.73×10^{-5}

By solving this system of equations:

$$\text{mg/l Chl a} = \frac{0.29A660 - 0.04A642 + 0.0011}{0.137}$$

$$\text{mg/l Chl b} = \frac{0.140A642 - 0.02A660 - 0.0011}{0.04}$$

The concentrations of chlorophyll a and b were determined directly in the oils using 5 cm cuvettes. The transmittance was set to read 100% with diethyl ether.

Determination of Beta-carotene

Two grams of oil were weighed into 70 ml test tubes with screw caps. Twenty ml of 0.7 N alcoholic KOH solution were added, and the oil was allowed to saponify for 5 minutes in a beaker containing boiling water. The test tube was cooled at room temperature. Twenty ml of distilled water were added, and the tube was shaken for 1 minute. Fifteen ml of hexane were added, and the tube was shaken vigorously for 2 minutes. The layers were allowed to separate. The upper layer was removed with a 20 ml pipette by employing suction with a pipette bulb. This extraction was repeated four times with the same amount of solvent.

The hexane layers were collected in a round distilling flask. The solvent was evaporated in a rotary evaporator under vacuum at a temperature of 40°C.

A glass column, 40X2 cm, was prepared according to the procedure described in A.O.A.C. (1975). The packing material consisted of 2 cm of glass wool at the bottom, 12 cm of a pre-prepared mixture of 1:1 Seasorb 43 (MgO) and diatomaceous earth. A 1 cm layer of anhydrous sodium sulfate at the top completed the packing material in the column. The packing was carried out under reduced pressure produced by a water aspirator. Thirty ml of 9:1 hexane-acetone mixture was added to wet the column. The extract was transferred to the column with 20 ml of hexane-acetone mixture. Fifty ml of the same mixture of solvents were used to elute the beta-carotene fraction contained in the sample. The solvent mixture contained in the eluate was evaporated as described before in order to reduce the volume to 15-20 ml of beta-carotene

extract. This extract was transferred to a 25 ml volumetric flask and brought to volume with the same mixture of solvents.

To prepare a beta-carotene standard curve, 5.0 mg of beta-carotene were dissolved with 9:1 hexane-acetone in a 250 ml volumetric flask. From this solution, 0.25, 0.75, 1.25, 1.75, 2.25, and 2.75 ml were placed separately into 50 ml volumetric flasks and brought to volume with the same mixture of solvents. Each one of these solutions contained 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 mg beta-carotene/l.

The percentage transmittance of these solutions was read at 450 nm in a Beckman DU spectrophotometer with 5 cm cuvettes. The percentage transmittance was set to read 100% with 9:1 hexane-acetone mixture. The regression line obtained for this beta-carotene standard curve was $Y = 0.6437X + 0.0014$; $r^2 = 0.999$.

Determination of Phosphatides

The official AOCS method (1973) was used for phosphorus determination. Three grams of sample were weighed into a Vycor crucible. One-half gram of ZnO was added. The sample was heated on a hot plate until the mass was completely charred. The crucible was held in a muffle furnace at 550-600°C for 2 hours. After removing the crucible from the furnace and cooling at room temperature, 5 ml of distilled water and 5 ml of concentrated HCl were added to the ash. The crucible was covered with a watch glass and heated to gentle boiling for 5 minutes. The solution was transferred quantitatively into a filter paper and the filtrate received into a 100 ml volumetric flask.

Drops of 50% KOH solution were added to neutralize to a faint turbidity. Concentrated HCl was added drop-wise until the ZnO precipitate was dissolved. The flask was brought to volume with distilled water and mixed thoroughly. Ten ml of this solution were placed in a 50 ml volumetric flask. Eight ml of hydrazine sulfate solution and 2 ml of sodium molybdate solution were added. After mixing, the solution was heated for 10 minutes in a boiling water bath. After cooling at room temperature, the flask was brought to volume with distilled water and mixed thoroughly. One cm cuvettes were filled with this solution, and the transmittance was read at 650 nm in a Beckman DU spectrophotometer. The transmittance was adjusted to read 100% with a cuvette containing distilled water. A reagent blank was prepared in the same way as described above.

A phosphorus standard solution containing 0.0, 0.01, 0.02, 0.04, 0.06, 0.08, and 1.0 mg of phosphorus was prepared by dissolving dry potassium dihydrogen phosphate in distilled water. To each of these solutions, 8 ml of hydrazine sulfate solution and 2 ml of sodium molybdate solution were added and then heated for 10 minutes as described before. The percentage transmittance was also read in the same way as described earlier. The regression line obtained for this phosphorus standard curve was $Y = 0.285X + 0.0169$, $r^2 = 0.995$. The phosphorus content of the sample was calculated by using the following formula:

$$\text{Phosphorus } \% = \frac{10\ (A - B)}{W\ V}$$

A = Phosphorus content of sample aliquot in mg

B = Phosphorus content of blank aliquet in mg

W = Weight of sample in g

V = Volume of aliquet taken (= 10)

The percentage of phosphatides was calculated by multiplying the percentage of phosphorus by 30.

Determination of Color

The official AOCS method (1973) was used for color determination in crude, refined, and laboratory bleached soybean oils. One hundred fifty grams of crude and refined soybean oils were treated separately with 0.25 g of diatomaceous earth. The samples were agitated for 2.5 minutes at 250 rpm at room temperature and filtered. This treatment was not given to laboratory bleached oil. The percentage transmittance was read in a DU spectrophotometer at 460, 550, 620, and 670 nm using 5 cm cuvettes. The transmittance was set to read 100% with CCl_4.

Determination of Alpha and Gamma Tocopherols

The method described by Carpenter (1978) was used to determine alpha and gamma tocopherols in crude, refined, and bleached oils. Five grams of each oil were weighed separately into a 100 ml volumetric flask. The sample was brought to volume with 1.5% iso-propyl alcohol (IPA) in hexane. The chromatographic separation was performed by a Waters Associates Liquid Chromatograph on a 4 mm X 30 cm μ-Porasil column. The mobile phase was constituted of 1.5% IPA in

hexane (HPLC grade) at a flow of 2 ml/minute and pressure of 600 psi. The UV detector was set at 280 nm.

To prepare alpha and gamma tocopherol standards, 4.5 mg (reagent grade) of alpha tocopherol and 5.5 mg (reagent grade) of gamma tocopherol were weighed separately into 50 ml volumetric flasks. The flask was brought to volume with 1.5% IPA in hexane. For alpha tocopherol, 16, 32, 48, 64, 80, 96, 128, and 160 µl of standard solutions were injected into the HPLC. A regression line $Y = 0.099X - 0.0733$, $r^2 = 0.993$ was obtained by plotting peak height vs. alpha tocopherol concentration. With gamma tocopherol, 4, 8, 12, 16, 20, 24, 32, and 40 µl of standard solution were injected. The regression line obtained by plotting peak height vs. gamma tocopherol concentration was $Y = 0.033X - 0.0415$, $r^2 = 0.997$.

Measurement of Oxidation

Peroxide Value (PV)

Peroxide values were determined by the official AOCS method (1973) and were reported as milliequivalents/kg oil or as equivalents/kg oil. One to two grams of oil accurately weighed were taken from 100 ml beakers containing the sample before starting irradiation and at regular intervals during irradiation. The sample was dissolved in a 250 ml Erlenmeyer flask with 30 ml of glacial acetic acid-chloroform (3:2). One ml 50% (W/V) KI solution was added. The mixture in the flask was shaken and allowed to stand for exactly 1 minute. It was then diluted with 50 ml of distilled water and

titrated with 0.01N sodium thiosulfate solution. One ml aqueous 1.0% (M/V) of amylose instead of soluble starch was used as indicator.

Conjugated Diene Absorption

About 50 mg of oil were accurately weighed into a 50 ml volumetric flask. The sample was brought to volume with spectral grade iso-octane (2,2,4-trimethylpentane) and shaken thoroughly for 1 minute to assure complete solution. The absorption by conjugated dienes was measured at 233 nm in a Beckman DU spectrophotometer with 1 cm cuvette. The transmittance was set to read 100% with iso-octane. Conjugated diene was calculated as a molar absorption. The molar absorbance of 26,000 was assumed for conjugated diene hydroperoxides. Also the molecular weight of the oil was calculated based on the molecular weight of linoleic acid.

TBA Test

The 2-thiobarbituric acid (TBA) test of Sidwell et al. (1954) was used in this study. Three grams of oil were accurately weighed into a 125 ml Erlenmeyer flask and dissolved with 10 ml benzene. Ten ml of TBA reagent were added. The flask was stoppered and shaken frequently for 4 minutes. The content of the flask was transferred to a 30 ml separatory funnel and shaken for 1 minute. The lower aqueous layer was transferred into a test tube. The tube was heated in a boiling-water bath for 30 minutes. After cooling to room temperature, 1 cm cuvette was filled and the percentage transmittance read at 530 nm in a Beckman DU spectrophotometer. The transmittance

was set to read 100% with distilled water. The result of the TBA test was reported as absorbance.

RESULTS AND DISCUSSION

It was noted in the literature review that chlorophyll can act as a sensitizer to initiate, in the presence of light, oxidation of vegetable oils by singlet oxygen. On the other hand, β-carotene may inhibit the process of photooxidation in the presence of chlorophyll. One of the purposes of this investigation was to study the roles of chlorophyll and of β-carotene, which occur naturally in soybean oil, in the photooxidation of soybean oil.

Most of the research carried out in fats and oils or fatty materials in the presence of sensitizers, quenchers, or both, used conditions where the fat or fatty materials were diluted with organic solvents. Under diluted conditions, different rates in the pattern of oxidation may result as compared to those of the natural systems; so in this work the effect of chlorophyll a and b and β-carotene was studied in concentrations that are expected to be found in crude soybean oil. Because other pigments and substances that may affect oxidation can be present in crude and refined oils, bleached oil, prepared from crude oil, was used as a substrate so that a better control of the effect of adding chlorophyll or β-carotene could be achieved.

Fatty acid composition was examined by gas liquid chromatography (GLC) in order to determine if the fatty acid composition was the same for crude, refined, and bleached oil or if possibly it was

altered by the refining and bleaching process. The data in Table 2 reveal the fatty acid composition of these oils. (The results in this table and those presented throughout this study are the average of duplicate determinations.) The usual range of values in percentage for fatty acid composition of soybean oil from soybeans cultivated in the United States is: myristic (14:0) tr-0.5, palmitic (16:0) 7-11, stearic (18:0) 2-6, oleic (18:1) 15-33, linoleic (18:2) 43-56, and linolenic (18:3) 5-11 (Collins & Sedwick, 1959).

Table 2: Fatty acid composition of soybean oils.

Fatty Acid[a]	Fatty Acid (%)		
	Crude Oil	Refined Oil	Bleached Oil
14:0	tr.	tr.	tr.
16:0	10.2	10.3	9.8
18:0	3.6	4.2	3.9
18:1	22.5	24.0	24.9
18:2	55.1	54.6	54.4
18:3	8.6	6.9	7.0

[a]The notation used to describe fatty acids is number of carbon atoms; ":" denotes number of double bonds.

The fatty acid composition of the soybean oils used is within the range of values reported in the literature. Refined oil, as shown in Table 2, had slightly lower values of 18:2 than in the crude oil. This small difference in fatty acid composition is not significant.

The severe bleaching conditions used in the laboratory did not appear to affect the fatty acid composition appreciably. The similarity in fatty acid composition among the oils indicated that a difference in unsaturation is not a factor that would influence their pattern of oxidation.

The efficiency of bleaching in removing the pigments from crude soybean oil was monitored by measuring the color and the content of chlorophyll and β-carotene before and after bleaching the oil.

The color of the oils was determined spectrophotometrically. The percentage transmittance at 460, 550, 620, and 670 nm is given in Table 3.

Table 3: Transmittance of soybean oils at different wavelengths.

λ (nm)	Transmittance (%)		
	Crude Oil	Refined Oil	Bleached Oil
460	0.0	0.0	97.0
550	49.0	59.0	98.0
620	81.0	91.0	100.0
670	96.5	98.5	100.0

As was expected, refined oil had higher transmittance at 550 and 620 nm than crude oil. The higher transmittance in refined oil is due to the partial removal of chromogenic pigments during the refining operation. The relatively low transmittance at 550 nm is an indication that ordinary refining is not completely effective in removing the chromogenic pigments present in soybean oil. On the

other hand, the transmittance of bleached oil as shown in Table 3 indicated that the laboratory bleaching conditions were very efficient in removing the chromogenic pigments from the oil. The bleached oil appeared to be completely colorless by visual inspection.

The spectrophotometric method for evaluating color was developed by the American Oil Chemist's Society as a possible replacement for the visual Lovibond Glass method for trading purposes, but the Lovibond method continues as the dominant method for commercial grading of oils although spectrophotometric measurements are used frequently in controlling refining and bleaching operations (Formo, 1979).

Finally, to verify if the chlorophylls and β-carotene were removed from crude oil during the bleaching operation, an analysis of the chlorophyll and β-carotene content was performed. The results of this analysis are presented in Table 4.

Table 4: Content of chlorophyll *a* and *b* in soybean oil.

Oil	chl *a* (mg/l)	chl *b* (mg/l)
Crude	0.55	0.42
Refined	0.29	0.17
Bleached	0.08	0.04

As shown in Table 4, both chlorophyll *a* and *b* were present in the oils. The content of chlorophyll *a* was in all cases greater than that of chlorophyll *b*. Pritchett et al. (1974) reported that normal soybean oil contains about 1.5 mg of chlorophyll per liter. No data reporting

the proportion of chlorophyll a and b present in soybean oil were found in the literature. The relatively high values of chlorophyll found in refined oil indicate that ordinary refining is not efficient in removing chlorophyll from the oil. The very low amounts of chlorophyll found in bleached oil indicate that activated carbon adsorbed this pigment very well. This result is in agreement with the study carried out by Hinner et al. (1946), who reported that activated carbon materials adsorb chlorophyll very efficiently.

The results of β-carotene analysis are presented in Table 5. Smith and Circle (1972) reported that the β-carotene content of mature soybeans varies from 0.2 to 2.4 mg/kg. The β-carotene contents of the crude and refined oils are within the range of values reported.

Table 5: β-carotene content of soybean oils.

Oil	β-carotene (mg/l)
Crude	1.25
Refined	1.52
Bleached	0.07

Soybean oil, like the majority of edible oils, after the refining process is subjected to a treatment with bleaching earth or clay to reduce the color of the oil. Because carotenoids are readily adsorbed by fuller's earth or activated carbons (Sonntag, 1979), lower values of β-carotene were expected to be found in the refined oil than in the crude. The higher content of β-carotene in refined

oil than in crude soybean oil indicated that the bleaching conditions were not adequate to remove carotenoid pigments. It also suggested that the refined oil may have come from a different lot than the crude oil.

The very low amount of β-carotene found in bleached oil indicated that, as in the case of chlorophyll, the laboratory bleaching conditions efficiently removed pigments from the crude soybean oil.

Irradiation of the Oils With UV Radiation

The effect of chlorophyll a and b at concentrations of 0.5 ppm and 1.0 ppm as well as the effect of β-carotene at concentrations of 1, 5, and 10 ppm, in absence or in presence of chlorophyll a or b, was studied in bleached oil. Crude, refined, and bleached oil were also used in these experiments to compare the pattern of oxidation in these, as measured by peroxide values, to those of the bleached oil in which chlorophyll or β-carotene was added. The UV radiation used was mainly in the 366 nm region. Although this source of radiation is not used in public displays, it is well known from the works of Coe and LeClerc (1934b, 1935), Lundberg (1962), and Sattar et al. (1976a) that ultraviolet, violet, and blue spectral regions below 450 nm promote oxidation more rapidly than those of longer wavelengths. In order to evaluate the effect of higher energy radiation on the development of peroxides in the oils, UV was chosen as an irradiation source.

Effect of β-carotene on Oxidation

The effect of β-carotene at concentrations of 6 and 11 ppm was studied in the bleached oil. Samples containing crude and refined

oil were irradiated at the same time and under the same conditions. Aliquots were taken at fixed intervals for peroxide analysis. The peroxide value of the controls (shielded by a cover of aluminum foil) was determined at the end of the 90 hr period. To verify whether or not UV radiation would affect the β-carotene added to the bleached oil, the β-carotene content was analyzed before and after the irradiation period. The same analyses were carried out on the controls. The results of peroxide values obtained are presented in Table 6 and the β-carotene content in Table 7.

Except for the crude oil, all samples developed relatively high peroxide values at the end of this period. The controls, except for that of crude oil, developed peroxide values of about one-half of those for samples that were irradiated. As can be seen in Figure 2, bleached oil containing 11 ppm of β-carotene had the highest peroxide values, followed by bleached oil with 6 ppm. Carotene at a concentration of 11 ppm failed to act as an antioxidant for bleached oil in the presence of UV radiation, but rather it promoted oxidation. This promoting effect on oxidation is in agreement with the observations of Thompson and Steenback (1944), who found β-carotene to be an active pro-oxidant in plant fats as contrasted to the finding by McConnell and Esselen (1947) that β-carotene at concentrations higher than 0.0013% would retard development of rancidity. The peroxide values indicated that concentrations higher than 0.0013% enhanced the oxidation. One possible explanation for this can be found in the β-carotene analyses run at the end of the irradiation period. These

Table 6: Effect of UV radiation on the PV of soybean oils with and without added β-carotene.

Samples	Irradiation Time (hr)										Control[b]
	0	8	16	24	32	40	50	60	70	90	90
	PV (meq/kg)										
Crude	1.0	2.5	3.0	4.0	10.0	10.0	14.0	18.0	21.0	22.0	5.0
Refined	7.0	12.0	14.0	23.0	25.0	29.0	46.0	48.0	48.0	54.0	31.0
Bleached	7.0	13.0	19.0	34.0	34.0	34.0	44.0	45.0	51.0	60.0	27.0
B·[a] + 6 ppm β-carotene	7.0	15.0	17.0	30.0	33.0	34.0	42.0	42.0	50.0	61.0	25.0
B·[a] + 11 ppm β-carotene	7.0	15.0	21.0	28.0	34.0	36.0	45.0	50.0	57.0	75.0	31.0

[a]On this and succeeding tables, B· refers to bleached oil.

[b]On all tables, samples shielded with aluminum foil are referred to as control.

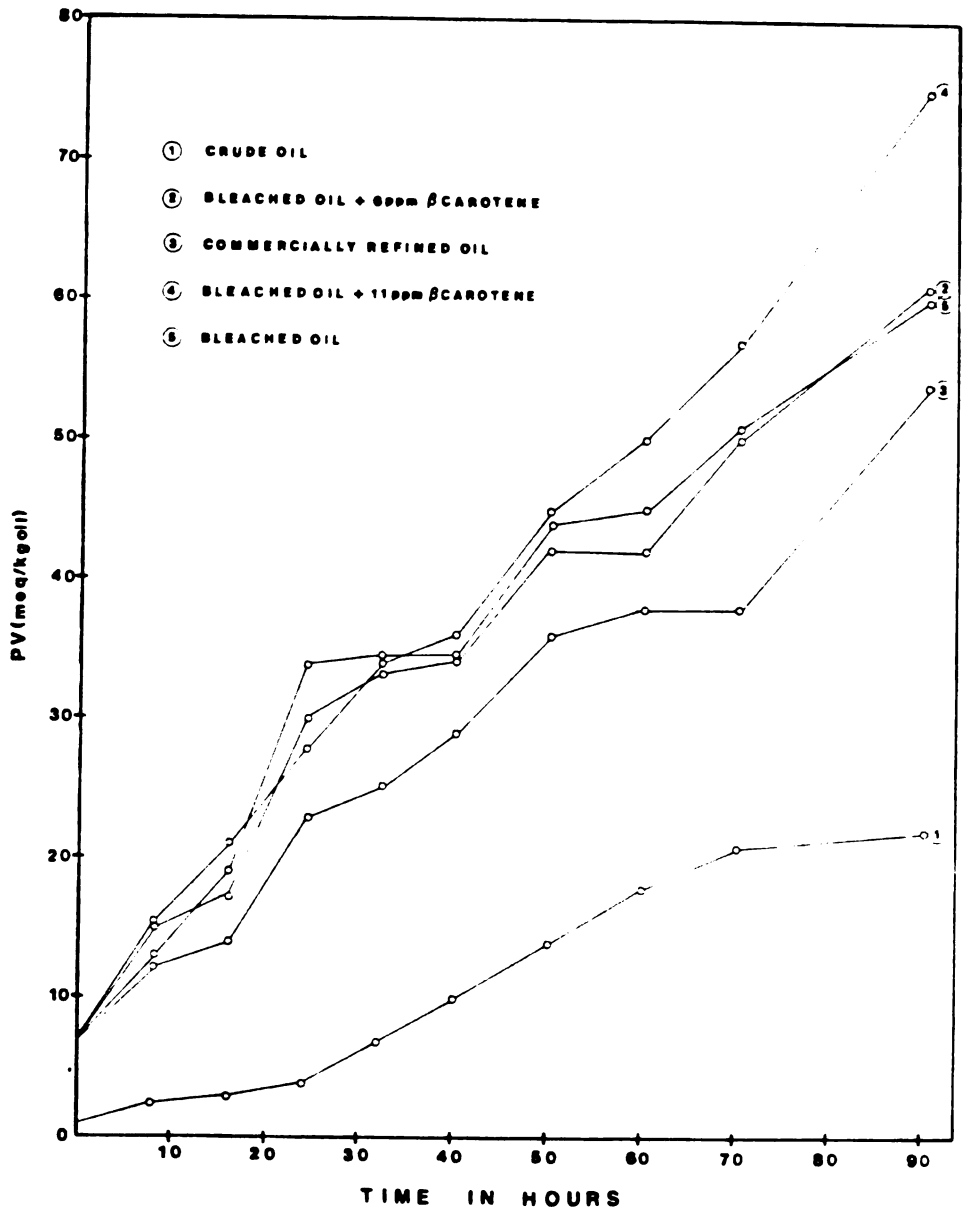


Fig. 2. The effect of UV radiation on peroxide value of crude, refined, and bleached soybean oil and bleached oil with added β-carotene.

shows that the greater the amount of β-carotene that is present, the greater is the amount destroyed by UV irradiation.

