EFFECT OF SELECTED ANTIOXIDANTS ON THE STABILITY OF VIRGIN OLIVE OIL

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ABSTRACT

EFFECT OF SELECTED ANTIOXIDANTS
ON THE STABILITY OF VIRGIN
OLIVE OIL

By

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This is a study to assess the effect of the antioxidants butylated hydroxyanisole (BHA), butylated hydroxytolyene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG), on the stability of virgin olive oil obtained from Greece. Three different samples were used.

The oil was treated with the above antioxidants which were used either alone or in different combinations, at different levels of concentration. Samples were then stored at room temperature in the dark for 22 weeks and at 50°C for 24 weeks, and were analyzed at regular intervals. In addition, samples containing antioxidants were stored at room temperature in light for a six month period. The peroxide value, diene conjugation and thiobarbituric acid (TBA) tests were conducted throughout the storage period to measure the oxidation of olive oil.

The Schaal Oven Test was also employed. In this test the peroxide value was used as a measure of oxidation.

The fatty acid composition of the olive oil was determined by gas liquid chromatography.

Under accelerated conditions, it was found that antioxidants increased the stability of the oil and their effectiveness varied between the three different samples of olive oil used. In the oven test, where the sample of oil used was the same as that used for storage conditions (room temperature and 50°C), the relative inhibition effect of the antioxidants used was in the following order TBHQ = BHA > BHT. The antioxidant combination which was found to be the most effective in the oven stability studies was 0.01% BHA + 0.01% TBHQ.

Olive oil stored at room temperature in the dark did not undergo any oxidative deterioration during the 22 weeks storage period. Therefore the antioxidants used in this case had no effect. On the other hand, olive oil stored without antioxidant at room temperature in light underwent a high degree of oxidation. Results obtained from peroxide value, diene conjugation and TBA tests correlated well in this experiment. The presence of antioxidants in the samples stored at room temperature in light had no effect with respect to retarding the peroxide formation.

The addition of antioxidants to olive oil stored at 50°C exhibited a beneficial effect. Citric acid used alone, however, showed a prooxidant effect. Potency of the antioxidants under these conditions was in the following order: TBHQ > BHT > BHA. Combinations of TBHQ with BHA and BHT provided good results, but they never exceeded the results obtained by using TBHQ alone.

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To my parents, my wife and

my son

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INTRODUCTION

Olive oil is the oil extracted from the fruits of the tree Olea eurapaea and it is one of the very few, if not the only, plant oil which can be consumed in its natural state without being chemically treated.

The olive tree probably originated in Mesopotamia and it has been cultivated for many centuries in the countries bordering the Mediterranean. For more than 3,000 years, the olive tree has been planted in Greece. While most of the world supply of olive oil is produced in the Mediterranean countries, some olive oil is also produced in the United States (California). Before World War I, Greece was the premier olive producing country in the entire Mediterranean region. Now Greece ranks third among the world's olive producing countries with Spain and Italy being number one and two, respectively.

Many different cultivated varieties have been developed over the centuries and they differ from each other in various ways such as the size of the fruit, its color, and the percentage oil content. These characteristics determine what the fruits will be used for--pickling or oil production.

Olive oil is mainly used in its natural form in salads and in the preparation of foods and is considered to be the finest edible oil. Part of it is also used in the production of margarine in the olive producing countries.

Officially reported production figures are usually concerned with edible oil. There is, however, a considerable amount of inedible oil, extracted by solvent methods from the residue remaining after pressing the olive paste. The oil which comes from the first pressing is called "virgin" olive oil and it is considered to be of highest quality. Virgin olive oil is the type of oil used in this study.

Like the rest of the vegetable oils, olive oil undergoes oxidative deterioration as a result of several factors. The autoxidation of olive oil results in the modification of its organoleptic characteristics and some of its physical properties such as viscosity. The prevention of autoxidation in olive oil is recognized as a problem of great importance from the standpoint of both health and economy.

The development of synthetic antioxidants has played a vital role in the marketing of other vegetable oils by retarding oxidation.

The present study was designed to gain knowledge on the effect of antioxidants in olive oil obtained from Greece. This work was based on storage stability tests at room temperature (both in the dark and in light) and at 50°C. The Schaal oven test was also employed. Peroxide values, diene conjugation studies and thiobarbituric acid (TBA) determinations were conducted as chemical tests to evaluate the effect of antioxidants on the stability of the olive oil.