Table 7: β-carotene (mg/l) content of soybean oil before irradiation and after 90 hr irradiation with UV.

Samples	Before Irradiation	After Irradiation	Control After 90 hr
Crude	1.25	1.02	1.15
Refined	1.52	1.02	1.15
Bleached	0.08	0.00	0.00
B· + 6 ppm β-carotene	6.00	1.53	5.70
B· + 11 ppm β-carotene	11.00	2.51	10.37

The degradation of β-carotene may result in inducing the production of free radicals that contribute to acceleration of the oxidation process. Also, the higher peroxide values in the refined oil as compared to those in the crude oil used may be due in part to the higher content of β-carotene in this oil.

Effect of Chlorophyll a and b and β-carotene on Oxidation

The effect of chlorophyll a at concentrations of 0.5 ppm and 1.0 ppm and at a concentration of 0.5 ppm with 5 ppm of β-carotene was studied in bleached oil. To verify whether or not UV radiation would affect the chlorophyll a added to bleached oil, the chlorophyll a content was analyzed before and after the irradiation period. The same analyses were carried out in the controls. The peroxide values are given in Table 8, and the chlorophyll a and β-carotene

Table 8: Effect of UV radiation on PV development in soybean oil containing added chlorophyll a and β-carotene.

Samples	Irradiation Time (hr)										Control
	0	8	16	24	32	40	50	60	70	90	90
	PV (meq/kg)										
Crude	1.0	2.5	3.0	4.0	7.0	10.0	14.0	18.0	21.0	22.0	5.0
B· + 0.5 ppm chl a	7.0	16.0	19.0	26.0	31.0	33.0	43.0	43.0	51.0	52.0	29.0
B· + 1.0 ppm chl a	7.0	15.0	20.0	30.0	35.0	35.0	43.0	44.0	54.0	66.0	40.0
B· + 0.5 ppm chl a + 5 ppm β-carotene	7.0	13.0	18.0	28.0	35.0	38.0	44.0	51.0	54.0	68.0	28.0

content are presented in Table 9. Figure 3 demonstrates that bleached oil containing 1.0 ppm of chlorophyll a produced higher values of peroxide throughout the irradiation period than bleached oil containing 0.5 ppm of chlorophyll a. The bleached oil and the bleached oil to which 0.5 ppm of chlorophyll a was added had similar patterns of oxidation.

Table 9: Effect of chlorophyll a and β-carotene content of soybean oil under UV radiation.

Samples	chl a (mg/l)			β-carotene (mg/l)		
	0 hr	90 hr	Control (90 hr)	0 hr	90 hr	Control (90 hr)
Crude	0.55	0.18	0.25	1.25	1.02	1.15
B· + 0.5 ppm chl a	0.50	0.02	0.08	..	..	..
B· + 1.0 ppm chl a	1.00	0.02	0.08	..	..	..
B· + 0.5 ppm chl a + 5 ppm β-carotene	0.50	0.03	0.10	5.00	1.00	4.00

Although β-carotene is referred to in the literature as having $^1O_2$ quenching ability (Foote & Denny, 1968), it did not inhibit the rate of oxidation in the presence of chlorophyll in this case. As shown in Table 9, after 90 hr of irradiation, the content of β-carotene was reduced from 5.0 to 1.0 ppm. As noted earlier, the oxidation of β-carotene may induce the formation of free radicals that enhance the rate of lipid oxidation.

The higher rate of oxidation in the presence of β-carotene and chlorophyll suggests that the production of free radicals by the

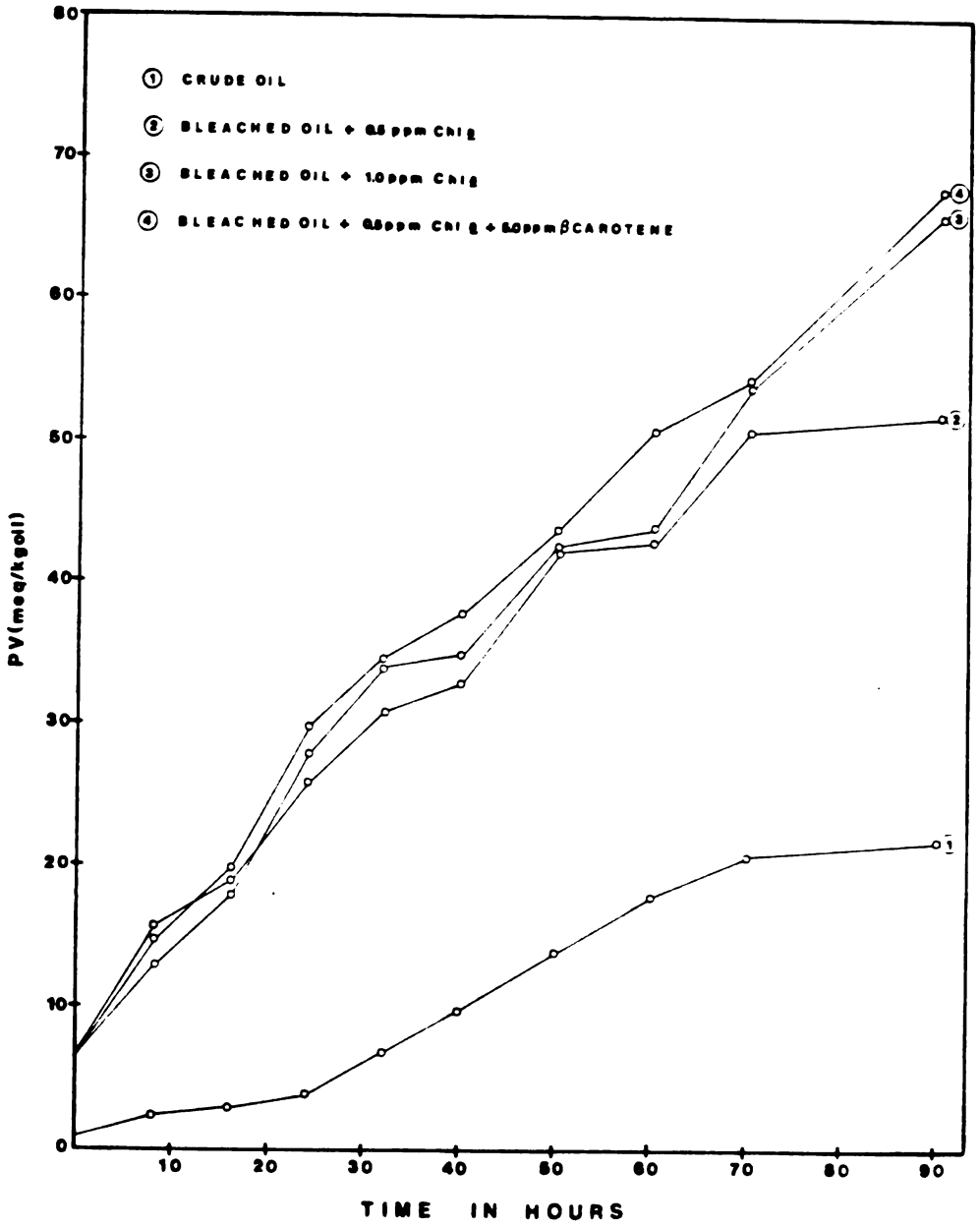


Fig. 3. The effect of UV radiation on peroxide value of soybean oil with added chlorophyll a and β-carotene.

degradation of β-carotene exceeds the effect of β-carotene as a quencher of $^1O_2$. This is in agreement with the work of Seely and Meyer (1971), who reported that β-carotene itself is oxidized by photooxidation to produce oxidation products.

The data in Table 9 indicate that chlorophyll a was destroyed during the irradiation period. The low values of chlorophyll a in the control samples indicate that chlorophyll a was destroyed even in the absence of light. The relatively high peroxide values in the controls reveal that oxidation has promoted destruction of the chlorophyll. Based on these results, higher concentrations of chlorophyll than those normally found in soybean oil (refer to Table 4) are needed to accelerate the oxidation process.

Effect of Chlorophyll b and β-carotene on Oxidation

The analyses for chlorophyll content of the oils, reported in Table 4, showed that both chlorophyll a and b were present in soybean oil. To verify if chlorophyll a and b had the same behavior in soybean oil, an experiment was carried out with chlorophyll b using the same concentration and conditions described for the experiments with chlorophyll a. The peroxide values are presented in Table 10, and the chlorophyll b and β-carotene content are shown in Table 11.

The pattern of peroxide values during irradiation in the presence of chlorophyll b was similar to that found with chlorophyll a. As shown in Figure 4, chlorophyll b at a concentration of 1.0 ppm induced higher peroxide values than 0.5 ppm. Similar to the findings with

Table 10: Ultraviolet radiation effect on oxidation of soybean oil containing added chlorophyll b and/or β-carotene.

Samples	Irradiation Time (hr)										Control
	0	8	16	24	32	40	50	60	70	90	90
	PV (meq/kg)										
Crude	1.0	2.5	3.0	4.0	7.0	10.0	14.0	18.0	21.0	22.0	5.0
B· + 0.5 ppm chl b	7.0	18.0	18.0	26.0	30.0	34.0	42.0	44.0	48.0	53.0	30.0
B· + 1.0 ppm chl b +	7.0	15.0	22.0	29.0	36.0	38.0	47.0	50.0	56.0	72.0	30.0
B· + 0.5 ppm chl b + 5.0 ppm β-carotene	7.0	14.0	16.0	27.0	34.0	38.0	45.0	50.0	55.0	68.0	32.0

chlorophyll a, the chlorophyll b was also destroyed in the presence of UV radiation. The same happened in the absence of light.

Table 11: Chlorophyll b and β-carotene content in UV radiated soybean oil.

Samples	chl b (mg/l)			β-carotene (mg/l)		
	0 hr	90 hr	Control (90 hr)	0 hr	90 hr	Control (90 hr)
Crude	0.55	0.18	0.25	1.25	1.02	1.15
B· + 0.5 ppm chl b	0.50	0.00	0.09	..	..	..
B· + 1.0 ppm chl b	1.00	0.00	0.20	..	..	..
B· + 0.5 ppm chl b + 5 ppm β-carotene	0.50	0.00	0.12	5.0	1.83	3.2

Both Wagenknecht et al. (1952) and Walker (1964) observed degradation of chlorophyll in peas and beans to nonchlorophyll compounds and attributed this to the action of lipoxygenase producing free radicals, which in turn degraded chlorophyll. Holden (1961) created a system that contained legume seed extracts and long-chain fatty acids and observed the degradation of chlorophyll to non-chlorophyll-like products. This suggests that the destruction of chlorophyll in absence of light occurs when free radicals are produced during autoxidation, which degrade chlorophyll a and b.

In the absence of light, bleached oil developed peroxide values (Table 6) similar to those in samples containing chlorophyll a or b. This indicated that neither chlorophyll acted as prooxidants in the absence of light. Taufel et al. (1959) reported that, in the dark,

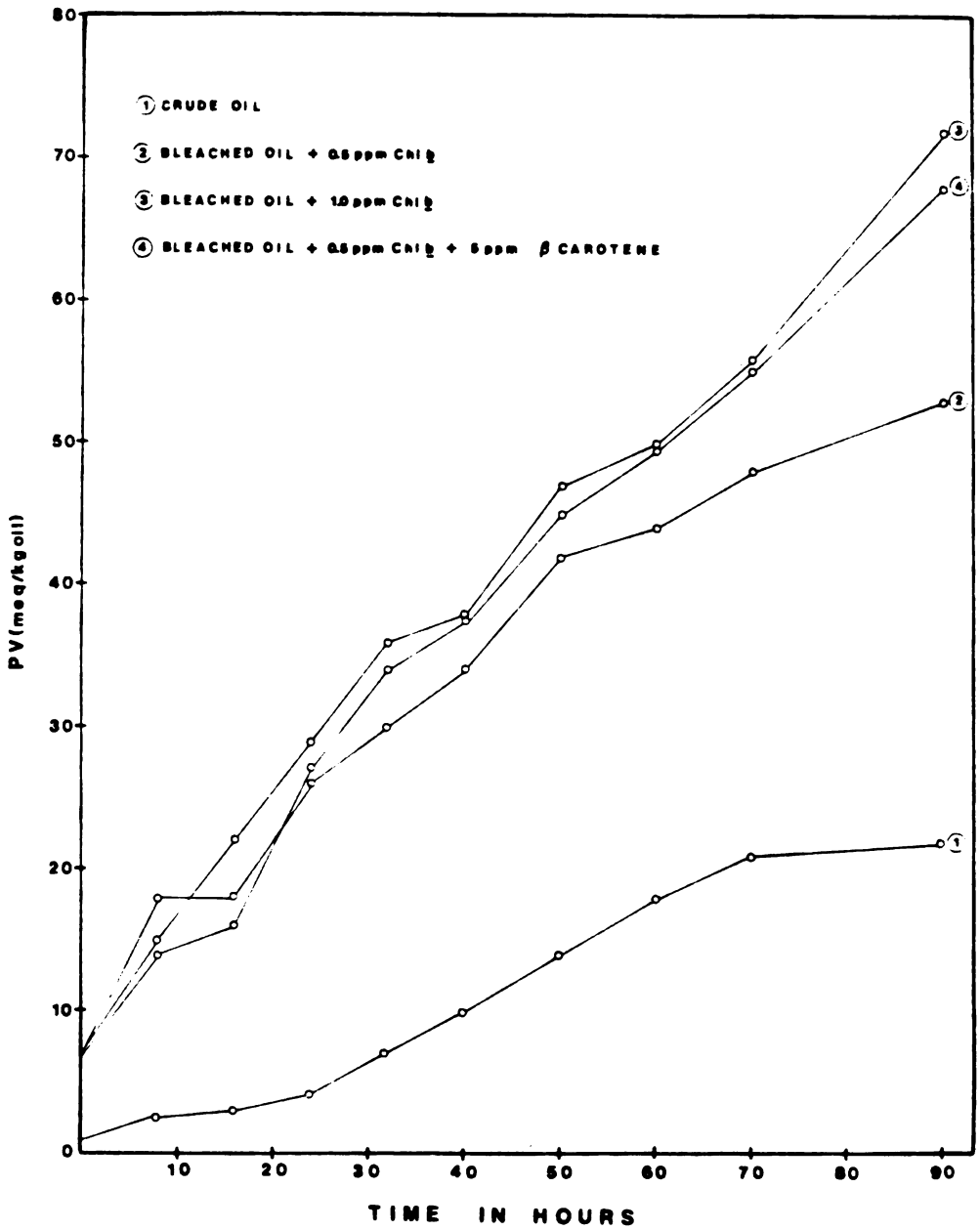


Fig. 4. Effect of UV radiation on peroxide value of soybean oil with added chlorophyll b and β-carotene.

chlorophyll acted synergistically as antioxidant with phenolic compounds. Since chlorophyll did not act synergistically as an antioxidant in the bleached oil in the absence of light and because the tocopherols also act as antioxidants, it suggests that tocopherols could have been removed from the oil during the laboratory bleaching process.

Addition of 5 ppm of β-carotene to the bleached oil containing 0.5 ppm of chlorophyll b did not inhibit the rate of oxidation, but, as in the presence of chlorophyll a, the rate of oxidation was enhanced. Chlorophyll a and b at the same concentrations appear to behave in the same way in the bleached oil.

Effect of Chlorophyll and β-carotene in Bleached Oil Irradiated With UV

An experiment was conducted to study the effect of β-carotene at concentrations of 1, 5, and 10 mg in the presence of 0.6 ppm of chlorophyll in which the samples were irradiated for a longer period of time. Crude and refined oil were also subjected to the same conditions. The peroxide values of the controls and of the samples at the end of a 192 hour irradiation period are presented in Table 12.

Bleached oil and bleached oil in which β-carotene and/or chlorophyll were added, as shown in Figure 5, developed much higher peroxide values throughout the 192 hour irradiation period than crude and refined oil. This experiment makes more evident the fact that β-carotene increased the rate of oxidation since higher concentrations of β-carotene promoted the development of higher peroxide values. It also makes more clear that small amounts of chlorophyll

did not increase the peroxide values appreciably in the presence of UV radiation.

Table 12: Peroxide values of soybean oil at the end of a 192 hour irradiation by UV.

Samples	PV (meq/kg oil)	
	Control	Irradiated Sample
Bleached	28	219
B· + 0.6 ppm chl	27	221
B· + 0.6 ppm chl + 1 mg β-carotene	64	241
B· + 0.6 ppm chl + 5 mg β-carotene	53	243
B· + 0.6 ppm chl + 10 mg β-carotene	49	257
Crude	4	50
Refined	16	74

Effect of Chlorophyll, β-carotene, and α- and γ-tocopherols

The experiments discussed in the previous section indicated that chlorophylls at concentrations of 0.5 and 0.6 ppm did not accelerate the rate of oxidation of bleached oil appreciably when irradiated by UV, nor did β-carotene decrease the rate of oxidation in the presence of chlorophyll. Also, bleached oil developed higher peroxide values throughout the irradiation period than did the crude and refined oils. Since chlorophyll is reported in the literature to act as a photosensitizer, lower peroxide values would be expected to be found in bleached oil than in crude and refined oil that contained measurable quantities of chlorophyll. Both Nakano (1975) and King et al. (1975) have concluded that $^1O_2$ can be formed during the

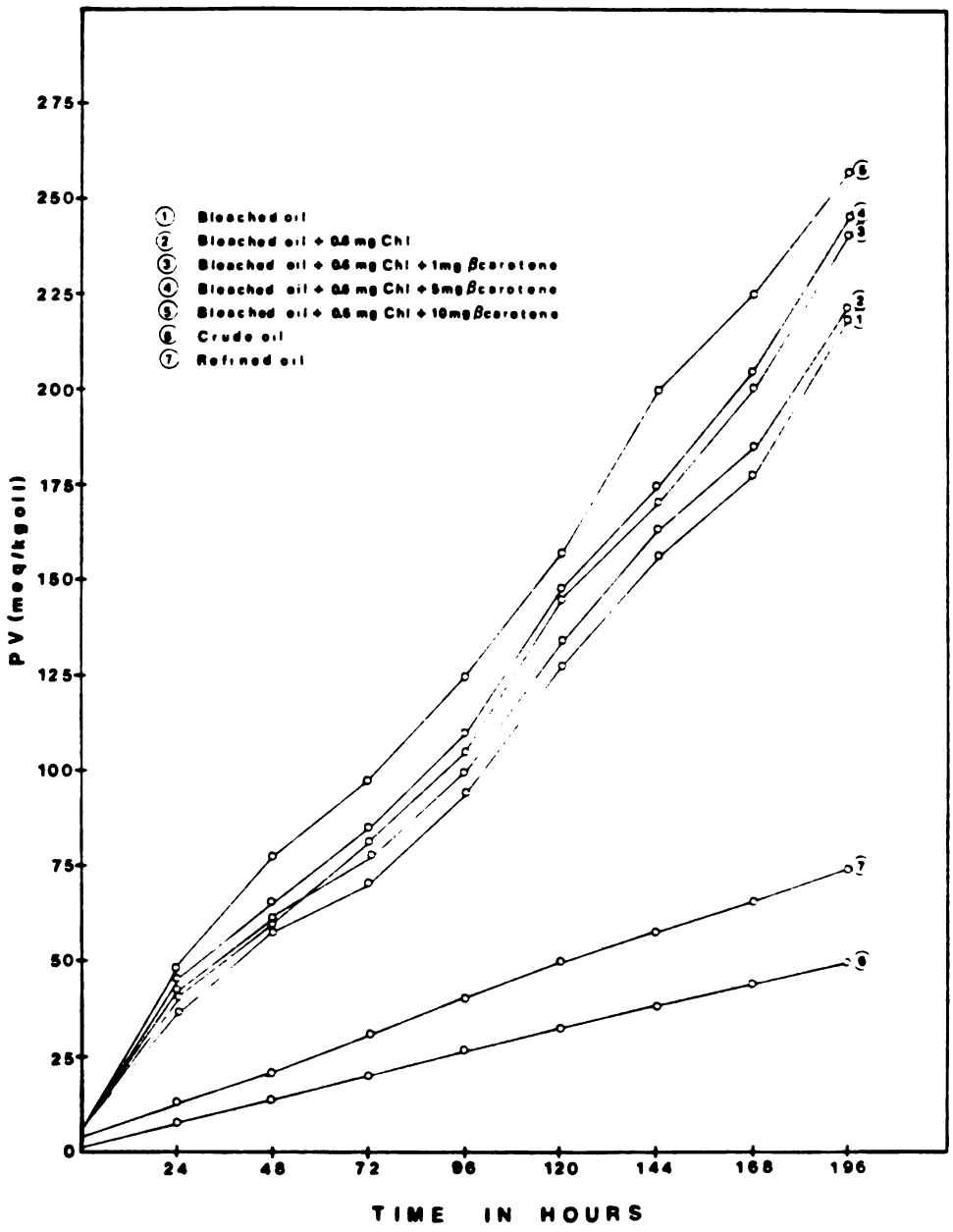


Fig. 5. Effect of added β-carotene and chlorophyll in UV induced oxidation of soybean oil.

breakdown of lipid hydroperoxides generated from liver microsomes oxidizing NADPH. Ultraviolet light accelerates markedly the decomposition of peroxides (Lundberg, 1962), so it is possible that $^1O_2$ may be generated with UV radiation without the presence of sensitizers.

Decolorization of soybean oil would be expected to increase the stability against oxidation by eliminating chromophoric pigments. The higher peroxide values of bleached oil than of crude and refined suggested the possibility that tocopherols, the natural antioxidants of vegetable oils, could have been removed from the oil during the bleaching process. In order to determine the α- and γ-tocopherol content of the oils, analyses by high-performance liquid chromatography (HPLC) were carried out. The results of these analyses are presented in Table 13.

Table 13: Content of α- and γ-tocopherol of soybean oils.

Oils	α-tocopherol (mg/100 g)	γ-tocopherol (mg/100 g)
Crude	4.08	116.5
Refined	4.50	110.0
Bleached	1.55	11.3

Carpenter (1979) found values of 7.0 and 92.4 mg/100 g, respectively, for α- and γ-tocopherols in soybean oil by HPLC analysis. Yuki and Ishikawa (1976) found that a typical soybean oil (I.V. 113.2)

contained 4.8, 47.1, and 12.7 mg/100 g, respectively, of α-, γ-, and δ-tocopherols. The values of α- and γ-tocopherols shown in Table 13 are similar to those reported by Carpenter (1979). The tocopherol profile of crude, refined, and bleached oil as determined by HPLC is shown in Figure 6. Although the β- and δ-tocopherols were not analyzed quantitatively because sources of analytical grade of these were not found, they were identified by using a vitamin E mixture that contained the four tocopherols. The HPLC profiles of crude and refined oils, as shown in Figure 6, are very similar.

The refined oil used in this experiment was subjected to commercial refining, bleaching, and deodorizing processes. The similarity in tocopherol profile between crude and refined soybean oil indicates that the tocopherols are not substantially removed from oils by these processes. This result is in agreement with Sherwin (1976), who reported that the tocopherols survive oil-processing steps and thus are found in good quantities in finished vegetable oils. On the other hand, the tocopherol content was appreciably reduced by the laboratory bleaching conditions. In addition to lowering the concentration of α- and γ-tocopherols, β- and δ- appeared to be removed completely from the oil during the bleaching operation. Besides the antioxidant properties, tocopherols have been reported (Yamauchi & Matsushita, 1977) to be able to act as $^1O_2$-quenchers. The results of the tocopherol analyses may explain the higher values of peroxides found in bleached oil than in crude and refined oil during the irradiation process.

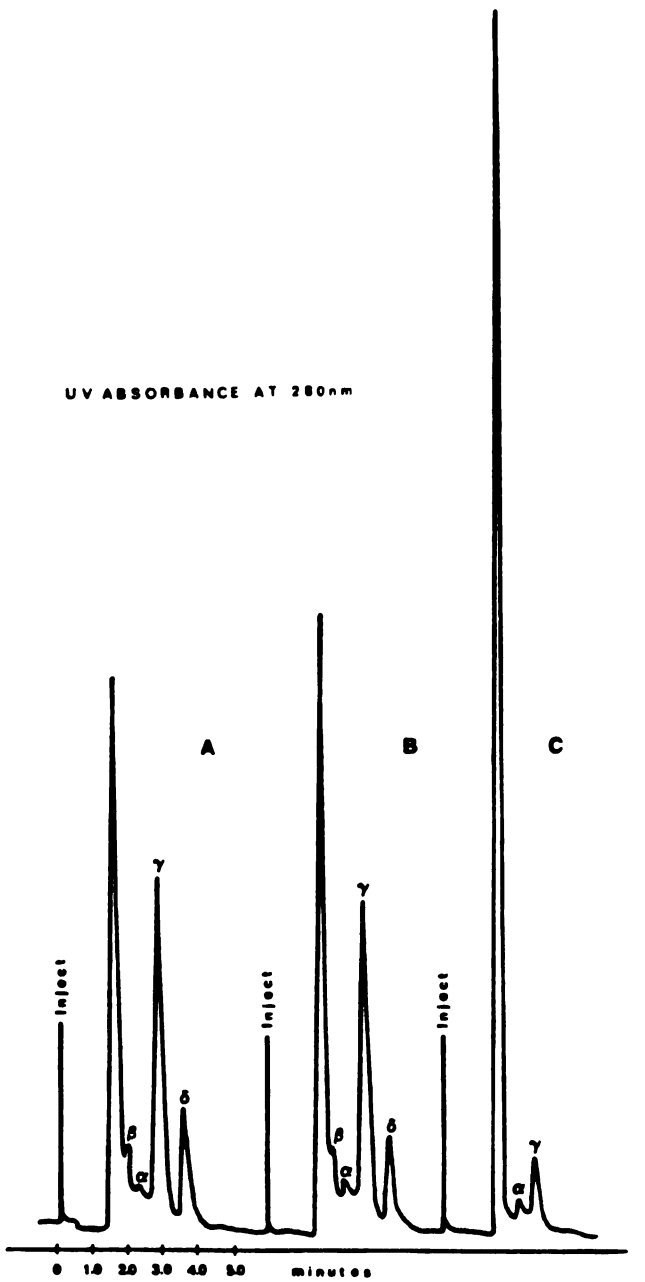


Fig. 6. HPLC tocopherol profile of (A) crude, (B) refined, and (C) bleached soybean oil.

A new experiment was developed to learn if the addition of α- and γ-tocopherols could inhibit the oxidation of bleached oil. The effect of combining α- and γ-tocopherol with chlorophyll and/or β-carotene also was studied under the same conditions. The samples were irradiated with UV radiation for a period of 192 hr. The peroxide values of the sample were determined at 24 hr intervals. The peroxide values of the controls were determined at the end of 192 hr. Both α- and γ-tocopherols were effective in protecting bleached oil from oxidation in the absence of radiation, as shown in Table 14. The combination of these tocopherols was more effective than each one alone. On the other hand, when α- and γ-tocopherol were combined with 0.6 ppm of chlorophyll, the highest peroxide values among the control samples were developed. In the absence of radiation, the tocopherols neither inhibited the rate of oxidation in the presence of chlorophyll nor in the presence of β-carotene.

In the presence of UV radiation, as shown in Figure 7, the combination of α- and γ-tocopherol failed to decrease the oxidation rate of the oil throughout the 192 hr irradiation period. Oxidation was also enhanced when either chlorophyll or β-carotene was present. Systems containing α- and γ-tocopherols and chlorophyll behaved in the same way as those containing either α- or γ-tocopherol alone.

The results of this experiment, contrary to what was expected, showed that neither α- nor γ-tocopherol protected bleached oil from oxidizing in the presence of UV radiation. This is in agreement with Parkhurst et al. (1968), who reported that tocopherols are much less effective antioxidants in the presence of light than in the dark.

Table 14: Peroxide values of the soybean oil controls containing tocopherols at the end of 192 hr.

Samples	PV (meq/kg oil)
B· + 1 mg α-tocopherol (0.004%)	11
B· + 1 mg γ-totopherol (0.004%)	15
B· + 1 mg α-tocopherol + 1 mg γ-tocopherol	9
B· + 1 mg α-tocopherol + 0.6 mg chl	19
B· + 1 mg γ-tocopherol + 0.6 mg chl	18
B· + 1 mg α-tocopherol + 1 mg γ-tocopherol + 0.6 mg chl	25
B· + 1 mg α-tocopherol + 1 mg β-carotene + 0.6 mg chl	18
B· + 1 mg γ-tocopherol + 1 mg β-carotene + 0.6 mg chl	18
B· + 1 mg α-tocopherol + 1 mg γ-tocopherol + 1 mg β-carotene + 0.6 mg chl	17
Crude oil	2

Sherwin (1976) reported that if tocopherols are added back to stripped oil at levels normally present in refined vegetable oils, the oxidative stability of the oil will be restored to a level typical of normally processed vegetable oil. Our results showed that this is not true if the oil is subjected to UV radiation. A possible explanation for the ineffectiveness of α- and γ-tocopherol in inhibiting the oxidation of bleached oil could be based on the fact that tocopherols in the presence of UV radiation are degraded to form other products, and it may be that during this degradation free radicals are produced that enhance the overall oxidation.

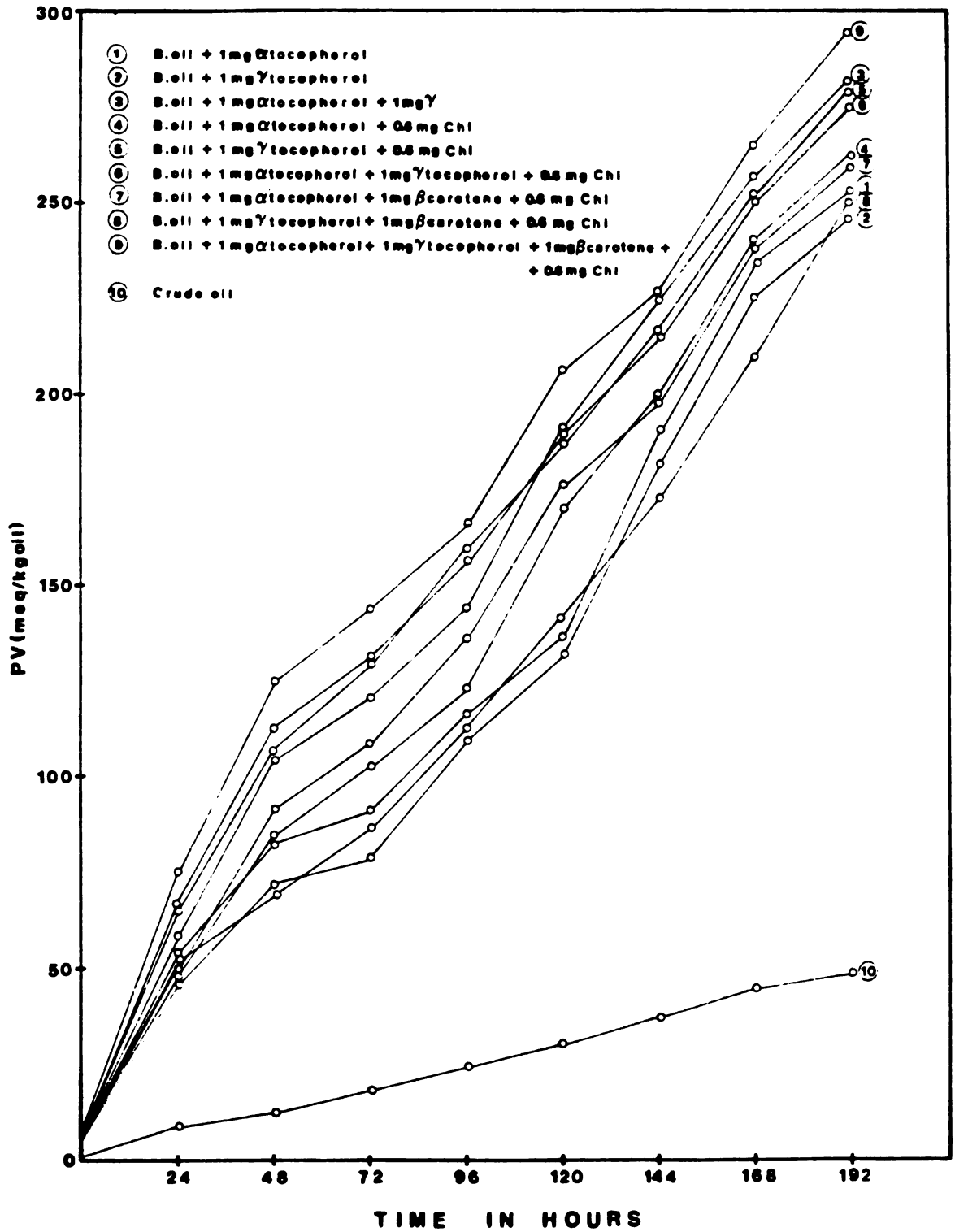


Fig. 7. Effect of UV radiation on peroxide value of soybean oil with added tocopherol, β-carotene, and chlorophyll.

Effect of TBHQ, BHA, α- and γ-tocopherols, Chlorophyll, and β-carotene in Bleached Oil Irradiated With UV

TBHQ and BHA were selected to study their effects on the oxidation of soybean oil in the presence of UV radiation. Their effects alone or in combination with chlorophyll, α- and γ-tocopherols, or β-carotene on peroxide values are shown in Figure 8. Table 15 gives the peroxide values of the controls at the end of 192 hr. In the absence of radiation, TBHQ was much more effective in inhibiting the oxidation of bleached oil than was BHA. Also, TBHQ proved to be superior to BHA in controlling the stability of the oil in the presence of chlorophyll. The combinations of TBHQ with either α- or γ-tocopherols were more effective than the combination of TBHQ and β-carotene.

Table 15: Peroxide values of the soybean oil controls containing TBHQ, BHA, and other components at the end of 192 hr.

Samples	PV (meq/kg oil)
B· + 1 mg TBHQ (0.004%)	4
B· + 1 mg BHA (0.004%)	10
B· + 1 mg TBHQ + 0.6 mg chl	6
B· + 1 mg BHA + 0.6 mg chl	13
B· + 1 mg TBHQ + 1 mg β-carotene + 0.6 mg chl	8
B· + 1 mg BHA + 1 mg α-tocopherol + 0.6 mg chl	14
B· + 1 mg TBHQ + 1 mg α-tocopherol + 0.6 mg chl	5
B· + 1 mg TBHQ + 1 mg γ-tocopherol + 0.6 mg chl	5
B· + 1 mg BHA + 1 mg α-tocopherol + 0.6 mg chl	15
Crude oil	2

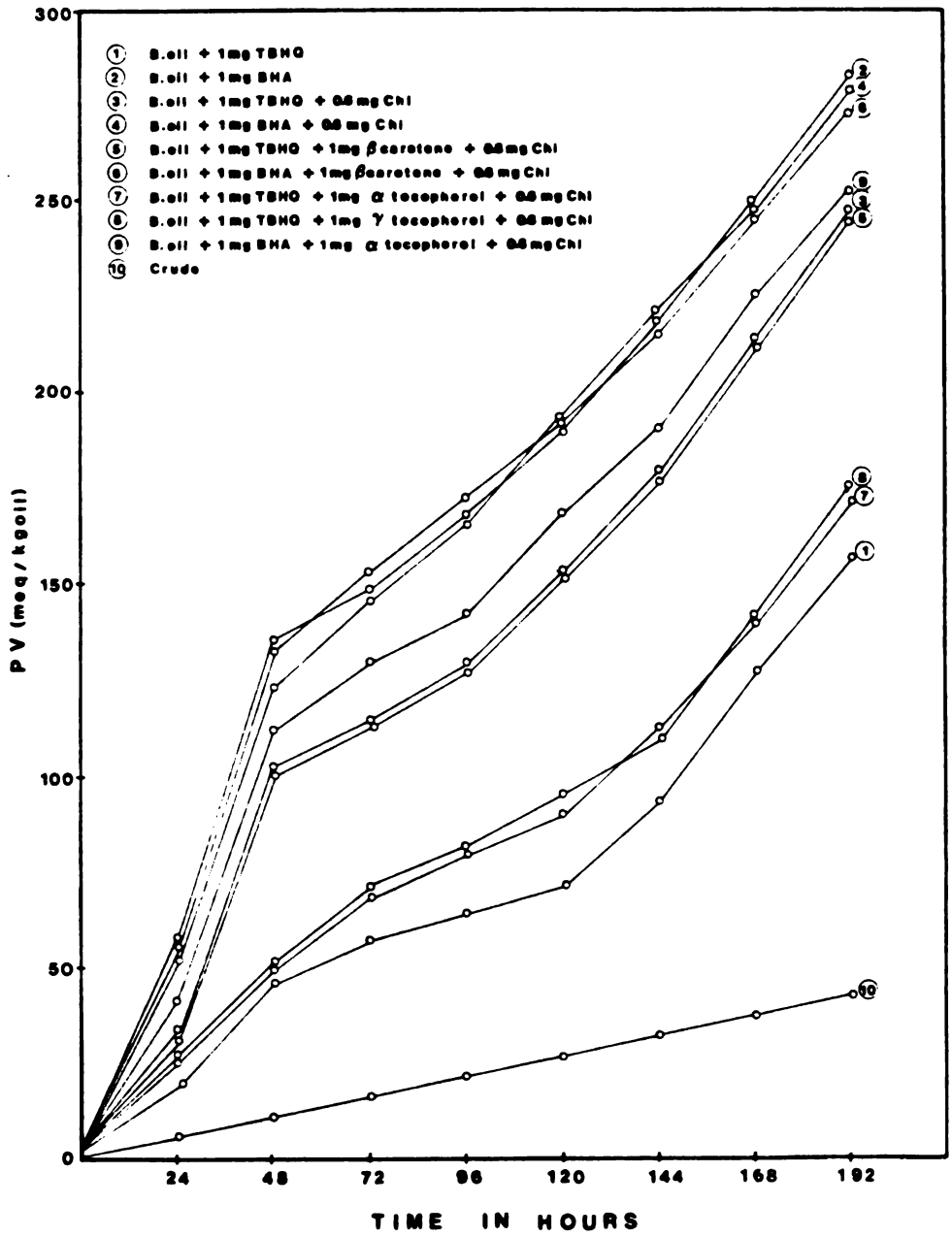


Fig. 8. Effect of UV radiation on peroxide value of soybean oil containing TBHQ, BHA, β-carotene, and chlorophyll.

TBHQ and, to a lesser degree, BHA were effective even in the presence of UV radiation unless a sensitizer was present. Although systems containing α- or γ-tocopherol and TBHQ afforded some protection toward photooxidation in the presence of chlorophyll, relatively high peroxide values were developed at the end of 192 hr in all systems containing sensitizer.

TBHQ has been reported (Sherwin & Thompson, 1967; Sherwin & Luckadoo, 1970) to be a potent antioxidant against the autoxidation of soybean oil and other polyunsaturated fats and oils. The fact that TBHQ did not inhibit the oxidation of bleached soybean oil in the presence of chlorophyll may indicate that an oxidation mechanism different from autoxidation was involved in the presence of chlorophyll. In the absence of radiation where autoxidation might be the only mechanism involved, TBHQ was a very effective antioxidant.

Another interesting fact is that in radiation-induced oxidation, the rate decreased when either α- or γ-tocopherols were added to the bleached oil, TBHQ, and chlorophyll. The presence of tocopherols either enhanced the inhibiting effect of TBHQ or acted by quenching singlet oxygen.

Effect of Lecithin, α-tocopherol, Vitamin E Mixture, β-carotene, and Chlorophyll in Bleached Soybean Oil Irradiated With UV

It has been shown so far that β-carotene, α- and γ-tocopherols, TBHQ, and BHA were ineffective in inhibiting the photooxidation of soybean oil in the presence of chlorophyll. In all cases, crude oil

developed much lower peroxide values throughout the irradiation period than the others.

The phosphatide content of the oils was determined to examine the possibility that the difference in the pattern of oxidation could be caused by a difference in phosphatide content. These results are presented in Table 16. The removal of phosphatides from the oil is carried out industrially by a process known as degumming. Crude soybean oil is reported to contain 1.1 to 3.2% of phosphatides, 20% lecithins, 31% cephalins, and 40% inositol (Sonntag, 1979).

Table 16: Phosphatide content of soybean oils.

Oil	Phosphatide (%)
Crude	1.16
Refined	0.00
Bleached	0.00

The results presented in Table 16 indicate that the commercial degumming process and the laboratory bleaching conditions were efficient in removing phosphatides from crude soybean oil. An experiment was developed to learn if addition of lecithin could inhibit the photooxidation of bleached soybean oil. Also, the effect of a vitamin E mixture (containing 50% of α-tocopherol and equal proportions of β-, γ-, and δ-tocopherols) was studied at the same time.