The antioxidants used in this work were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG).

REVIEW OF LITERATURE

Mechanism of Autoxidation

Autoxidation occurs when oxygen reacts with the double bonds in the unsaturated fatty acids. It is an autocatalytic mechanism in which the rate of reaction increases with time due to the formation of products acting as catalysts. The mechanism of autoxidation involves the following three steps (Dugan, 1961).

1. Initiation

$$RH + O_2 \longrightarrow R\cdot + \cdot OOH \qquad (I)$$

2. Propagation

$$R\cdot + O_2 \longrightarrow ROO\cdot \qquad (II)$$

3. Termination

$$R\cdot + R\cdot \longrightarrow RR \qquad (IV)$$

$$R\cdot + ROO\cdot \longrightarrow ROOR \qquad (V)$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2 \qquad (VI)$$

RH refers to any unsaturated fatty acid in which the H is labile by reason of being on a carbon atom adjacent to a double bond. R· refers to a free radical formed by removal of a labile hydrogen. The oxidation 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).

The autoxidation of fats is affected by a number of factors. These factors are: (1) saturation of the fat, (2) heat, (3) light, (4) ionizing radiation, (5) enzymes, (6) catalysts, (7) presence of oxygen, and (8) use of antioxidants (Lea, 1962).

In the autoxidation of fats, unsaturated fatty acids are oxidized to hydroperoxides which consequently undergo decomposition yielding a number of volatile compounds including unsaturated esters, 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).

Besides the oxidative rancidity formed during the autoxidation of fats, there is another type of oxidative deterioration which is detectable in the very early stages of oxidation and is called flavor reversion. This reversion is termed "grassy," "beany," "fishy," etc. depending on the resemblance of the oil flavor. The term reversion is a misnomer because the objectionable flavor and odor is not characteristically normal to the fresh fat (Spannuth, 1949).

Although reversion occurs principally in vegetable oils containing polyunsaturated fatty acids (Lundberg, 1962), Gutierrez (1963) observed the phenomenon of reversion in olive oil.

Flavor Compounds of Oxidized Olive Oil

Studying the rancidity of olive oil Corrao (1966) demonstrated that the organoleptic threshold of this oil is independent

of the peroxidation level. Swern (1964) suggested that oleic acid is probably responsible for the odor and the flavor of the true oxidative rancidity, while Badings (1970) was able to isolate seven aldehydes responsible for the off-flavor in autoxidized oleic acid at 20°C.

Gutierrez and Romero (1960) observed that during the oxidation of olive oil different compounds are produced in different ways and in different proportion, and it is difficult to determine which groups are responsible for the off-flavor of this oil. Foresti (1964), however, found that the off-flavor in olive oil is caused by saturated aldehydes and dienals as well as α,β-unsaturated aldehydes.

Role of Chlorophyll on the Oxidation of Olive Oil

Taufel et al. (1959) observed that chlorophyll in the presence of light acts as a prooxidant for methyl oleate. This pigment, however, has no prooxidant effect in the dark; on the contrary it acts 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. Interesse et al. (1971) found that, under the action of light, the four pigments chlorophylls a and b and pheophytins a and b develop an oxidizing activity while in the dark they act as antioxidants.

Due to the chlorophyll content, which according to Vitagliano (1960) varies from 0-9.7 ppm, virgin olive oil is easily oxidized and is very sensitive to light. Studying the effect of light on the oxidative stability of olive oil, Pretzch (1970) observed that olive oil exposed to light and air undergoes higher oxidation than that stored in the dark. Valentinis and Romani (1960), however, proved