In the absence of radiation (Table 17), the highest peroxide value was developed in the system containing lecithin, β-carotene,

and chlorophyll. A relatively high peroxide value was also developed in the bleached oil containing only lecithin. This is in agreement with Olcott and Mattil (1936), who reported that phosphatides alone were not effective as antioxidants, but were effective in combination with phenolic inhibitors.

Table 17: Peroxide values of the soybean oil controls containing lecithin or vitamin E at the end of 120 hr.

Samples	PV (meq/kg oil)
B· + 0.5% lecithin	23
B· + 0.5% lecithin + 1 mg α-tocopherol	8
B· + 0.5% lecithin + 0.6 mg chl	13
B· + 0.5% lecithin + 1 mg β-carotene	13
B· + 0.5% lecithin + 1 mg β-carotene + 0.6 mg chl	44
B· + 0.5% lecithin + 1 mg α-tocopherol + 0.6 mg chl	15
B· + 2 mg vitamin E mixture + 1 mg β-carotene	7
B· + 2 mg vitamin E mixture + 1 mg β-carotene + 0.6 mg chl	10
Crude oil	2

The two systems containing the vitamin E mixture had a low peroxide value in the absence of radiation, even when chlorophyll was present. The pattern of peroxide development of the samples irradiated with UV radiation was similar to that of the controls (Figure 9).

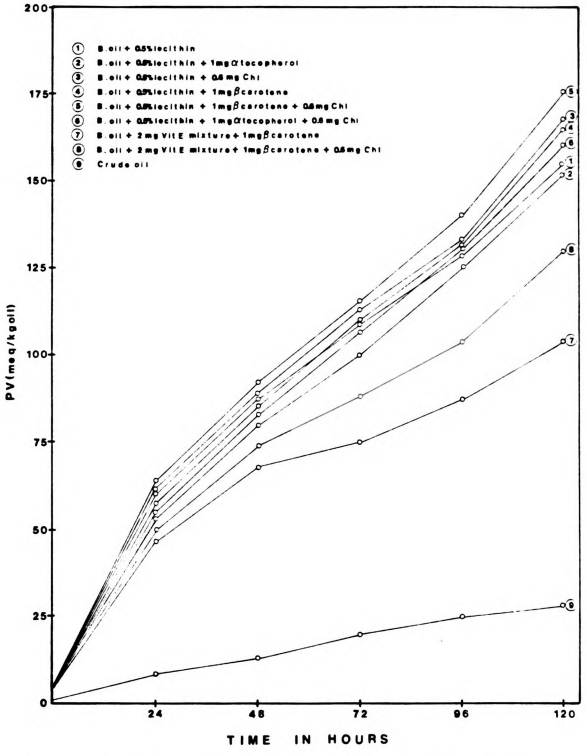


Fig. 9. Effect of UV radiation on peroxide value of soybean oil with added lecithin, vitamin E mixture, β-carotene, and chlorophyll.

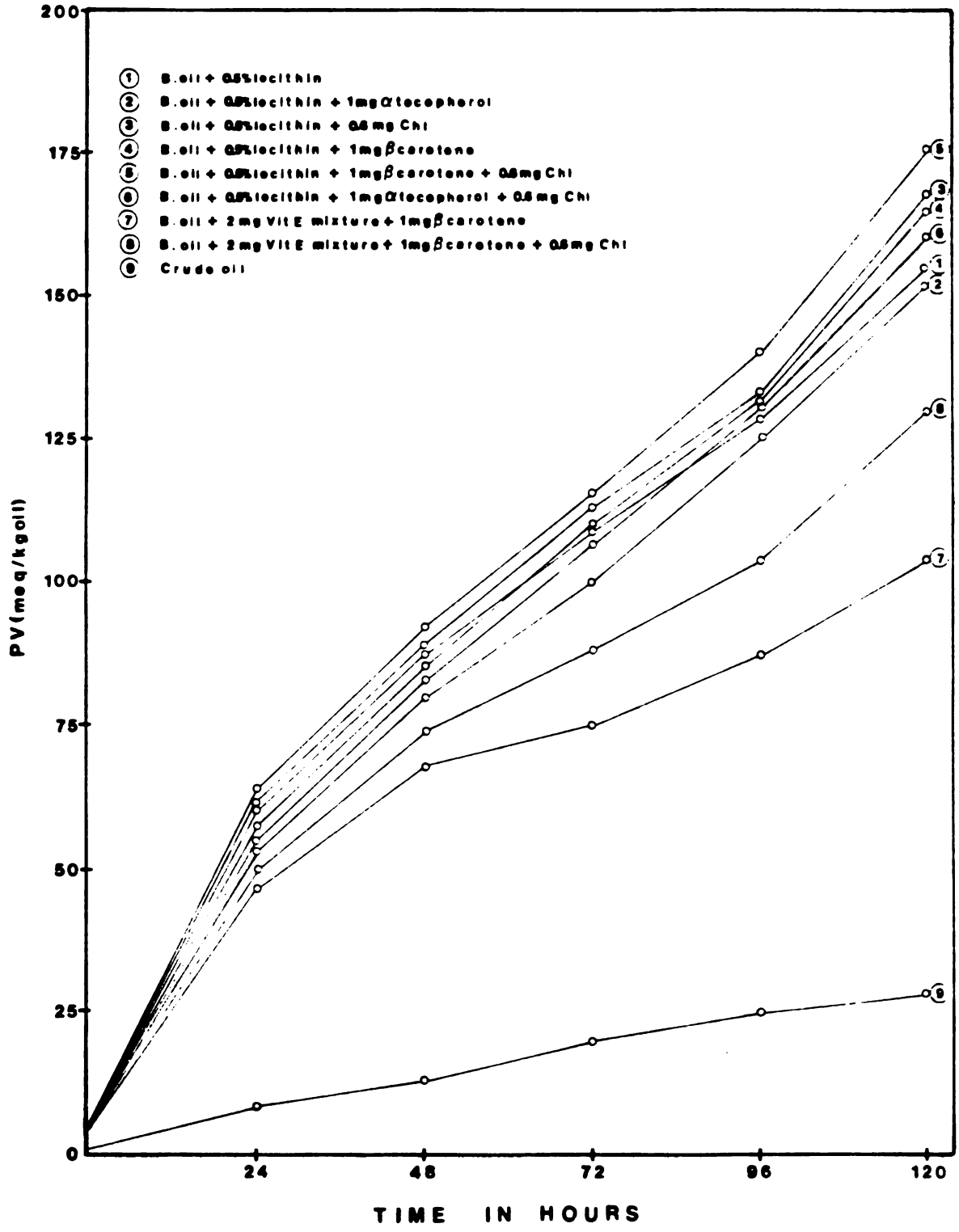


Fig. 9. Effect of UV radiation on peroxide value of soybean oil with added lecithin, vitamin E mixture, β-carotene, and chlorophyll.

Samples containing the vitamin E mixture developed the lowest peroxide values throughout the 120 hr irradiation period. On the other hand, samples containing either lecithin and chlorophyll or lecithin and β-carotene developed the highest peroxide values. The lower peroxides developed in those systems containing the vitamin E mixture, in presence or absence of light, indicate that the combined effect of the four tocopherols was more efficient in inhibiting oxidation of bleached oil than the use of each tocopherol separately. The relative effectiveness of tocopherols as antioxidants is greater for δ- and γ- than β- and α-tocopherols (Daubert, 1950; Sherwin, 1976; Parkhurst et al., 1968).

The relatively high peroxide values developed throughout the 120 hr irradiation period in the presence of chlorophyll indicate that the vitamin E mixture was not an efficient oxidation inhibitor in the presence of sensitizers. The combination of β-carotene and the vitamin E mixture did not appreciably inhibit the oxidation of bleached soybean oil in the presence of chlorophyll and UV radiation.

Based on the results obtained so far, no evident reason was found to explain the very high resistance of crude soybean oil toward photooxidation. It is possible that the other phosphatides or their combination with the natural antioxidants and β-carotene may interact to protect the crude soybean oil from the photooxidation process.

Effect of UV Radiation on Peroxide Value and Conjugated Diene of Soybean Oil With Added Chlorophyll a or b

It has been suggested that singlet oxygen, generated by sensitizers such as chlorophyll, can initiate oxidation of vegetable oils (Rawls & Van Santen, 1970). Although in this study chlorophyll at low concentrations did not accelerate the rate of oxidation appreciably, we tried to determine to what extent this mechanism could have participated in and affected the photooxidation of soybean oil.

It is well known from the literature (Khan et al., 1954; Foote, 1976; Terao & Matsushita, 1977; Frankel et al., 1979) that in photosensitized oxidation in which singlet oxygen is involved, both conjugated and unconjugated hydroperoxides are formed. Conjugated hydroperoxides absorb in the region of 233-234 nm. Because unconjugated hydroperoxides do not absorb in this region of the spectrum, lower values of absorption at 233-234 nm are expected in those reactions in which singlet oxygen is involved than in those reactions in which autoxidation is the only mechanism.

In the present experiment, either chlorophyll a or b was added to the bleached oil, and their effect on conjugated diene and peroxide values was followed during a 48 hr irradiation period. Crude, refined, and bleached oil were also used.

Addition of either chlorophyll a or b to bleached oil did not increase the peroxide values appreciably (Table 18). The molar absorbance shown in Table 19 increased with greater time of exposure to UV radiation. This increase in molar absorbance was expected because, even in the presence of singlet oxygen, autoxidation might

be the main mechanism that was involved, especially in the presence of UV radiation. Also, some conjugated dienes may be formed in linolenate by singlet $^1O_2$ oxidation. Bleached oil and bleached oil with either chlorophyll a or b developed higher molar absorbance than crude and refined oils. The higher values of molar absorbance for these oils correspond to higher peroxide values.

Table 18: Peroxide values of soybean oil irradiated 48 hr with UV radiation.

Samples	PV (meq/kg)				
	0 hr	12 hr	24 hr	36 hr	48 hr
Crude	5	8	12	16	19
Refined	5	8	13	18	21
B·	5	35	51	68	72
B· + 4 ppm chl a	5	40	56	73	78
B· + 4 ppm chl b	5	40	55	72	75

Table 19: Molar absorbance of soybean oil irradiated with UV radiation.

Samples	Molar Absorbance ($x10^{-3}$) at 233 nm				
	0 hr	12 hr	24 hr	36 hr	48 hr
Crude	2.016	2.660	2.830	3.630	3.690
Refined	2.055	2.770	3.167	3.790	4.120
B·	1.607	5.475	7.270	9.450	10.170
B· + 4 ppm chl a	1.495	5.594	7.830	9.000	10.330
B· + 4 ppm chl b	1.585	5.530	7.540	9.360	10.070

Since conjugated and unconjugated hydroperoxides are formed in the presence of singlet oxygen, the molar absorbances obtained were divided by the peroxide values (meq/kg oil) and plotted against time as shown in Figure 10. In the first 12 hours of irradiation, the decrease of the molar absorbance/PV ratio was more pronounced in bleached soybean oil with added chlorophylls than in crude and refined oils. It was observed visually that the green color of the samples containing chlorophyll disappeared in the first 10 hr of irradiation concurrently with the accentuated decrease in molar absorbance/PV ratio. This may be an indication that singlet oxygen participated, at least in the first hours of irradiation, in the formation of unconjugated hydroperoxides. After this period, as shown in Figure 10, the molar absorbance/PV ratio leveled off. Although the drop in molar absorbance/PV ratio of bleached oil was not as high as when chlorophyll was present, it was still high and suggests that perhaps singlet oxygen was also involved in the oxidation of bleached oil in the presence of UV radiation.

The very similar values obtained for conjugated diene and peroxides with chlorophyll a and b throughout the 48 hr irradiation period indicate once more that these chlorophylls behave similarly in the photooxidation of soybean oil.

Irradiation of the Oils With Fluorescent Light

Fluorescent light is widely used in stores and supermarkets to illuminate display cases of many kinds of foods, including fats and oils. Transparent and translucent packing are quite often used for

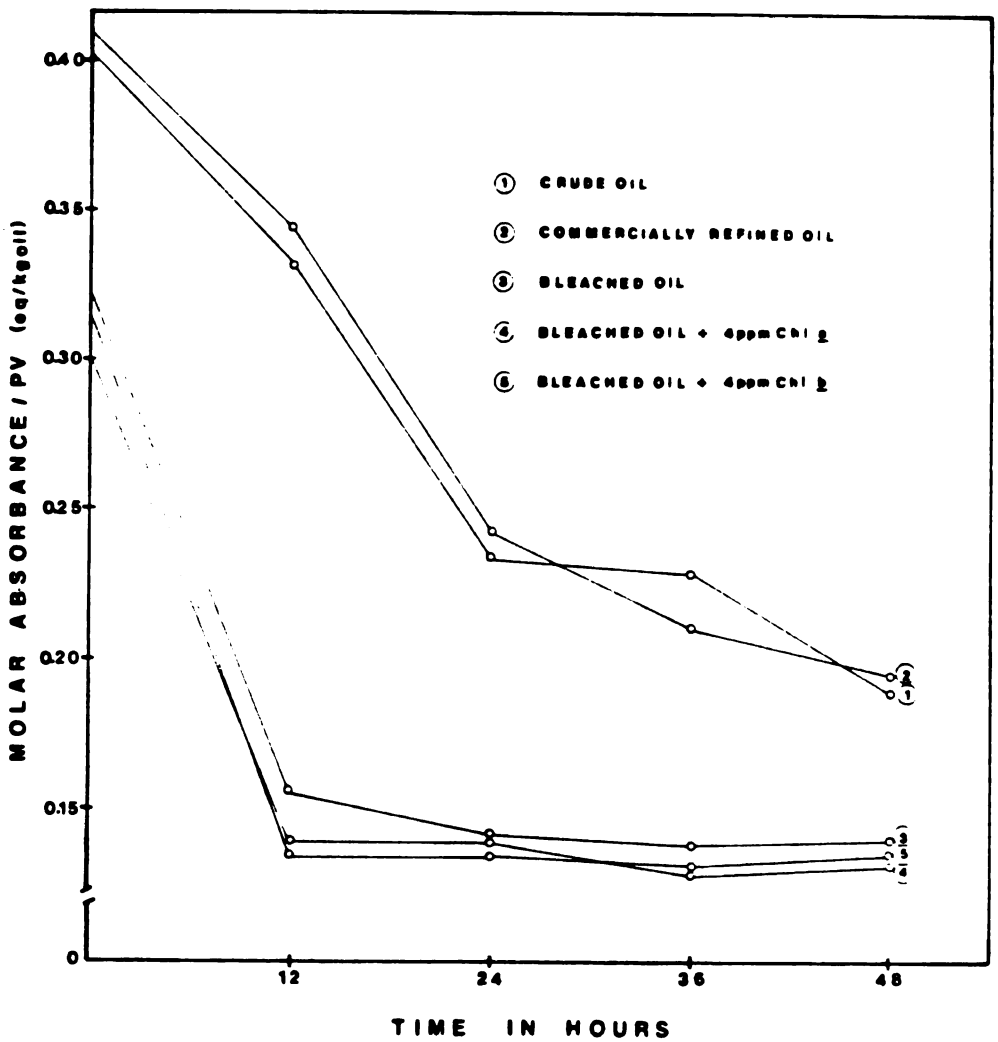


Fig. 10. Molar absorbance/PV ratio vs. time of soybean oil irradiated with UV radiation.

fats and oils and fat-containing food products. These packages allow the passage of considerable amounts of light energy, and this may be sufficient to initiate photooxidation of fats or fat-containing food products if other conditions are favorable.

Effect of Fluorescent Light on PV of Crude, Refined, and Bleached Soybean Oil With Added Chlorophyll *a* and β-carotene

It was demonstrated in the UV radiation experiments that chlorophyll at low concentrations did not accelerate the photooxidation of soybean oil appreciably, nor did β-carotene inhibit the oxidation in the presence or absence of chlorophyll.

To verify if wavelengths longer than those of UV radiation could have a different effect on the pattern of oxidation of soybean oil, samples of crude, bleached, and bleached oil with added chlorophyll and/or β-carotene (at a concentration of 0.004%) were irradiated with fluorescent light. The peroxide values were determined throughout a 120 hr irradiation period (Figure 11). Bleached oil containing either chlorophyll *a* or β-carotene developed the highest peroxide values during the irradiation period.

Higher peroxide values were developed in bleached oil than in crude oil. Addition of chlorophyll *a* at concentrations of 1 ppm did not increase the peroxides appreciably over those formed in bleached oil. Addition of β-carotene to the bleached oil containing chlorophyll *a* did not inhibit oxidation; on the contrary, it was stimulated. A possible explanation for this again would be the degradation of these pigments (chlorophyll and β-carotene) in the presence of light.

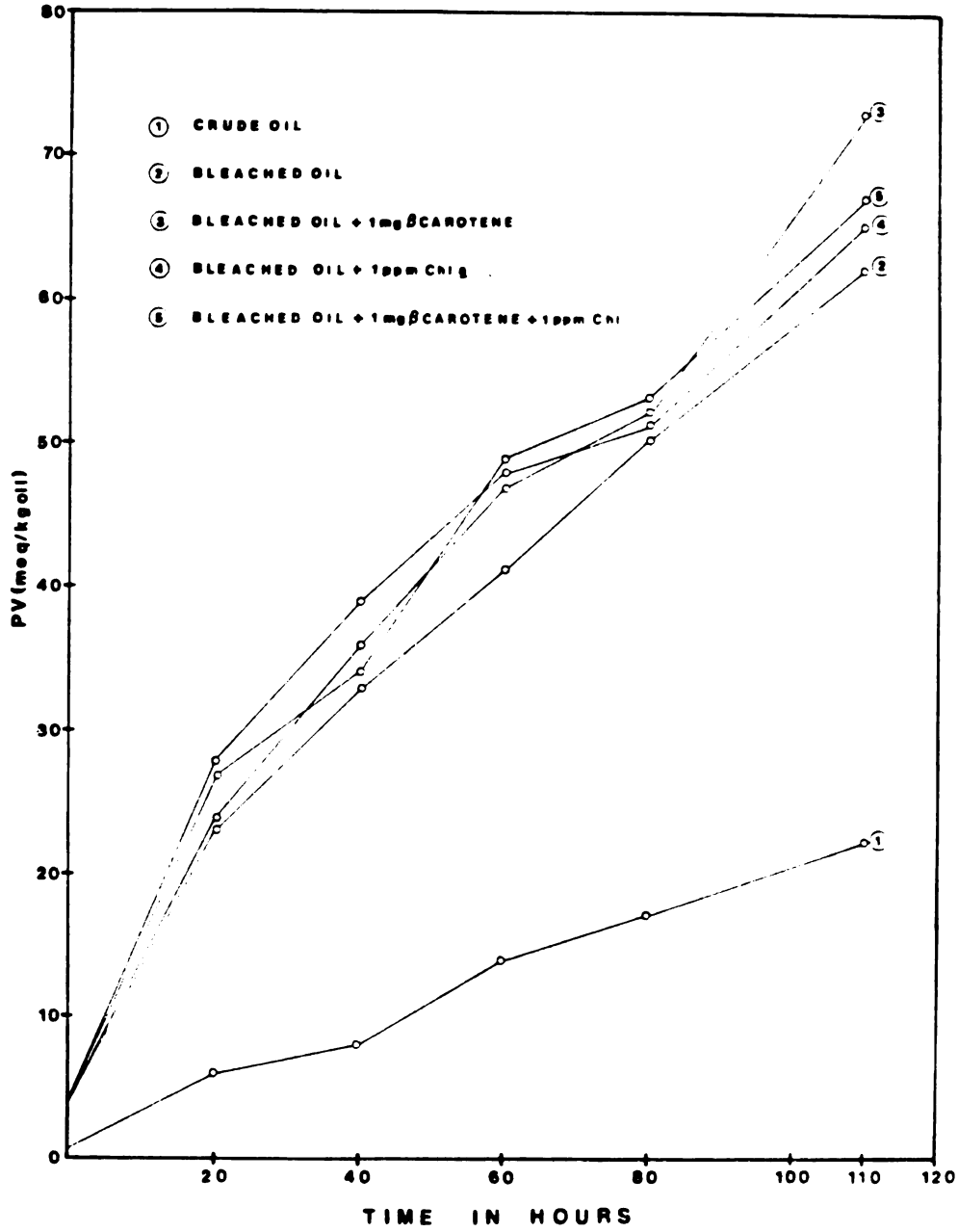


Fig. 11. Effect of fluorescent light on the peroxide value of soybean oil with added chlorophyll a and β-carotene.

In general, the patterns of oxidation as measured by peroxide values were similar to those obtained in samples irradiated with UV radiation (Figure 3) except that lower peroxide values were developed for the same time of irradiation with fluorescent light. This was expected because of the longer wavelengths and lesser energy of fluorescent radiation than UV.