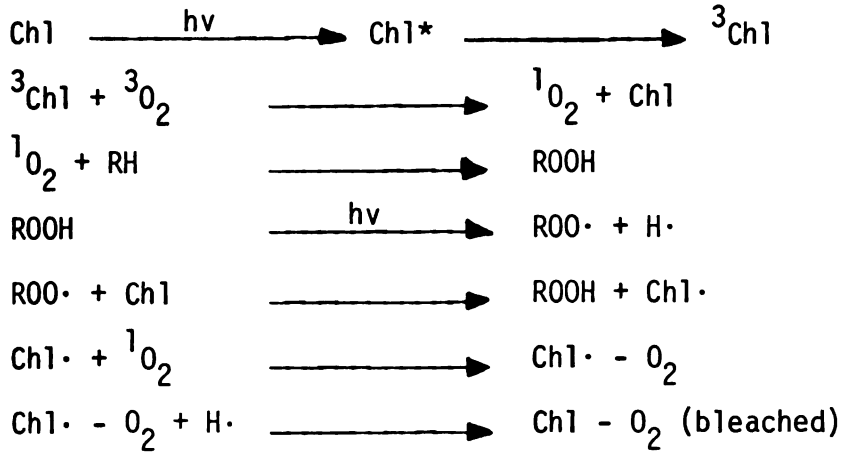
that, in the absence of air, direct sunlight causes a decrease of peroxide and Kreis value during the storage of olive oil. It was also observed by Gutierrez and Jimenez (1970) that virgin olive oil stored in polyethylene and exposed to light undergoes higher oxidation than that stored in dark.

Radiations of wave length greater than 630 nm present a maximum of absorption in virgin olive oils corresponding to the absorption of chlorophyll and are very active, while the zone between 530-630 nm has minimum activity (Borbolla et al., 1963). Francesco (1961) was more specific in finding that virgin olive oils have a chlorophyll absorption peak at 665 nm. A very important observation made by Vazquez et al. (1960) is that, because of their chlorophyll content, olive oils are very sensitive to radiation of wavelength between 320 and 720 nm whether in the presence or the absence of antioxidants.

Bleaching of Chlorophyll

The different types of chlorophyll have the ability to sensitize, in the existence of an excited state, the oxidation of organic substances by molecular oxygen (Seely, 1966). Rawls and Van Santen (1970) demonstrated that, in chlorophyll catalyzed photooxidation, a mechanism which would supply singlet O_2 is necessary for the formation of the original hydroperoxides. These hydroperoxides, formed by singlet O_2, are produced at a much faster rate (1450 times) than by triplet O_2.

During photooxidation, bleaching of chlorophyll occurs. Sastry et al. (1973) proposed the following mechanism for the bleaching of chlorophyll as the effect of visible light.



Chlorophyll when exposed to visible light gets excited and is converted to the triplet state of chlorophyll. This triplet state of chlorophyll reacts with triplet oxygen resulting in singlet oxygen, which in turn reacts with substrate to form hydroperoxides (Rawls and Van Santen, 1968).

Hydroperoxides give rise to peroxy radicals on exposure to light. These peroxy radicals abstract hydrogen atoms from chlorophyll, thus disturbing its conjugated electron system. Abstraction of a proton from chlorophyll causes a free radical. The resulting radical reacts with oxygen to give a peroxy free radical of chlorophyll. This combined with a proton stabilizes itself to a stable peroxide.

Effect of Free Fatty Acids (FFA) on Oxidation

Among the factors that might promote oxidation and influence the effectiveness of antioxidants used is the presence of free fatty acids.

Olcott (1958) observed that the addition of oleic acid to refined olive oil and other vegetable oils decreased the stability of the oils at 60°C. A similar observation was made by Chahine and El-Shobaki (1966) in unpurified shark liver oil at 100°C.

Vega et al. (1960) and Catalano and Felice (1970) reported that the presence of FFA in concentrations as low as 0.5% have a strong prooxidant effect on olive oil and cause the stability of the oil to diminish. The later investigations pointed out that this prooxidant effect can be realized by means of a catalytic mechanism. They further demonstrated (1970) that the autoxidation of olive oil is related to the FFA concentration and that the chain length or the presence of a double bond plays a secondary role.

Olcott (1958) noted that the antioxidant action can be modified by the presence of free fatty acids in triglyceride systems and Catalano and Felice (1970) found that the antioxidant effect of NDGA, BHA and ascorbyl palmitate was reduced in the presence of FFA.

Metal Catalysts

The presence of small quantities of prooxidant metals such as iron, copper and manganese which occur in oils naturally or as a result of processing plays an important role in the stability of the oils. These metals catalyze the rate of formation and destruction of peroxides thus leading to the more rapid formation of substances with undesirable odors and flavors (Swern, 1964).