Effect of Fluorescent Light on PV of Soybean Oil With Added Chlorophyll and Varying Amounts of β-carotene

The effect of fluorescent light on peroxide values of crude, refined, bleached, and bleached oil with added chlorophyll and different amounts of β-carotene was studied.

In the absence of light, as shown in Table 20, bleached oil containing chlorophyll developed the highest peroxide value at the end of 192 hr. Low peroxide values were formed in bleached oil alone. These values were relatively near to those found in crude oil. Except for refined oil, the same tendency of peroxide development as that in the controls was developed in the samples irradiated with fluorescent light. With fluorescent radiation, the difference in the rate of oxidation of bleached oil containing chlorophyll as compared to bleached oil alone (Figure 12) was greater than the difference of these oils irradiated with UV light. This probably occurred because chlorophyll, acting as a sensitizer, lasted for a longer period of time in the presence of fluorescent radiation than with UV radiation (Figure 5).

Table 20: Peroxide values in soybean oil after 192 hr.

Sample	PV (meq/kg oil)
B·	12
B· + 0.6 mg chl	41
B· + 0.6 mg chl + 1 mg β-carotene	31
B· + 0.6 mg chl + 5 mg β-carotene	34
B· + 0.6 mg chl + 10 mg β-carotene	26
Crude	7

Contrary to what happened in the presence of UV radiation (Figure 5), increasing the amount of β-carotene in samples containing chlorophyll and irradiating with fluorescent light decreased the rate of oxidation. The possibility that β-carotene could have performed more effectively as a $^1O_2$ quencher in the presence of fluorescent light may help to justify the lower rate of oxidation with this source of radiation.

All samples developed lower peroxide values throughout the 192 hr irradiation period with fluorescent light than with UV radiation (Figure 5). This was expected because it is well known from the work of Coe and LeClerc (1934) and Greenbank and Holm (1941) that light of shorter wavelength is more conducive to the formation of peroxides than at greater wavelength. The light emitted from fluorescent lamps, as explained before, is between 350 and 700 nm, with relatively greater power in the region of 500 to 650 nm (Allphin, 1973), whereas the UV light used in this work was in the region of 366 nm.

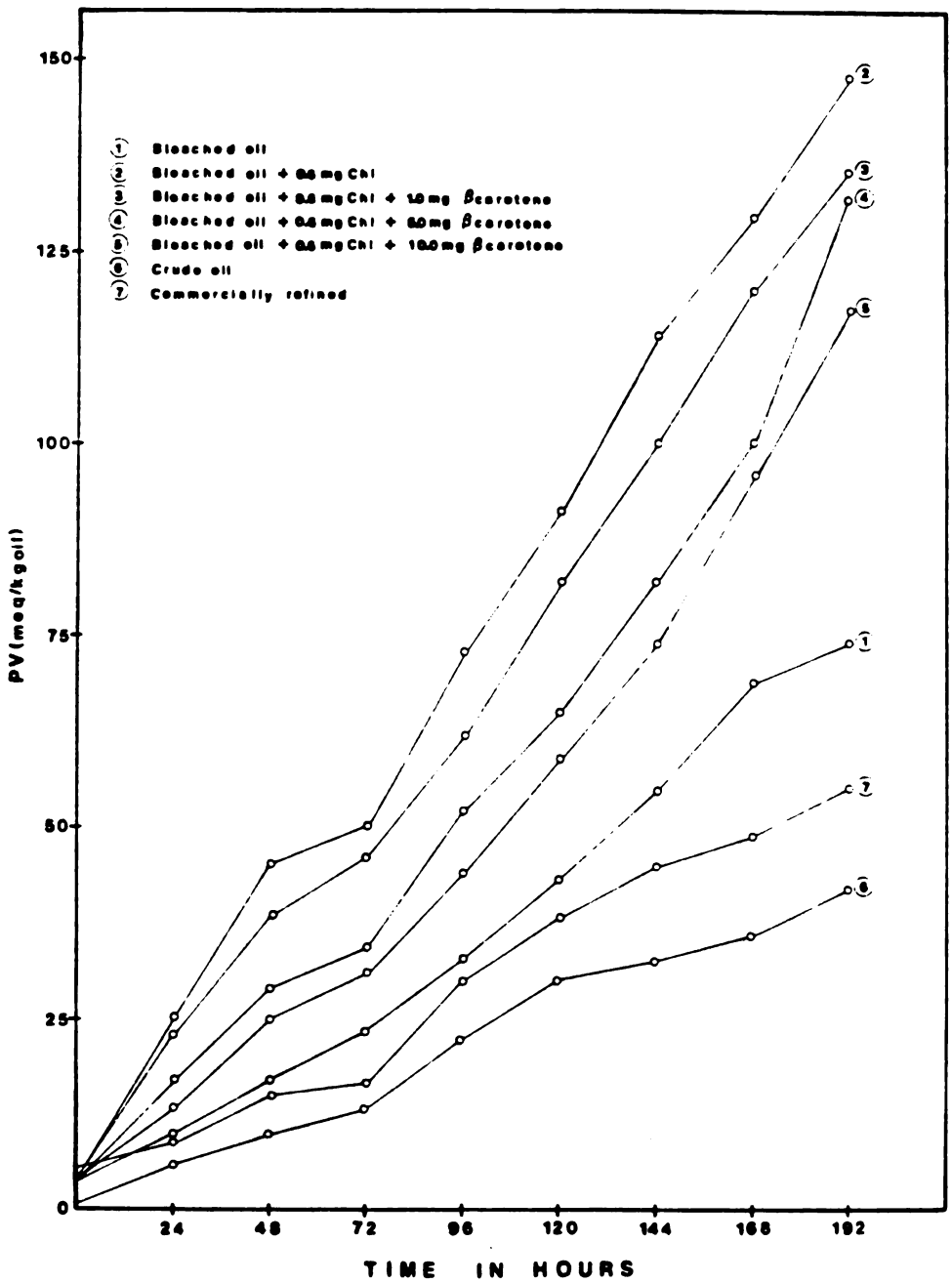


Fig. 12. Effect of fluorescent light on the peroxide value of soybean oil with added chlorophyll and varying amounts of β-carotene.

Effect of Fluorescent Light on PV of Soybean Oil With Added β-carotene, Chlorophyll, and α- and γ-tocopherols

The effect of fluorescent light on peroxide values was studied in crude, bleached, and bleached soybean oil with added β-carotene, chlorophyll, and α- and γ-tocopherols. In Table 21 are shown the peroxide values of the controls at the end of 192 hr. Alpha and γ-tocopherols or their combination were effective in preventing oxidation of bleached oil in the absence of light but were not effective in the presence of chlorophyll. Addition of carotene to the system containing chlorophyll and either α- or γ-tocopherols failed to inhibit the rate of oxidation in absence of light.

Table 21: Peroxide values of soybean oil with α-tocopherol after 192 hr.

Samples	PV (meq/kg oil)
B· + 1 mg α-tocopherol (0.004%)	6
B· + 1 mg γ-tocopherol (0.004%)	8
B· + 1 mg α-tocopherol + 1 mg γ-tocopherol	8
B· + 1 mg α-tocopherol + 0.6 mg chl	18
B· + 1 mg γ-tocopherol + 0.g mg chl	17
B· + 1 mg α-tocopherol + 1 mg γ-tocopherol + 0.6 mg chl	15
B· + 1 mg α-tocopherol + 1 mg β-carotene + 0.6 mg chl	21
B· + 1 mg γ-tocopherol + 1 mg β-carotene + 0.6 mg chl	16
B· + 1 mg α-tocopherol + 1 mg γ-tocopherol + 1 mg β-carotene + 0.6 mg chl	14
Crude oil	5

Figure 13 shows that α- and γ-tocopherols were very efficient in inhibiting oxidation of bleached oil in the presence of fluorescent light. Their combination was more effective than each one separately. The greater protection provided by α- and γ-tocopherol in the oxidation of bleached oil indicates, as in the case of β-carotene, that tocopherols are less affected by fluorescent light than by UV radiation. In the presence of fluorescent light, α-tocopherol was more effective than γ-tocopherol in inhibiting oxidation of bleached oil. Sherwin (1976) reported that the order of antioxidant activity of tocopherols is found to be $\delta > \gamma > \alpha$, but this order of antioxidant potency in vegetable oils may be influenced significantly by light conditions. It may be that fluorescent light radiation affects more the degradation of γ- than α-tocopherol. Alpha and γ-tocopherol, on the other hand, were unable to protect the oil from the oxidizing effect in the presence of chlorophyll.

Addition of β-carotene to the system containing bleached oil, α-, γ-tocopherol or their combinations, and chlorophyll was quite effective in decreasing the oxidation rate in the presence of light. These results suggest that either the addition of β-carotene enhanced the effect of tocopherols in inhibiting autoxidation of bleached oil, or tocopherols combined with β-carotene exhibited some quenching effect on singlet oxygen generated by chlorophyll in the presence of light.

The pattern of peroxide values in samples irradiated with fluorescent light was quite different from the pattern obtained in

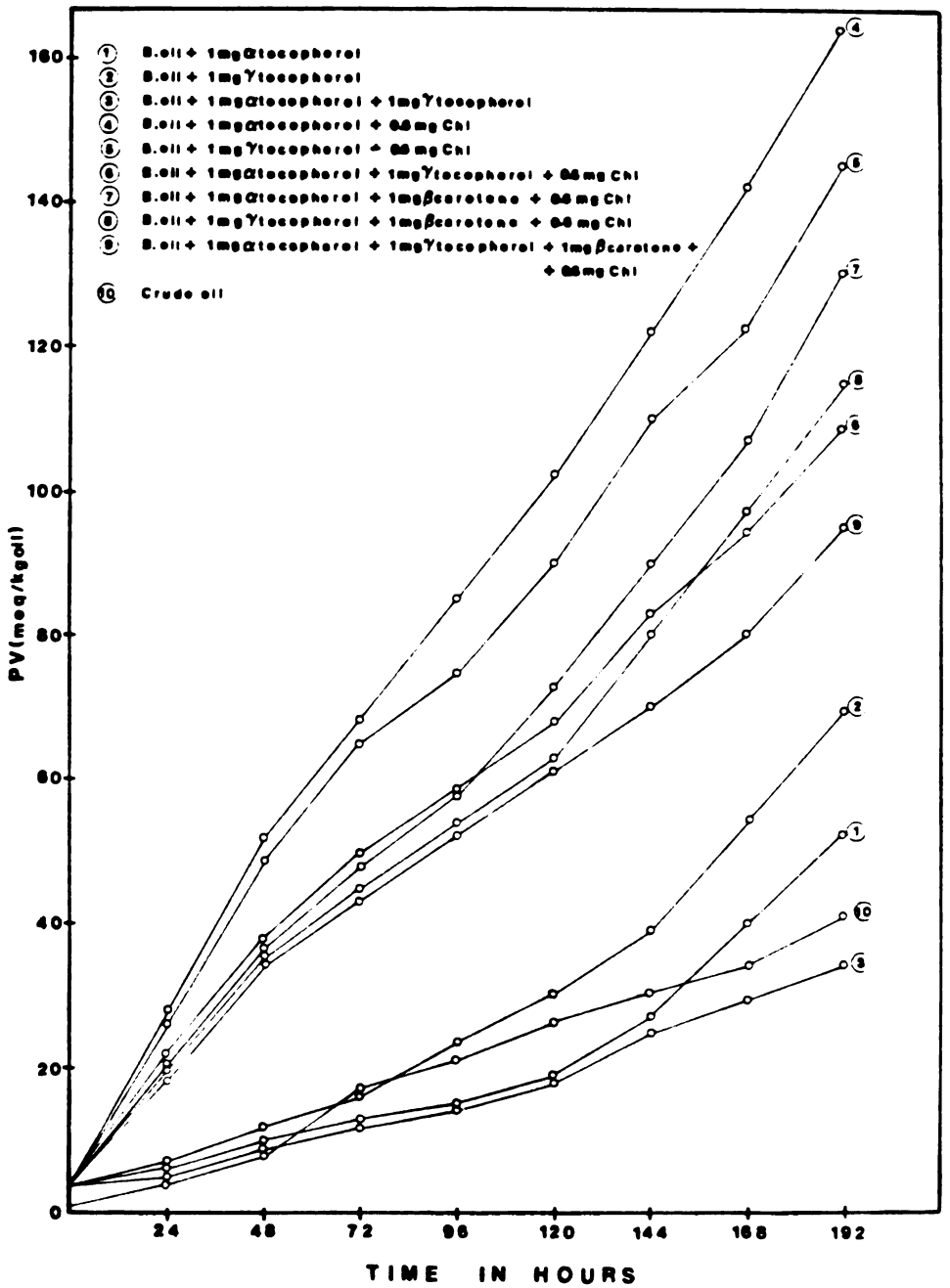


Fig. 13. Effect of fluorescent light on the peroxide value of soybean oil with added α- and γ-tocopherol, β-carotene, and chlorophyll.

the presence of UV radiation (Figure 7). In addition, lower peroxide values were obtained throughout the 192 hr irradiation with fluorescent light.

Effect of Fluorescent Light on the PV of Crude and Bleached Soybean Oil With Added TBHQ, BHA, β-carotene, Chlorophyll, and α- and γ-tocopherols

This study shows the effect of synthetic (TBHQ and BHA) as well as natural (α- and γ-tocopherols) antioxidants in the oxidation of bleached soybean oil in the presence and absence of fluorescent light radiation. Except for the radiation source, the conditions of the experiment were similar to those used when UV radiation was used. In Table 22 are shown the peroxide values of the controls after 192 hrs.

Table 22: Peroxide values of soybean oil controls with BHA and TBHQ after 192 hr.

Samples	PV (meq/kg oil)
B· + 1 mg TBHQ (0.004%)	6
B· + 1 mg BHA (0.004%)	8
B· + 1 mg TBHQ + 0.6 mg chl	11
B· + 1 mg BHA + 0.6 mg chl	26
B· + 1 mg TBHQ + 1 mg β-carotene + 0.6 mg chl	9
B· + 1 mg BHA + 1 mg β-carotene + 0.6 mg chl	22
B· + 1 mg TBHQ + 1 mg α-tocopherol + 0.6 mg chl	20
B· + 1 mg TBHQ + 1 mg γ-tocopherol + 0.6 mg chl	18
B· + 1 mg BHA + 1 mg α-tocopherol + 0.6 mg chl	24
Crude oil	5

In the absence of light with chlorophyll present, TBHQ was more efficient in preventing oxidation of bleached soybean oil than BHA. In the presence of fluorescent light radiation (Figure 14), TBHQ was very effective in protecting bleached soybean oil from oxidation. Its protection was more pronounced than that given by BHA. On the other hand, TBHQ offered little protection toward oxidation of bleached oil in the presence of chlorophyll. As was already mentioned, TBHQ is an effective antioxidant in systems containing polyunsaturated fatty acids. Its action here indicates that another type of oxidation mechanism besides autoxidation may be involved when chlorophyll is present.

The presence of β-carotene as well as α- and γ-tocopherols in the systems containing bleached oil with TBHQ and chlorophyll contributed in some degree to a decrease in the rate of oxidation. In systems differing only by the presence or absence of TBHQ or BHA, those containing TBHQ were even more effective in preventing oxidation than those with BHA. Compared to the effects found under UV irradiation (Figure 8), TBHQ was a more effective antioxidant in fluorescent light than with UV radiation. In all samples, lower peroxide values were developed throughout the 192 hr irradiation period with fluorescent light than those irradiated with UV radiation.

Effect of Fluorescent Light on the PV of Crude and Bleached Oil With Added Lecithin, Chlorophyll, β-carotene, α-tocopherol, and Vitamin E Mixture

This study shows the effect of lecithin and a vitamin E mixture on peroxide values of bleached soybean oil under fluorescent light

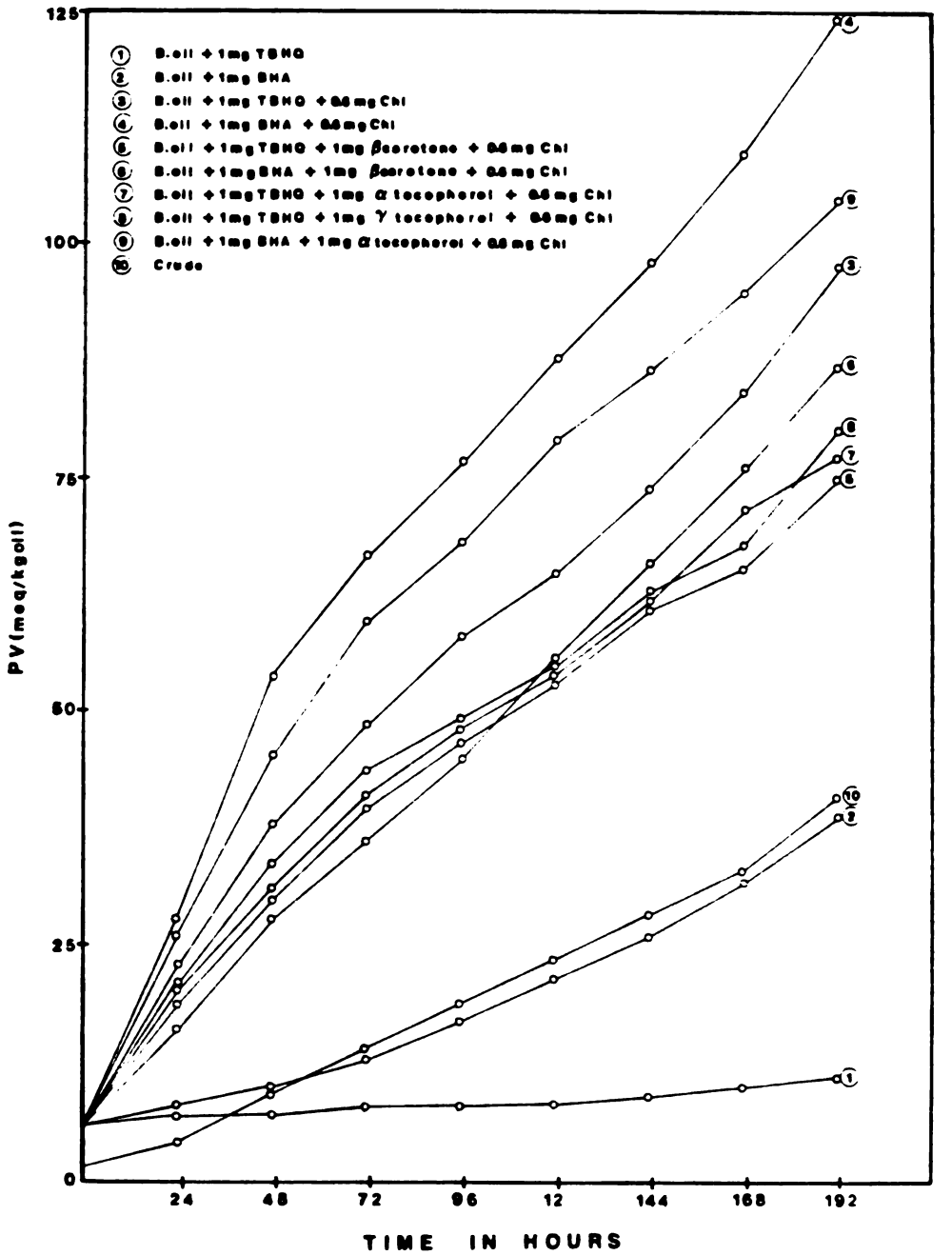


Fig. 14. Effect of fluorescent light on the peroxide value of soybean oil with added TBHQ, BHA, α- and γ-tocopherol, β-carotene, and chlorophyll.

with or without addition of chlorophyll, β-carotene, and α-tocopherol. The experimental conditions, except for the light source, were similar to those of the UV experiment. The vitamin E mixture (containing 50% of α-tocopherol and equal proportions of β-, γ-, and δ-tocopherols) in the presence of β-carotene, as well as lecithin combined with α-tocopherol, was very effective in preventing the development of peroxides in bleached oil (Figure 15). On the other hand, the oxidation was not inhibited in the presence of chlorophyll. The ineffectiveness of tocopherols in bleached oil containing chlorophyll may be an indication of the presence of a different oxidation mechanism, which in conjunction with autoxidation took place during irradiation. In all systems in which chlorophyll was present, high peroxide values were developed throughout the 120 hr irradiation period.

Matsushita (1979) reported that β-carotene combined with δ-tocopherol was effective in inhibiting oxidation of bleached oil in the presence of chlorophyll and light. The vitamin E mixture used in this experiment also contained δ-tocopherol, but there was no effective inhibition of soybean oil oxidation with the vitamin E mixture and β-carotene in the presence of chlorophyll. A possible explanation for the different results may be the different amounts of β-carotene and δ-tocopherol that were present in the systems.

The pattern of peroxide values in samples irradiated with fluorescent light was quite different from that in samples irradiated with UV radiation (Figure 9). In all cases, lower peroxide values were obtained throughout the 120 hr in the presence of fluorescent light.

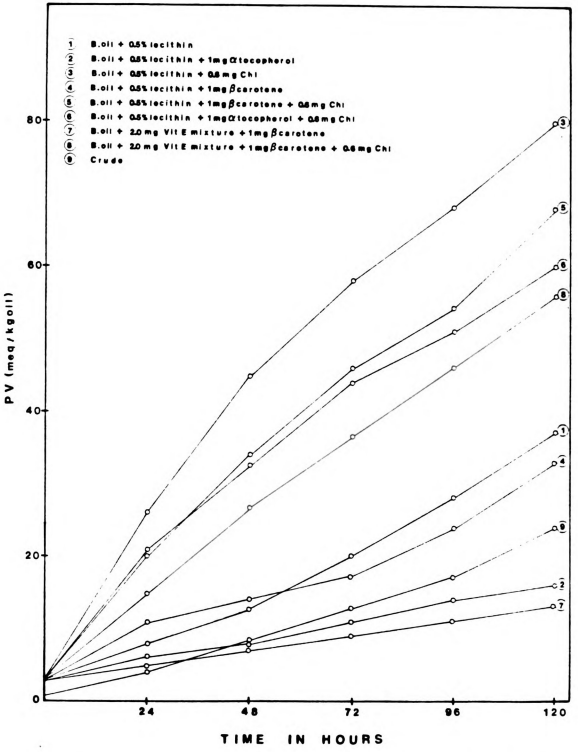


Fig. 15. Effect of fluorescent light on the peroxide value of crude and bleached soybean oil with added lecithin, α-tocopherol, vitamin E mixture, β-carotene, and chlorophyll.

Effect of Fluorescent Light on PV and Conjugated Diene in Crude, Refined, and Bleached Soybean Oil and Bleached Oil With Added Chlorophyll

The effect of fluorescent light on peroxide values and conjugated diene formation was studied in crude, refined, and bleached soybean oil with added chlorophyll *a* and *b* (40 g instead of 25 g of oil were used). The peroxide values and molar absorbances are presented in Tables 23 and 24, respectively.

Table 23: Peroxide values of soybean oils irradiated with fluorescent light.

Samples	PV (meq/kg)				
	0 hr	12 hr	24 hr	36 hr	48 hr
Crude	4	8	13	15	17
Refined	4	7	11	15	17
B·	3	7	10	17	21
B· + 4 ppm chl *a*	3	22	30	38	45
B· + 4 ppm chl *b*	3	23	30	37	46

Table 24: Molar absorbance of soybean oils irradiated with fluorescent light.

Samples	Molar Absorbance ($x10^{-3}$) at 233 nm				
	0 hr	12 hr	24 hr	36 hr	48 hr
Crude	1.91	2.43	2.63	2.89	3.17
Refined	2.22	2.49	2.73	3.00	3.31
B·	1.26	1.86	2.27	2.82	3.35
B· + 4 ppm chl *a*	1.21	2.90	3.48	4.36	4.96
B· + 4 ppm chl *b*	1.24	2.96	3.60	4.62	5.20

Bleached oil containing either chlorophyll a or b had higher peroxide values throughout a 48 hr irradiation than bleached oil alone (Table 23). The same happened with the molar absorbance of the oils containing either chlorophyll a or b (Table 24). The peroxide values and molar absorbances demonstrate that the effects of chlorophyll a and b are the same in oxidizing bleached soybean oil.

The ratio of molar absorbance/PV was plotted against time as shown in Figure 16. The bleached oils containing either chlorophyll a or b, as compared to crude, refined, and bleached oils, had at the first 12 hrs of irradiation a drop in molar absorbance/PV ratio more pronounced than in the others. The drop in molar absorbance/PV ratio was coincidentwith the disappearance of the green color caused by the degradation of chlorophyll.

If unconjugated and conjugated hydroperoxides are formed, lower values in the ratio of molar absorbance of conjugated diene/PV are expected than in those where only conjugated hydroperoxides are present. The lower values of molar absorbance/PV ratio found in bleached oil containing added chlorophyll a or b are an indication that singlet oxygen was involved in the photooxidation of bleached soybean oil in the early stages of irradiation-induced oxidation. Based on the fact that the drop in molar absorbance/PV ratio was pronounced in the early stages of irradiation, an experiment was conducted using 50 g of oil and analyzing the peroxide values, conjugated diene, and TBA test each 2 hr period for 10 hrs. The results are shown in Table 25.

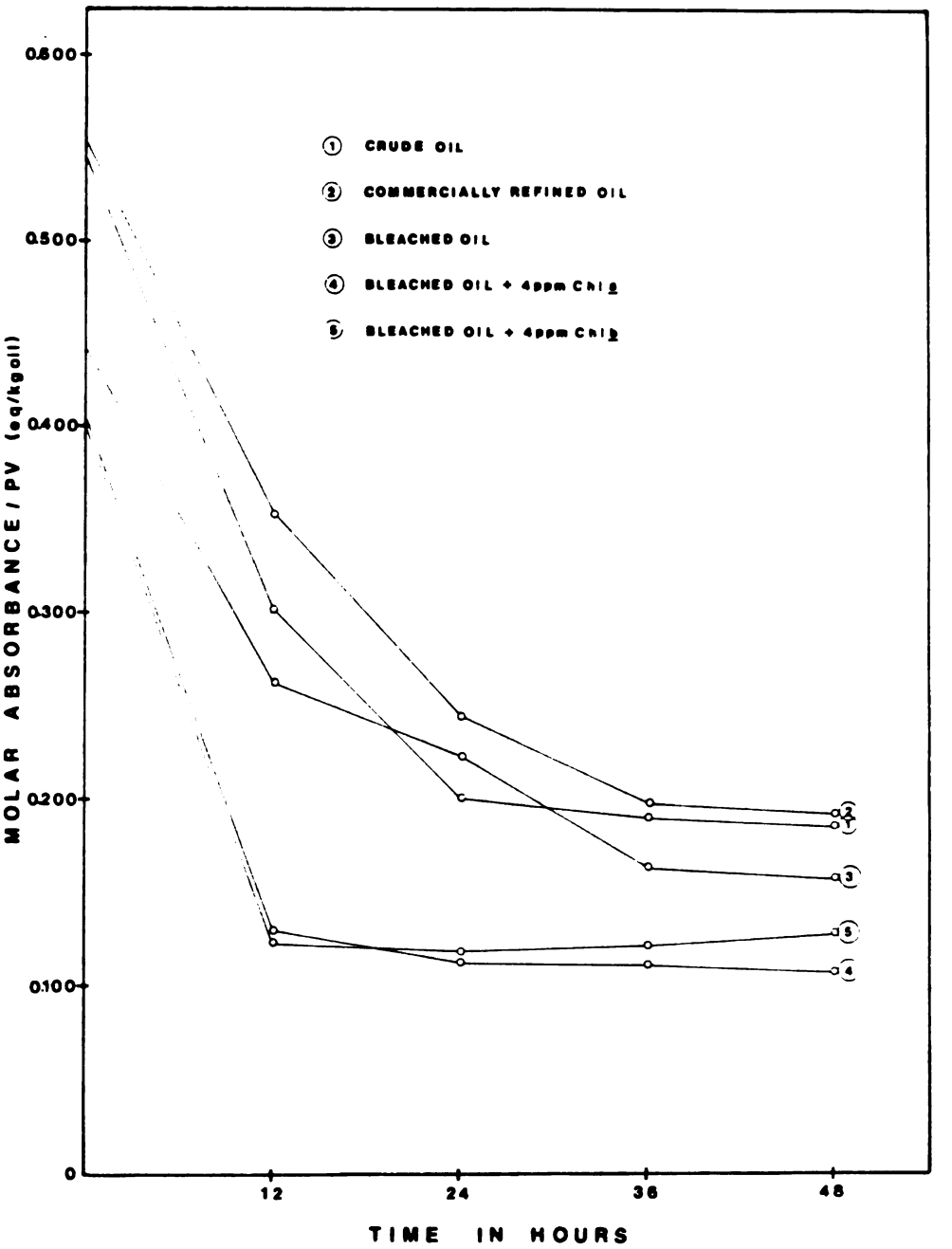


Fig. 16. Molar absorbance/PV ratio vs. time of soybean oils with added chlorophyll a and b.

Table 25: Molar absorbance, PV, and TBA of soybean oils irradiated with fluorescent light.

Hours	Bleached Oil			Bleached Oil, Added chl			Crude Oil		
	MA $x10^{-3}$[a]	PV[b]	TBA[c]	MA $x10^{-3}$	PV	TBA	MA $x10^{-3}$	PV	TBA
0	1.59	1.4	0.128	1.74	2.5	0.148	2.00	1.0	..
2	1.80	2.1	0.133	1.96	4.2	0.173	2.21	1.6	..
4	1.83	2.8	0.140	2.00	5.0	0.184	2.21	2.0	..
6	1.92	4.0	0.170	2.33	8.8	0.236	2.23	2.6	..
8	1.93	4.2	0.236	2.52	10.3	0.270	2.23	3.0	..
10	2.07	4.6	0.450	2.83	12.0	0.530	2.23	3.6	..

[a]Molar absorbance at 233 nm.

[b]Peroxide value in meq/kg oil.

[c]Expressed as absorbance.

The absorbance of the TBA values for crude oil is not presented in Table 25 because the presence of phosphatides in this oil prevented a clear separation between the organic and aqueous layer, which contained the products resulting from the TBA and malonaldehyde reaction.

Bleached oil containing chlorophyll produced higher values of peroxide and molar absorbance than crude and bleached oils throughout the irradiation period (Table 25). Also, the TBA values of bleached oil with chlorophyll were higher than from bleached oil alone. Figure 17 shows the effect of irradiation time on peroxide and TBA values. Dahle et al. (1962) reported that only peroxides that possessed double bonds β, γ to the peroxide group were capable of undergoing cyclization with the ultimate formation of malonaldehyde.

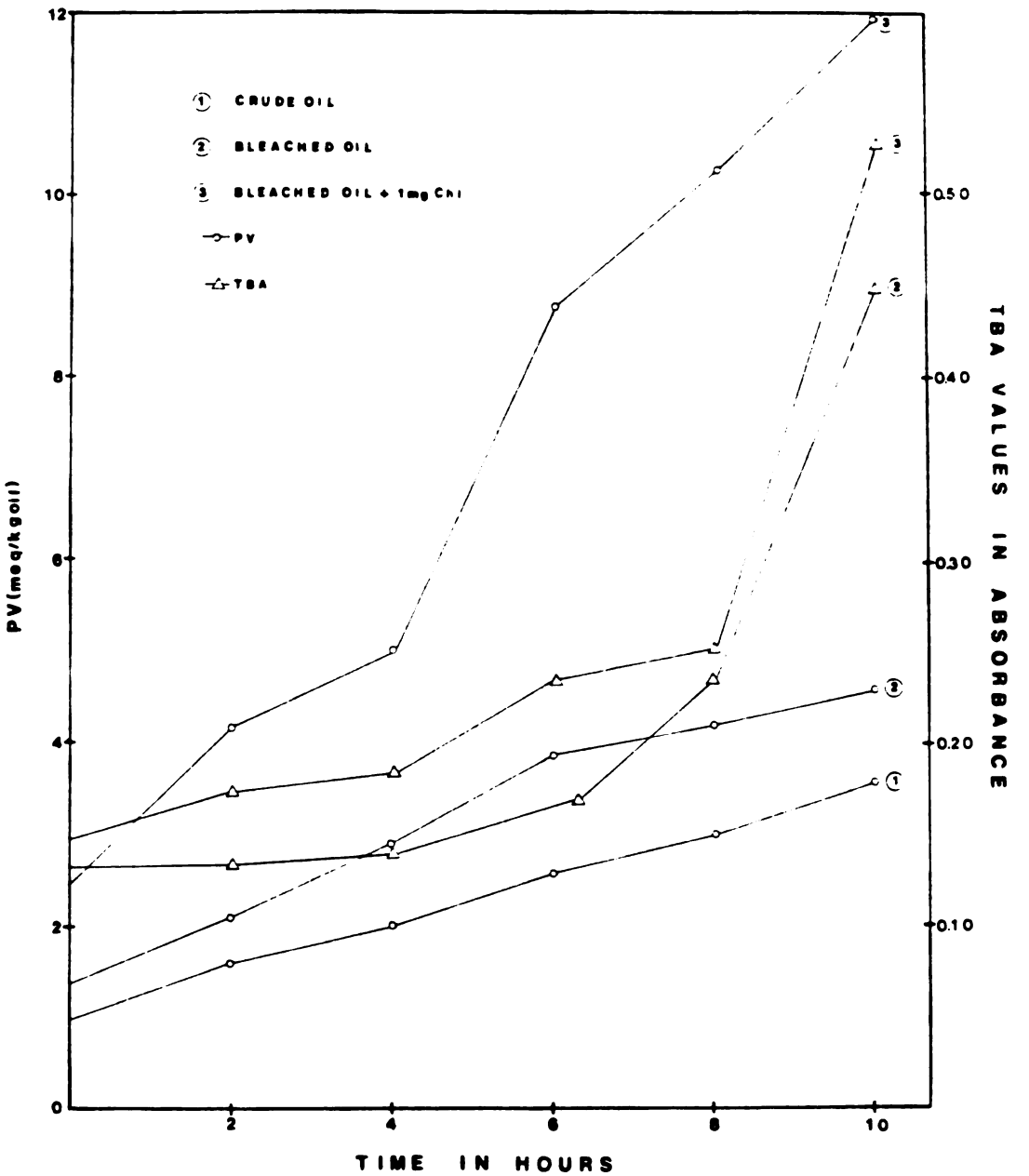


Fig. 17. Peroxide value and malonaldehyde production in soybean oil irradiated with fluorescent light.

Unconjugated hydroperoxides, which can be formed with linoleic and linolenic acid in the presence of singlet oxygen, may lead to the formation of β, γ systems. Although the absorbance by TBA products of the bleached oil containing chlorophyll was higher than that of the bleached oil, how much this difference accounts for the participation of singlet oxygen is not clear. More studies attempting to correlate the TBA value and singlet oxygen participation in oxidation reactions of soybean oil are required.

In Figure 18 is shown the molar absorbance/PV ratio during a 10 hr irradiation period. The values of the molar absorbance/PV ratio at the beginning of irradiation were 2.0, 1.59, and 0.69, respectively, for crude, bleached, and bleached oil with added chlorophyll. It is interesting to note that although the bleached oil to which chlorophyll was added came from the same lot as the bleached oil, the peroxide value of the bleached oil with chlorophyll was higher in the bleached oil alone (Table 25). This difference in peroxides in the oil containing chlorophyll was probably caused by the light from the laboratory environment during the time required to run the peroxide values (about 20 min). This suggested that chlorophyll sensitized the oxidation reaction of the oil at a very early stage of exposure to light or other radiation energy. The value 0.69 of the molar absorbance/PV ratio obtained with bleached oil in the presence of chlorophyll was the same as reported by Chan (1977) in his study of photosensitized oxidation of methyl linolenate using erythrosine as a sensitizer. This result, as shown in Figure 18,

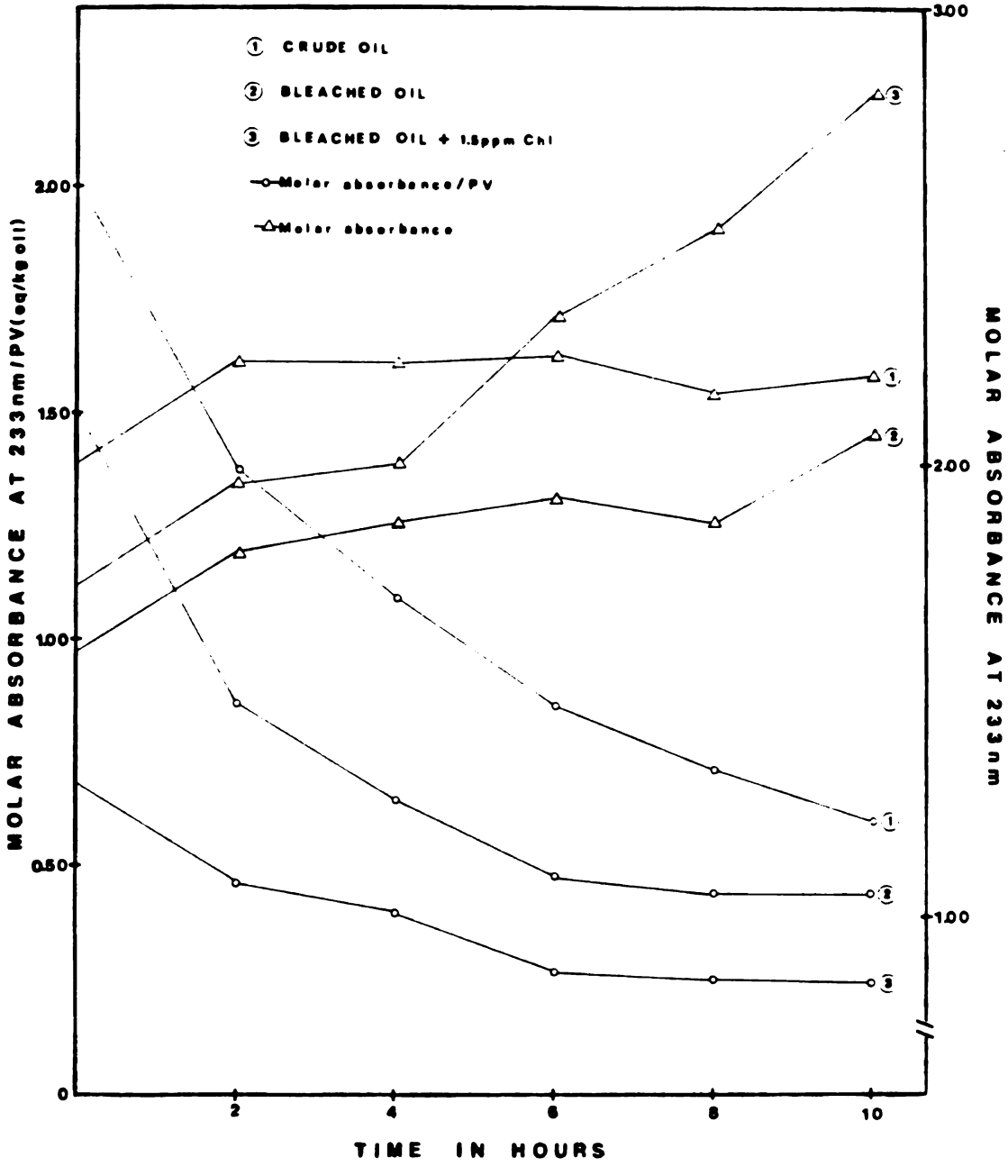


Fig. 18. Molar absorbance and molar absorbance/PV ratio vs. time of soybean oils irradiated with fluorescent light.

reinforces the concept that singlet oxygen participated in the oxidation of bleached oil containing chlorophyll.

Irradiation of the Oils With Incandescent Light

A floodlight (150 w) as an irradiation source of incandescent light was also used in this study. This radiation source was chosen because most of the radiant energy of incandescent light is concentrated in the region of 650 to 1,500 nm (Alphin, 1973). Thus the effect on the pattern of oxidation of soybean oil of wavelengths longer than those of UV and fluorescent light could be studied. Incandescent lamps have long been one of the most widely used artificial light sources. Because fluorescent lamps produce much less radiant heat per lumen than incandescent light (Sattar & Deman, 1975), fluorescent light has replaced incandescent lamps in stores and supermarkets. Incandescent light (100-150 w floodlight type) has been widely used in certain regions as a heat source, to warm fried food displayed on a glass shelf.

Effect of Incandescent Light on the PV of Crude, Refined, Bleached Soybean Oil, and Bleached Oil With Added Chlorophyll and β-carotene

The effect of β-carotene at concentrations of 1, 5, and 10 mg (this corresponds to 0.004, 0.02, and 0.04% w/w in the oil sample) in the presence of 0.6 mg of chlorophyll (this corresponds to 0.0024% w/w or 24 ppm in the oil sample) was studied in bleached soybean oil. Samples of crude, refined, bleached, and bleached oil with 0.6 ppm of chlorophyll were also used at the same time and under the same

conditions. Similar conditions (except for the light source and period of irradiation) were used with UV and fluorescent light.

The peroxide values of the control samples after 84 hr are presented in Table 26. As in previous experiments (Tables 12 and 20), β-carotene had no inhibiting effect on the oxidation of aluminum-foil-covered samples that received no light.

Table 26: Peroxide values of soybean oil after 84 hr under incandescent light.

Samples	PV (meq/kg oil)
B·	12
B· + 0.6 mg chl	24
B· + 0.6 mg chl + 1 mg β-carotene	28
B· + 0.6 mg chl + 5 mg β-carotene	28
B· + 0.6 mg chl + 10 mg β-carotene	28
Crude	6
Refined	10

In the presence of incandescent radiation (Figure 19), as in the UV experiment (Figure 5), bleached oil containing chlorophyll developed high peroxide values but lower than those containing chlorophyll and β-carotene. The high peroxide values in the presence of greater amounts of β-carotene indicate that incandescent light also affected the β-carotene. The destruction of β-carotene may produce oxidation products that would be expected to catalyze free radical oxidation.