Some of the metals are such extremely powerful catalysts of oxidation that their effect is high even in concentrations as low as one part in 100 million (Ingold, 1968).

Fedeli et al. (1973) found that the oxygen absorption rate in autoxidized olive oil was related to the amount of catalytic metals (Ca, Na, Co, Ni, Fe, Cu and Mg) present in the oil. It was also found (Vioque et al., 1959 and Vioque, 1968) that upon removal of most

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of the catalytic metals present in olive oil by passing it through cation exchange resins the stability of the oil was increased. He further found (1968) that there is a correlation between olive oil stability and iron concentration as is shown below:

$$K = (Fe)^{a} \cdot S$$

where K and a are constants

$$(Fe) = ppm$$

and S = stability (A.O.M.)

Measurement of Oxidative Rancidity

Many methods have been developed for measuring the oxidative rancidity or the stability of fats and oils. From these the most commonly used methods are: the active oxygen method (A.O.M.), Schaal oven test, peroxide value, thiobarbituric (TBA) test, and carbonyl test (Dugan, 1955).

Peroxide Value (PV)

For peroxide value determination a number of analytical procedures have been developed, such as Wheeler (1932), Lea (1939) and Stine et al. (1954). The peroxide value method involves the quantitative measurement of the primary products of oxidation (peroxides), expressed as milliequivalents of reactice oxygen per kilogram of fat.

For determining the peroxide value of virgin olive oil Maurikos et al. (1972) used a new polarographic method, where the supporting electrolyte was LiCl in methanol-benzene with a dropping mercury electrode.

The peroxide value is the most important method in evaluating the present condition of olive oil. Montedoto and Petruccioli (1972), however, observed that the peroxide values that are computed during the test are usually affected by the presence of quinones which are formed by the phenolic substances during storage.

Diene Conjugation

During the oxidation of polyunsaturated fatty acids, an increase in ultraviolet absorption occurs due to the formation of conjugated diene and triene hydroperoxides. The change of ultraviolet absorption, however, is not easily related to the degree of oxidation, since the effects upon the various unsaturated acids (oleic, linoleic, linolenic, etc.) are different in quality and magnitude (Holman and Burr, 1946).

Bartolomeo and Sergio (1969) observed that the primary oxidation products of olive oil are hydroperoxides with an absorption peak at 232 nm and that the greater the absorbance the greater the degree of primary oxidation. For the secondary oxidation products--aldehydes, ketones, etc.--they found an absorption peak at 270 nm. Jimenez and Gutierrez (1970) and Bartolomeo and Sadini (1959) found that the rancidity of olive oil causes the ratio A232/A270 to decrease according to the degree of alteration. This ratio remains constant in virgin olive oils stored in the dark and decreases rapidly in virgin oils stored in sunlight due to the rapid increase of oxidation (Jiminez and Gutierrez, 1970).

A quasilinear relation was found (Montefredine and Luciano, 1968) between the absorbance at 232 nm and the peroxide value and

between the absorbance at 270 nm and the acidity in virgin olive oil. Olive oil, like all oils free of conjugated double bonds, shows a slow increase in absorptivity at 232 and 268 nm during the induction period which is followed by a sharp, sudden increase. The absorbance at these wavelengths can be used to predict the thermal stability of the oil (Ninnis and Ninni, 1968).

Ninnis and Ninni also demonstrated (1966) that ultraviolet spectrophotometric analysis can be used to detect the adulteration of virgin olive oil.

Thiobarbituric Acid (TBA) Test

The 2-thiobarbituric acid (TBA) test has been used widely for measuring oxidative changes in foods containing unsaturated fatty acids. Kohn and Liversedge (1944) observed that animal tissues which had been incubated aerobically gave a red color when mixed with 2-thiobarbituric acid. Bernheim et al. (1947) found that the red color was formed from the oxidation products of unsaturated fatty acids and 2-thiobarbituric acid.

Many researchers have reported that the red color is a condensation product of one molecule of malonaldehyde with two molecules of 2-thiobarbituric acid. Sinnhuber et al. (1958) and Dahle et al. (1969), found that the original material formed is predominantly a nonvolatile substance and therefore is not malonaldehyde. This nonvolatile substance undergoes decomposition under the conditions of the TBA test and produces malonaldehyde which then reacts with TBA.