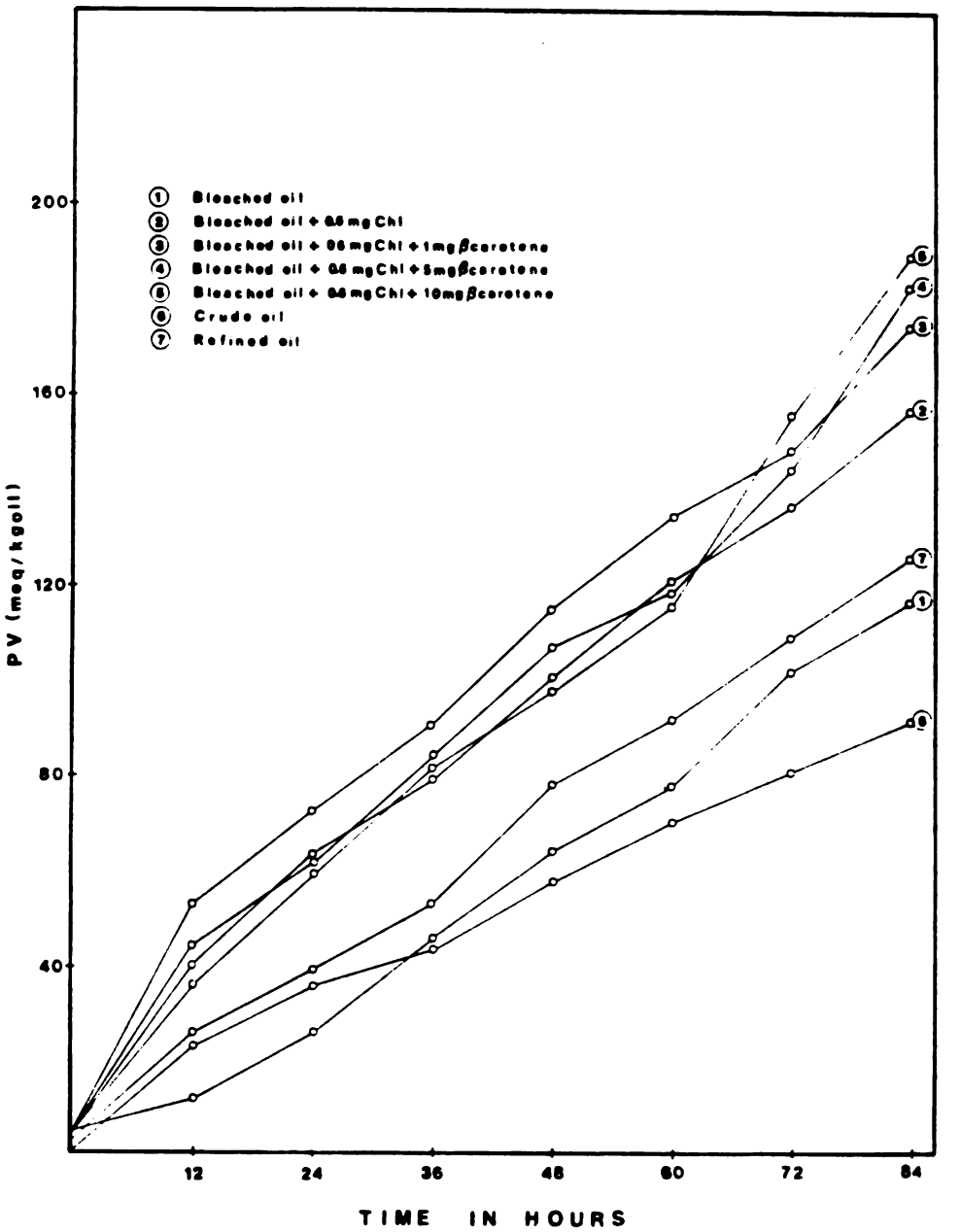


Fig. 19. Effect of incandescent light irradiation on the peroxide value of soybean oil with added β-carotene and chlorophyll.

Similar reasons can be given to explain the failure of β-carotene to inhibit oxidation of bleached oil in the presence of chlorophyll.

To compare the effect of incandescent light to that of systems irradiated with UV and fluorescent light is difficult because different amounts of radiation energy were produced by each source. The amounts of radiation energy for UV, fluorescent, and floodlight sources were 0.08, 11.4, and 177 W/m^2, respectively. Comparatively, the amount of radiant energy produced by incandescent light was 15.5 and 2528 times greater than fluorescent and UV light, respectively.

To exemplify the effect of the same radiant energy with different light sources, let us take the peroxide values of bleached oil at the end of the same time of irradiation (72 hr) with UV, fluorescent, and incandescent light. The peroxide values obtained were 71, 23, and 102, respectively, with UV, fluorescent, and incandescent light (Figures 5, 12, and 19). If the energy of 1 W/m^2 had been used in all radiation sources, these peroxide values, by a proportional calculation, would be 887, 2, and 0.6 for UV, fluorescent, and incandescent light sources, respectively, at the end of the 72 hr irradiation period. These results are in agreement with the works of Coe (1941), Greenbank and Holm (1941), and Sattar (1976a), who indicated that greater wavelengths of light energy promote oxidation the least, and that the most deleterious wavelengths are those shorter than 450 nm.

Effect of Incandescent Light on the PV of Crude and Bleached Soybean Oil With Added α- and γ-tocopherols, β-carotene, and Chlorophyll

Except for irradiation source and period of irradiation, the conditions of this experiment were similar to those previously described for UV and fluorescent light. The peroxide values of the controls after 96 hr are shown in Table 27.

Table 27: Peroxide values of soybean oil with added tocopherols after 96 hr under incandescent light.

Samples	PV (meq/kg oil)
B· + 1 mg α-tocopherol (0.004%)	5
B· + 1 mg γ-tocopherol (0.004%)	6
B· + 1 mg α- + 1 mg γ-tocopherol	5
B· + 1 mg α-tocopherol + 0.6 mg chl	20
B· + 1 mg γ-tocopherol + 0.6 mg chl	17
B· + 1 mg α- + 1 mg γ-tocopherol + 0.6 mg chl	17
B· + 1 mg α-tocopherol + 1 mg β-carotene + 0.6 mg chl	15
B· + 1 mg γ-tocopherol + 1 mg β-carotene + 0.6 mg chl	22
B· + 1 mg α- + 1 mg γ-tocopherol + 1 mg β-carotene + 0.6 mg chl	17
Crude	6

As was expected, the development of peroxides in the controls followed a pattern similar to those presented in the UV and fluorescent light experiments. Either α- or γ-tocopherols or their combination were effective in inhibiting the oxidation of bleached oil in the absence of light. Systems containing chlorophyll developed

higher peroxides than those in which chlorophyll was absent. In the presence of light, as shown in Figure 20, the bleached oil system containing α- and γ-tocopherol developed the lowest peroxide values among all the samples. Alpha and γ-tocopherol failed to inhibit the oxidation of the bleached oil when chlorophyll was present. Also, β-carotene in the presence of tocopherols and chlorophyll failed to inhibit the oxidation of bleached oil. Combinations of α- or γ-tocopherols or both with β-carotene did not inhibit oxidation in the presence of chlorophyll.

This experiment, like the others, showed that crude oil was quite stable as indicated by the development of peroxides. Sensitization by chlorophyll may be responsible for the higher peroxide values developed in the samples containing this pigment.

Effect of Incandescent Light on the PV of Crude and Bleached Soybean Oil With Added TBHQ, BHA, α- and γ-tocopherol, β-carotene, and Chlorophyll

Except for radiation source and time of irradiation, this experiment was similar to those described previously with UV and fluorescent light.

The peroxide values of the controls after 96 hr are presented in Table 28. As expected, the peroxide values of the controls were similar to those developed in other experiments in the absence of light.

In the presence of incandescent light (Figure 21), TBHQ proved to be effective in inhibiting photooxidation of bleached oil in the absence of chlorophyll. Also, TBHQ was more effective than BHA in

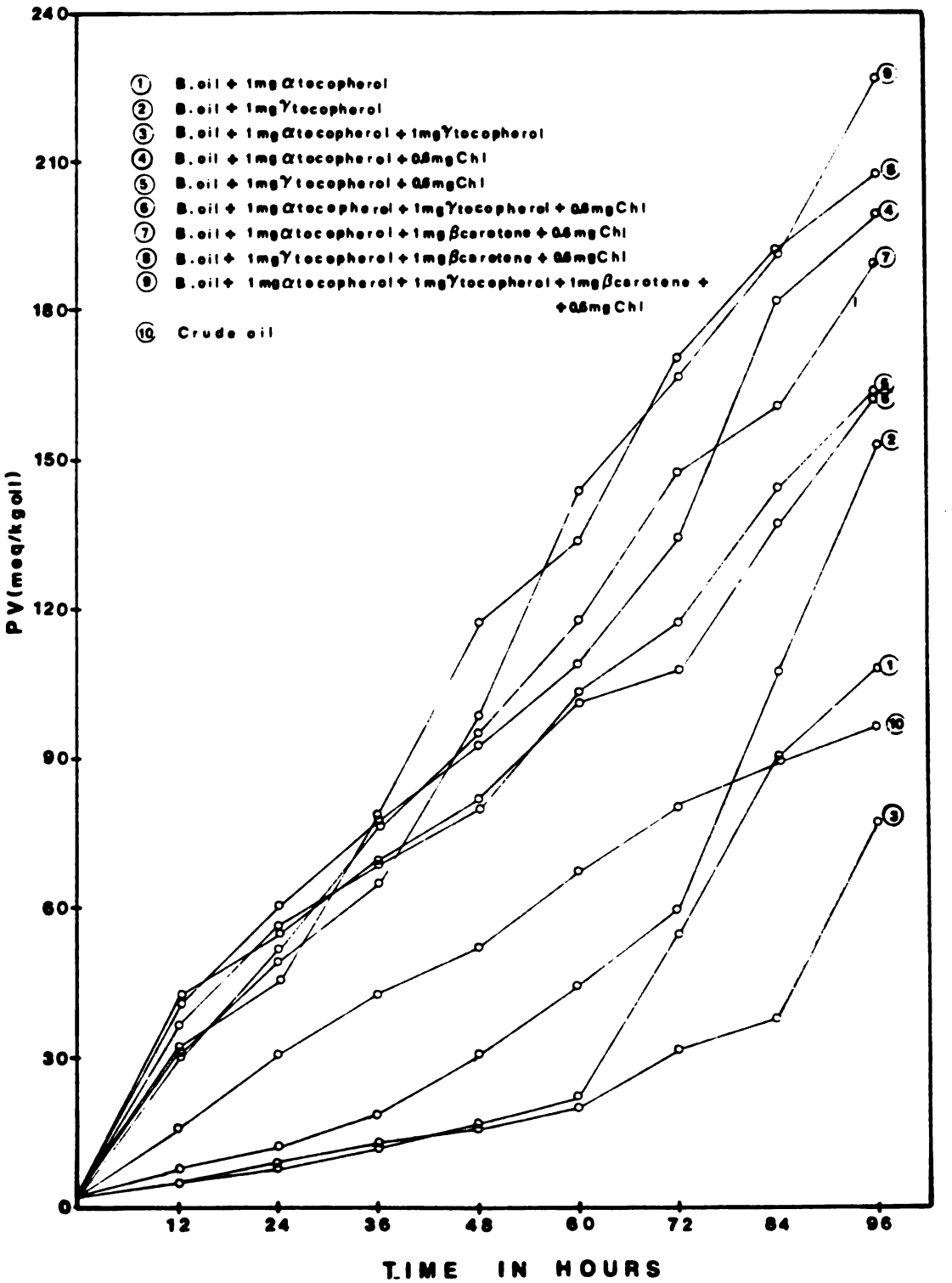


Fig. 20. Effect of incandescent light on the peroxide value of soybean oil with added α- and γ-tocopherols, β-carotene, and chlorophyll.

the absence of chlorophyll. In the presence of chlorophyll, contrary to what happened in the fluorescent light experiment, BHA was more effective than TBHQ.

Table 28: Peroxide values of soybean oil with added antioxidants after 96 hr under incandescent light.

Samples	PV (meq/kg oil)
B· + 1 mg TBHQ (0.004%)	4
B· + 1 mg BHA (0.004%)	5
B· + 1 mg TBHQ + 0.6 mg chl	11
B· + 1 mg BHA + 0.6 mg chl	20
B· + 1 mg TBHQ + 1 mg β-carotene + 0.6 mg chl	12
B· + 1 mg BHA + 1 mg β-carotene + 0.6 mg chl	14
B· + 1 mg TBHQ + 1 mg α-tocopherol + 0.6 mg chl	13
B· + 1 mg TBHQ + 1 mg γ-tocopherol + 0.6 mg chl	21
B· + 1 mg BHA + 1 mg α-tocopherol + 0.6 mg chl	21
Crude oil	7

Sherwin (1976) reported that the steric hindrance of BHA serves to protect the active hydroxyl group under some conditions. Although it is not a particularly effective antioxidant in vegetable oils, BHA is commonly used in combination with other antioxidants in order to take advantage of the synergistic effect. BHA is also reported (Dugan & Kraybill, 1956) as having a carry-through activity. It is possible that these properties of BHA were the reasons for the higher protection of BHA than that of TBHQ in bleached oil with chlorophyll and in the presence of incandescent radiation.

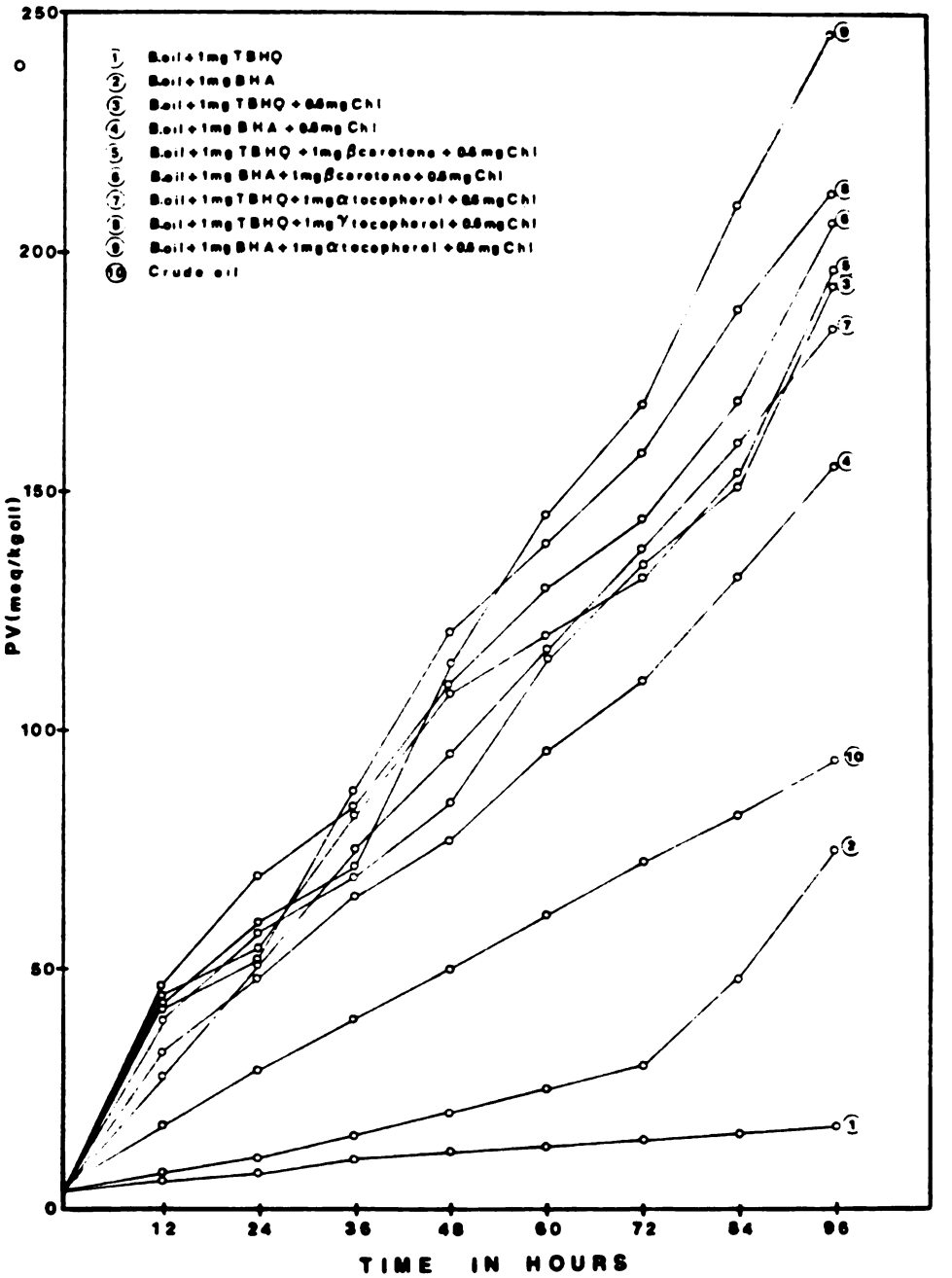


Fig. 21. Effect of incandescent light radiation on the peroxide value of soybean oil with added TBHQ, BHA, α- and γ-tocopherols, β-carotene, and chlorophyll.

In the presence of chlorophyll and under incandescent light, β-carotene and/or α- and γ-tocopherol failed to inhibit the oxidation of bleached oil. This may indicate either that β-carotene was degraded or was inefficient as an $^1O_2$ quencher. The same could have happened with α- and γ-tocopherols.

Effect of Incandescent Light on the PV of Crude and Bleached Soybean Oil With Added Lecithin, α-tocopherol, Vitamin E Mixture, β-carotene, and Chlorophyll

The conditions of this experiment, except for the irradiation source and time of exposure, were similar to those with UV and fluorescent light.

The systems containing either the vitamin E mixture and β-carotene or lecithin and α-tocopherol, as shown in Figure 22, were effective in inhibiting oxidation of bleached soybean oil. Apparently the retardation of peroxide formation in those systems was caused by the presence of tocopherols only. It seems that the addition of lecithin did not alter the pattern of oxidation of bleached oil. Although a sample containing only bleached oil was not run at the same time in this experiment, the peroxide values of bleached oil containing lecithin (Figure 22) showed that lecithin in combination with other components was quite effective in reducing the rate of peroxide value formation.

All systems containing chlorophyll, except for that one in which the vitamin E mixture was present, developed high peroxide values. The inhibitory effect (not very high) of the vitamin E mixture and carotene in the presence of chlorophyll is in agreement with Matsushita

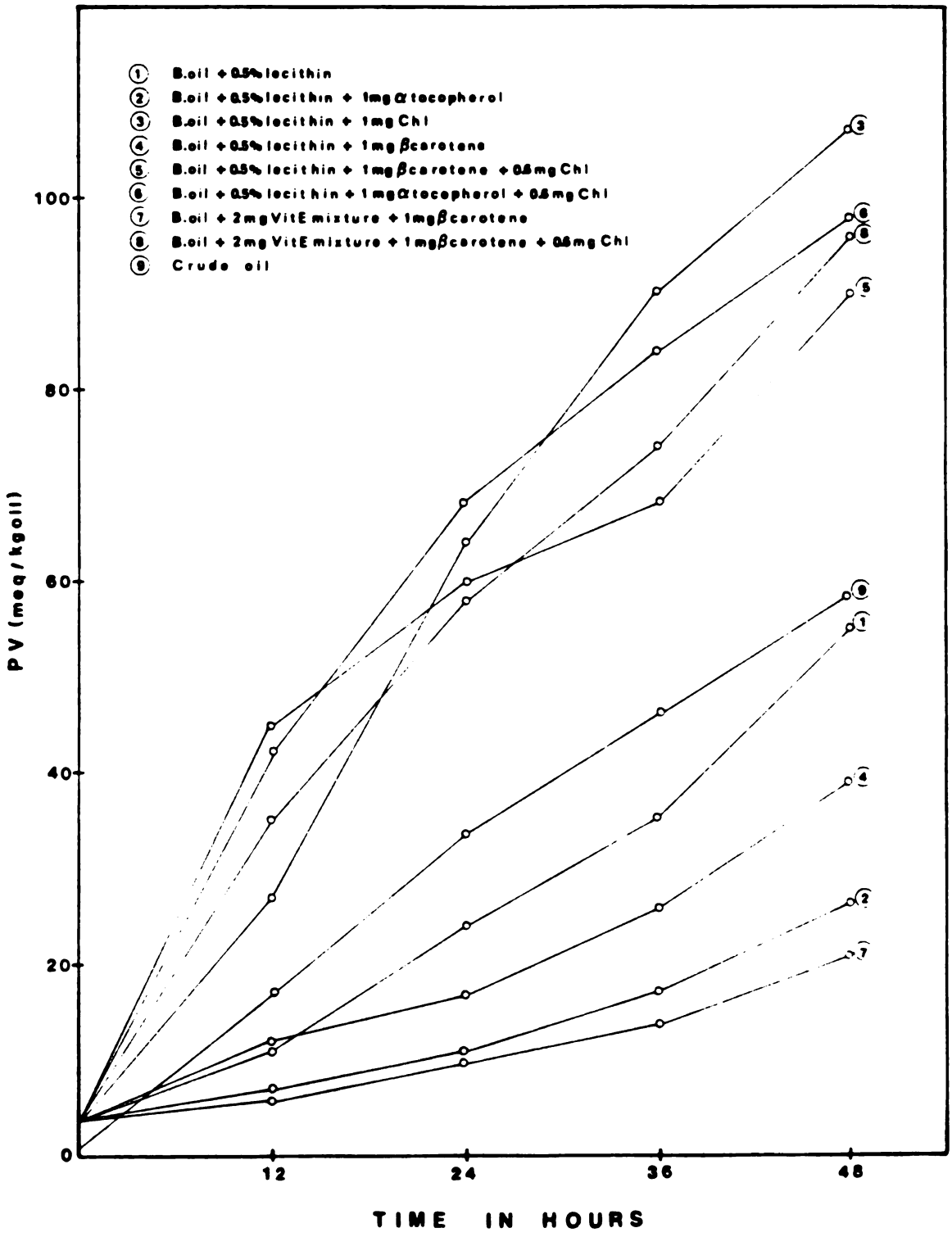


Fig. 22. Effect of incandescent light on the peroxide value of soybean oil with added lecithin, α-tocopherol, vitamin E mixture, β-carotene, and chlorophyll.