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's et al. (1969) 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.

Gutierrez and Romero (1960), Franjo (1963), and Casillo (1968) used the thiobarbituric (TBA) test to measure oxidative rancidity in olive oil. It was found by Casillo (1968) that TBA test detects the rancidity of olive oil at a lower level than other tests (peroxide value, Kreis test, acid number).

Role of Antioxidants in the Oxidation of Fats and Oils

The antioxidant role of various substances was known for a great number of years, but the technology of the antioxidants in relation with foods, fats and oils did not start until the late 1940s. Antioxidants are substances which in small quantities are able to prevent or to retard the oxidation of easily oxidizable materials such as fats (Chipault, 1962).

Higgins and Black (1944) summarized the requirements of an ideal antioxidant as follows:

1. It should exert no harmful physiological effect.
2. It should contribute no objectionable flavor, odor or color to the fat.
3. It should carry through and effectively protect from rancidity the foods made with the fat.
4. It should be sufficiently fat soluble.
5. It should be effective in low concentrations.

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6. It should be readily available.

7. It should be reasonable in cost.

Antioxidants may interfere with the process of autoxidation in two general ways, either as inhibitors of free radicals or as peroxide decomposers.

Uri (1961) proposed the following mechanism for the antioxidant function.

$$ROO\cdot + AH \text{ (antioxidant)} \longrightarrow ROOH + A\cdot$$

The radical A· may be stabilized by recombination in two ways:

$$A\cdot + A\cdot \longrightarrow A_2$$

$$ROO\cdot + A\cdot \longrightarrow ROOA$$

As peroxide decomposes, antioxidants act as catalysts in the decomposition of the peroxides which are initially present or 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 which are not free radicals.

Synergism is a phenomenon which occurs when two or more compounds used together give a more pronounced antioxidant effect than the sum of their individual effects. Synergists may be organic or inorganic compounds and they are usually acidic in character. These acids are only active in conjunction with primary phenolic antioxidants. Synergistic action was also observed by Mahon and Chapman (1953) and Dugan et al. (1954) when certain phenolic antioxidants were used in combination.

There are some theories on the action of the synergists. Synergists may work as metal scavengers, peroxide decomposers and sparing agents, as in the interaction of phenolic antioxidants or the interaction of other agents with phenolic antioxidants (Dugan, 1957).

Phenols and Tocopherols as Natural Antioxidants in Olive Oil

Phenols

The phenolic concentration of olive oil depends on cultivation procedures and environmental factors and is correlated to the stability of the oil. Vazquez et al. (1973, 1975) demonstrated that the polyphenol content of olive oil varies. They also found (1975) that the main polyphenols present in virgin olive oil are tyrosol and 3-hydroxytyrosol and observed some antioxidant effect in 3-hydroxytyrosol.

Polyphenols that were extracted from olive leaves were found to act as antioxidants in olive oil. These polyphenols in a concentration of more than 20 mg/100 g of oil inhibit the oxidative rancidity of the oil (Notte and Romito, 1971). Cantarelli and Montedoro (1969, 1972) observed that phenols extracted from olive oil with 80% MeOH acted as antioxidants and inhibited rancidity. These phenols demonstrated a high stabilizing effect in other oils while the extraction of phenols from olive oil caused its rapid oxidation.

Tocopherols

Tocopherols are natural antioxidants which are primarily responsible for the stability of vegetable oils. It is known that an optimum quantity of tocopherols must exist for stabilization. An

excess of this quantity will provide lesser stability through an apparent prooxidant effect (Oliver et al., 1944). Hove and Hove (1944), in their experiment with carotene and ethyl oleate, found that gamma tocopherol was more effective than beta which in turn was more effective than alpha.

Using dimensional paper chromatography, Grasian and Arevalo (1965) identified only α-tocopherol in olive oil. They noted that γ-tocopherol must be considered as a product of α-tocopherol oxidation. Vitagliano (1960) and Boatella (1975) both agreed that olive oil contains α-tocopherol but they reported different quantities; the first from 12-162 ppm and the second from 70-150 ppm.

Addition of Vitamin E at the level of 0.05% in the bottled olive oil retarded the oxidative rancidity of the oil (Fahmy and El Said, 1962). Vazquez et al. (1973) found that olive oil was stabilized against oxidative rancidity by adding tocopherols and other polyphenolic compounds.