(1979), who reported that β-carotene can act as an inhibitor of photo-oxidative deterioration of vegetable oils in the presence especially of δ-tocopherol. The low peroxide values presented by the sample containing vitamin E mixture and β-carotene indicate that vitamin E mixture (tocopherols) protected carotene from being degraded. Since β-carotene was not degraded and if it could be acting as a quencher of $^1O_2$, lower values of peroxide would be expected in the systems containing β-carotene and vitamin E mixture in the presence of chlorophyll. It seems that the products of the degradation of chlorophyll by light have a greater effect on the oxidation than the $^1O_2$ that would be generated in the presence of this pigment.

General Discussion

This study was designed to investigate the photooxidation of soybean oil including the effect of UV, fluorescent, and incandescent light radiations on crude, commercially refined, bleached and bleached soybean oil with added chlorophyll, β-carotene, and natural or synthetic antioxidants.

Several workers (Coe, 1941; Greenbank & Holm, 1941; Sattar, 1976a) have reported that different wavelengths of radiant energy can have more or less pronounced effects on photooxidation of fats, oils, and fatty acids. Rawls and Van Santen (1970) suggested that chlorophyll may act as a photosensitizer generating singlet oxygen by transfer of excitation energy. In contrast, β-carotene may inhibit the process of photooxidation in the presence of chlorophyll (Foote & Denny, 1968; Koka & Song, 1978; Matsushita, 1979). On the other hand,

tocopherols as well as TBHQ and BHA are effective as antioxidants in the autoxidation of fats and oils (Daubert, 1950; Sherwin, 1976; Sherwin & Thompson, 1967). Crude and refined soybean oil contain small amounts of chlorophyll and β-carotene. The presence of these pigments in soybean oil may alter the pattern of oxidation of this oil in the presence of UV, fluorescent, or incandescent light energy.

To avoid the interference of other substances that occur naturally in soybean oil, the effect of adding chlorophyll, carotene, and antioxidants was studied in refined and bleached soybean oil. An examination of the β-carotene and chlorophyll content as well as the percentage of transmittance at different wavelengths showed that the pigments were efficiently removed from soybean oil by the refining and bleaching process used. The similarity in fatty acid composition among crude, commercially refined, and bleached soybean oil indicated that a difference in unsaturation was not a factor that would influence their pattern of oxidation.

Photooxidation studies with UV radiation in crude, commercially refined, bleached, and bleached oil with added chlorophyll a and/or β-carotene showed the following pattern of oxidation as measured by peroxide values: crude and refined oil developed lower peroxide values than bleached oil, whereas the crude oil developed the lowest peroxide values among the samples.

As compared to bleached oil alone, addition of 0.5 ppm of chlorophyll did not increase the rate of oxidation appreciably. Greater increases in peroxide values were obtained with additions of 1.0 ppm of chlorophyll. This indicated that the photooxidation-promoting

effect of chlorophyll in soybean oil is related to its concentration and that small amounts of chlorophyll, such as those that can be present in refined soybean oil, do not appear to be an important contributor to the photosensitized oxidation of this oil. The process by which chlorophyll acts as a sensitizer to initiate photooxidation of vegetable oil seems to be important only when amounts of chlorophyll greater than those remaining after processing are present.

Beta-carotene at concentrations of 1, 5, and 10 ppm did not inhibit the rate of oxidation of bleached soybean oil and, in fact, the higher concentrations of β-carotene promoted the development of higher peroxide values. These results confirmed the finding of Heftman (1947), who reported that pure carotene at concentrations higher than 3 mg% accelerated the rate of oxidation of methyl esters obtained from olive oil. These also agree with Thompson and Steenback (1944), who found β-carotene to be an active pro-oxidant. On the other hand, these disagree with McConnell and Esselen (1947), who reported that β-carotene retarded the development of rancidity at concentrations higher than 0.0013%.

The fact that β-carotene itself is oxidized by photooxidation to produce oxidation products (Seely & Meyer, 1971) that may produce free radicals which enhance the rate of oxidation may explain the higher rate of photooxidation of bleached oil in the presence of higher concentrations of β-carotene, unless the production of $^1O_2$ requires the presence of a sensitizer.

Analyses of chlorophyll content also demonstrated that this pigment was destroyed during the irradiation period, justifying the

low initial rate of oxidation in the presence of small amounts of chlorophyll. Analysis of the samples shielded from radiation by aluminum foil demonstrated that chlorophyll and, to a lesser degree, β-carotene were also destroyed in the absence of UV radiation.

Since chlorophyll a and b were found to be present in soybean oil, these were compared in similar experiments. It was found that both chlorophylls had a similar behavior toward UV-induced photo-oxidation of bleached oil, including their effects in the presence of β-carotene.

Except for the lower peroxide values developed throughout the irradiation period by bleached oil containing chlorophyll and/or β-carotene under fluorescent and incandescent radiation, the behavior of this oil in the presence of these sources of radiation was similar in effects to those developed in the presence of UV radiation.

Bleached oil developed higher peroxide values than crude and refined oil. Because of the removal of chlorophyll and β-carotene, lower peroxide values would be expected to be found in bleached oil than in crude and refined oil, which contained measurable quantities of chlorophyll. These results suggested that tocopherols, the natural antioxidants of vegetable oils, could have been removed from the oil during the bleaching operation. A study was initiated to measure the quantity of α- and γ-tocopherols. An HPLC analysis revealed that the tocopherol profile of bleached oil as compared to crude and refined oil was greatly affected by the bleaching process. This result is consistent with the report by Matsushita (1979) that decolorization of soybean oil increased the stability against photo-irradiation by

eliminating chromophoric impurities but that decolorized oil is more susceptible to autoxidation because tocopherols are eliminated.

The studies with α- and γ-tocopherols under UV radiation demonstrated that the tocopherols were ineffective in inhibiting the photooxidation of bleached soybean oil. This noninhibiting effect could be attributed to the degradation of tocopherols under UV radiation, which provided a means for the formation of free radicals that enhanced the overall oxidation. On the other hand, the protection given by α- and γ-tocopherols to the bleached oil against photooxidation by fluorescent and incandescent radiation indicated that these radiation sources did not degrade these tocopherols, or if they did, it was in lesser degree than by UV radiation.

Experiments with a vitamin E mixture that contained the four tocopherols (α-, β-, γ-, and δ-) demonstrated that this mixture was more effective in inhibiting the photooxidation of bleached soybean oil in the presence of β-carotene than α- and γ-tocopherol or their combinations. Also, the vitamin E mixture with carotene in the presence of chlorophyll had some protective effect on the photooxidation of bleached oil with the three radiation sources. These results seem to support the finding of Matsushita (1979) that δ-tocopherol has a greater inhibitory effect on $^1O_2$-initiated photooxidation among the tocopherols. These experiments demonstrated that the complete inhibition of photooxidative deterioration of soybean oil cannot be achieved when a sensitizer is present.

Because TBHQ is known to have a strong antioxidant capacity for polyunsaturated oil (Sherwin & Thompson, 1967) and BHA is a

synergistic antioxidant with carry-through properties (Dugan & Kraybill, 1956), these were chosen for study in the photooxidation of soybean oil. TBHQ was very effective in inhibiting photooxidation of bleached soybean oil in those experiments irradiated with fluorescent and incandescent light. In the absence of chlorophyll, TBHQ was superior to BHA in protecting bleached oil from oxidation. However, TBHQ was ineffective in inhibiting photooxidation of bleached oil in the presence of chlorophyll. The autoxidation of the unsaturated fatty acid components of lipids proceeds by a free radical mechanism in which oxygen in the triplet state is added to form conjugated hydroperoxides, which are the main products of autoxidation. During the last few years there has been an accumulation of new knowledge to indicate the true nature and interaction of autoxidation and photooxidation processes. It is known that the photosensitized oxidation route provides a means for the formation of hydroperoxides without the necessity for a free radical mechanism. Oxygen in the singlet state generated by a photosensitized reaction can react directly with the double bonds of unsaturated fatty acids to produce hydroperoxides.

Because tocopherols and TBHQ in these experiments were effective in inhibiting photooxidation of soybean oil in the absence of a sensitizer and did not inhibit the photooxidation in the presence of chlorophyll, it may indicate that an oxidation mechanism different from autoxidation was involved in the presence of chlorophyll.

Studies of the ratio of molar absorbance of conjugated diene/PV in the crude, refined, bleached, and bleached oil with added

chlorophyll a or b suggested that $^1O_2$ was involved in the photooxidation of bleached oil in the early stages of irradiation. This result reinforced the observation that tocopherols and TBHQ did not inhibit the photooxidation of bleached oil in the presence of chlorophyll because another oxidation mechanism, besides autoxidation, took place at the same time, when a sensitizer was present.

Rawls and Van Santen (1970) reported that $^1O_2$ promoted the oxidation of methyl linoleate 1450 times faster than oxygen in the triplet state. The results of these experiments indicated that although chlorophyll at concentrations normally present in crude and refined soybean oil could have participated as a photosensitizer, at an early stage, to generate $^1O_2$, the oxidation did not proceed more rapidly than by autoxidation. This probably occurred because the components responsible for singlet oxygen formation were degraded and changed so that these no longer contributed to the process. The rate, then, would depend on the decomposition of hydroperoxides already accumulated in the system to provide radicals for continuing the oxidation process.

The results of phosphorus analysis indicated that phosphatides were absent in commercially refined and laboratory bleached soybean oil. Addition of lecithin apparently did not alter the photooxidation pattern of bleached oil with the three radiation sources.

Olcott and Van Veen (1963) reported that lecithin and cephalin are inactive as antioxidants for menhaden oil, but when present with ethoxyquin are very effective synergists. A similar synergistic effect could be the reason why lecithin in the presence of tocopherols

inhibited the photooxidation of bleached oil. It is possible that phosphatides that are present in crude soybean oil may play some synergistic effect with some of the nonglyceride constituents of this oil. Besides the higher content of β-carotene present in refined oil than in crude oil, the presence of phosphatides in crude oil may be one of the reasons why this oil withstood the radiation conditions better than refined soybean oil.

The peroxide values of the oils obtained with the three photooxidation-induced systems demonstrated that the shorter wavelength of the radiation source, the greater the effect in promoting oxidation. Thus, UV radiation had a much more pronounced effect on oxidation than did fluorescent and incandescent light.

The high energy concent of UV radiation brought about the degradation of chlorophyll, β-carotene, and possibly the added tocopherols. The degradation products of these substances in conjunction with autoxidation enhanced the overall oxidation of bleached soybean oil. Also, the UV radiation accelerates markedly the decomposition of peroxides (Lundberg, 1962), which in turn contribute to an increase in the rate of the autoxidation process.

One question that remains unanswered is why crude soybean oil withstood quite well the photooxidation with UV light. Speculation can be made that the nonglyceride fraction present in this oil acted as a whole as an energy absorber of UV radiation.

There was no appreciable difference in the pattern of oxidation of the oils irradiated with fluorescent and incandescent light. Apparently these radiation sources had a similar effect on crude,

refined, and bleached oil with added chlorophyll, β-carotene, antioxidants, or their combinations.

At equivalent times of irradiation, the greater amount of energy of incandescent light (177 W/m^2) induced the production of higher peroxide values in these samples than in those irradiated with fluorescent light (14.5 W/m^2). If the same amount of energy had been used for both light sources, lower peroxide values probably would have been obtained in the presence of incandescent light than fluorescent light. These results support previous studies which indicate that greater wavelengths of light energy promote oxidation the least and that the most deleterious wavelengths are those shorter than 450 nm.

SUMMARY AND CONCLUSIONS

Crude, commercially refined, bleached and bleached soybean oil with added chlorophylls, β-carotene, natural and synthetic antioxidants, and lecithin were irradiated separately with ultraviolet, fluorescent, and incandescent light.

Gas liquid chromatography (GLC) analysis indicated that the severe bleaching conditions did not affect appreciably the fatty acid composition of the bleached oil obtained from crude soybean oil. Analysis of β-carotene, chlorophyll, and color in the oil revealed that pigments were efficiently removed during the bleaching process. Both chlorophyll a and b were present in the crude and refined oil. Analysis of phosphorus indicated that phosphatides were not present in commercially refined and laboratory bleached soybean oil. Addition of chlorophyll a or b at a concentration of 0.5 ppm to the bleached oil did not increase the rate of oxidation appreciably in the irradiated oils. Higher oxidation rates were achieved by the addition of 1 and 24 ppm of chlorophyll. This indicated that the photooxidation-promoting effect of chlorophyll in soybean oil is related to its concentration.

Beta-carotene at several different concentrations did not inhibit the photooxidation of bleached oil in the presence of 0.5, 1.0, and 24 ppm of chlorophyll or in its absence. The higher concentrations of β-carotene actually stimulated higher rates of oxidation. The

analysis of β-carotene and chlorophyll before and after irradiation with UV showed that both chlorophyll and β-carotene were destroyed.

Chlorophyll and, to a lesser degree, β-carotene were also degraded in nonirradiated samples (controls).

High-performance liquid chromatography (HPLC) analysis indicated that severe bleaching conditions affected the tocopherol profile of the bleached oil. Addition of α- or γ-tocopherol or both failed to inhibit the oxidation of bleached oil in the presence or absence of chlorophyll under UV radiation. Good protection to the bleached oil toward photooxidation was afforded by α- and γ-tocopherol or both in the absence of chlorophyll with fluorescent or incandescent light radiation.

TBHQ, in the absence of chlorophyll, proved to be an effective inhibitor of photooxidation with all radiation sources except UV. TBHQ did not inhibit photooxidation of the bleached oil in the presence of chlorophyll.

A combination of a vitamin E mixture and β-carotene provided some protection against photooxidation of bleached oil irradiated with incandescent and fluorescent light in the presence of chlorophyll.

The values of the molar absorbance at 233/PV ratio indicated that both conjugated and unconjugated hydroperoxides were formed in bleached oil containing chlorophyll at the first hours of irradiation with both fluorescent and UV light.

Independently of the irradiation source, the order of resistance of the oils toward photooxidation was crude > refined > bleached oil.

The conclusions reached as a result of this study are summarized below:

1. The amount of chlorophyll that is normally present in crude and refined soybean oil is not enough to accelerate the photooxidation of this oil appreciably.

2. Chlorophyll a and b have the same effect in the oxidation of bleached soybean oil in the presence or absence of light.

3. Beta-carotene at concentrations of 1, 5, and 10 ppm in the presence or absence of chlorophyll acts as a prooxidant of bleached soybean oil in the presence of radiant energy. This suggests that the possible quenching effect of β-carotene on $^1O_2$ is exceeded by the prooxidant effects of its degradation products.

4. The more effective antioxidant effect provided by the vitamin E mixture than α- or γ-tocopherols alone toward photooxidation indicated that the combination of the four tocopherols is more effective than each tocopherol alone.

5. The four tocopherols or δ-tocopherol in the vitamin E mixture possibly protected β-carotene from photodestruction so that it was present to act as a $^1O_2$ quencher.

6. The presence of conjugated and unconjugated hydroperoxides as well as the noninhibition of oxidation by tocopherols and TBHQ in the presence of sensitizer indicated that $^1O_2$ could have been involved in the photooxidation of bleached oil when chlorophyll was present.

7. The noninhibition effect on photooxidation by tocopherols and TBHQ in the presence of sensitizer is probably related to the degradation of these antioxidants by $^1O_2$.

8. The higher peroxide values developed in samples irradiated with UV as compared to those samples irradiated with fluorescent and incandescent light might be attributed to the high energy content of UV radiation.

9. The destruction of low levels of chlorophyll in samples shielded with aluminum foil may be due to the production of free radicals during autoxidation that degrade chlorophyll.

10. It appears that lecithin can act synergistically with tocopherols to inhibit photooxidation of bleached soybean oil.

11. Bleached oil developed higher peroxide values than crude and refined oil due to the removal of tocopherols in the bleaching process. The higher peroxide values developed by refined oil compared to crude oil may be caused by the removal of phosphatides during the degumming operation, since commercial refining processes do not remove tocopherols appreciably from soybean oil.

12. Independently of the radiation sources, the resistance of the soybean oils toward photooxidation was: crude > commercially refined > bleached. These results indicate that the best way to store soybean oil is in the form of crude oil.

PROPOSALS FOR FURTHER RESEARCH

In the majority of studies, and this was no exception, questions are raised that cannot be adequately explained. Some unanswered questions and tantalizing topics that developed as a result of this study include:

1. The effect of other radiation sources such as that of sunlight on the photooxidation of soybean oil.

2. Crude oil withstood better the photooxidation than did refined oil. Studies can be undertaken to explain this phenomenon and to learn if the presence of phosphatides can play some role in the photooxidation of soybean oil.

3. The effect of the four tocopherols (α-, β-, γ-, and δ-) alone or in combination could provide information to enhance that obtained with the vitamin E mixture.

4. Studies identifying the degradation products of chlorophyll and their effects in promoting photooxidation of soybean oil could help to define if the effect of chlorophyll on the photooxidation is more pronounced due to the generation of $^1O_2$ or as acting as a prooxidant because of its degradation products.

5. In this study the possible involvement of $^1O_2$ was assayed by correlating conjugated diene absorbance/PV ratio. Both conjugated diene and peroxide value analyses are relatively easily accomplished. The identification of unconjugated hydroperoxides by other means such

as GLC-mass spectrometry could provide a better determination of the extent of involvement of $^1O_2$.

6. The reaction of $^1O_2$ with unsaturated fatty acid with two or more methylenic interrupted double bonds leads to the formation of conjugated and nonconjugated hydroperoxides. Because the TBA test requires the formation of β,γ to the peroxide group to be capable of undergoing cyclization with the ultimate formation of malonaldehyde, it is possible that the TBA test may be useful to help identify $^1O_2$ involvement in fats and oils of fatty materials. More studies trying to correlate TBA values with $^1O_2$ participation in fat oxidation are required.

7. In several areas the role of singlet oxygen has been a subject of intensive studies lately. There are several ways in which singlet oxygen might be generated, and one of them is the presence of sensitizer. It would be interesting to learn if chlorophyll is the major means by which $^1O_2$ is generated in vegetable oils to initiate oxidation.

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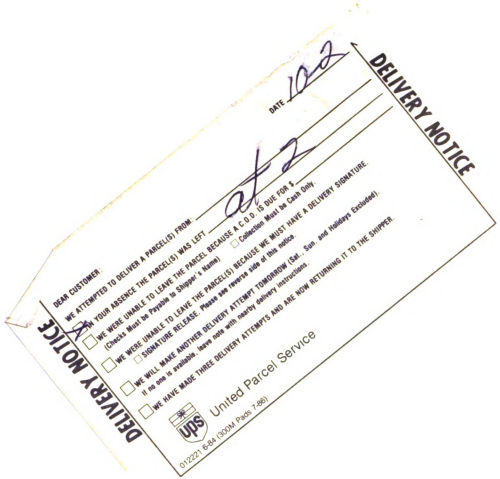
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DATE 12-2
DEAR CUSTOMER:
WE ATTEMPTED TO DELIVER A PARCEL(S) FROM
IN YOUR ABSENCE THE PARCEL(S) WAS LEFT
WE WERE UNABLE TO LEAVE THE PARCEL BECAUSE A C.O.D. IS DUE FOR $
(Checks Must be Payable to Shipper's Name) Collection Must be Cash Only
WE WERE UNABLE TO LEAVE THE PARCEL(S) BECAUSE WE MUST HAVE A DELIVERY SIGNATURE.
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