Ninnis et al. (1969) demonstrated that the tocopherol content of Greek olive oil can be used for the detection of its adulteration with other vegetable oils.

The Use of Synthetic Antioxidants in Olive Oil

The addition of various antioxidants as a means of preventing the oxidation of olive oil has been reported by many researchers. Gutierrez (1961, 1962, 1963) studied the effect of the antioxidants BHA, BHT, NDGA, octyl gallate and dodecyl gallate as well as the synergistic effect of citric acid on the olive oil.

Astudillo et al. (1968) found that antioxidants such as BHA and α-tocopherol used in low concentration (0.05%) did not have any effect on the oxidation of irradiated olive oil. Tadaaki and Kazuhito (1967) observed that citric acid added to olive oil stored at 50°C for 96 hours showed an antioxidant effect which was greater in concurrent use with antioxidants.

Studying the antioxidant action of ascorbyl palmitate in olive oil, Cerutti (1956) found that addition of 0.02% ascorbyl palmitate is sufficient to assure good preservation of olive oil. Miric et al. (1964) observed that isopropylidene-L-ascorbic acid is more effective than ascorbyl palmitate and NDGA in preventing oxidation of olive oil. It was observed by Schorderet (1969) that the stabilizing effect of ascorbyl palmitate was enhanced by 2-tert-butyl-4 hydroxyanisole and propyl gallate while a mixture of ascorbyl palmitate and NDGA caused greater peroxide formation than that in the unstabilized oil. Vitagliano and Vodret (1960) demonstrated that ascorbic acid was efficient in controlling the rise of peroxide formation by inhibiting lipase activity in olive pulp. The addition of 0.01-0.1% p-aminosalicylic acid in solution to unrefined olive oil showed a good antioxidant effect (Didenko et al., 1968).

Urakami et al. (1961) observed some antioxidant effect of egg yolk cephalin methyl oleate made from the oleic acid fraction of olive oil. An antioxidant effect also was observed by Hirahara et al. (1974) when alcohol or ether extract from clove was added to olive oil. Natural inhibitors of oxidation that are present in olive leaves were also found to favor the stability of olive oil (Notte and Romito, 1971).

Gutierrez (1962) found that the proprietary blend antioxidant Tenox II added to olive oil kept the oil in better conditions than BHT during storage at room temperature. He also observed (1963) that some antioxidants used in a mixture of olive oil and soy bean oil in a 50/50 ratio showed a good effect on the conservation of this blend.

MATERIAL AND METHODS

Raw Material: Virgin Olive Oil

The oil used in this study was obtained from Greece. Three different samples of virgin olive oil were shipped over, each coming from a different part of the island of Crete. In all cases the oil was extracted by hydraulic pressure from fruits of the variety tsounati. The samples from the three different regions were found to differ in fatty acid composition and initial peroxide value. They were numbered as olive oil No. 1, No. 2, and No. 3 and had initial peroxide value of 16, 35, and 12 respectively. To prevent the peroxide value from increasing, the oil was kept in a cool place (36.5°F) until the day it was used.

Dispersion of Antioxidants into the Samples

The antioxidants evaluated in these studies were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG) (Eastman Chemical Company). These antioxidants were used alone and in combinations. The type and the concentration of the antioxidants used in this study are shown in Table 1. Citric acid at 0.005% was also used, without the presence of antioxidants.

In order to insure complete solution and uniform dispersion of the antioxidants, the oil was placed in beakers and heated to 60°C before antioxidants were added. As for the citric acid it was first

Table 1.--Antioxidants and Synergist Used in Virgin Olive Oil Studies.

Antioxidant Added[a]	Concentration % (w/w)
BHA	0.01
BHA	0.02
BHT	0.01
BHT	0.02
TBHQ	0.005
TBHQ	0.01
TBHQ	0.02
PG	0.02
BHA + TBHQ	0.01 + 0.005
BHA + TBHQ	0.01 + 0.01
BHT + TBHQ	0.01 + 0.005
BHT + TBHQ	0.01 + 0.01
CA	0.005

[a]BHA = Butylated Hydroxyanisole

BHT = Butylated Hydroxytolyene

TBHQ = Tertiary Butylhydroquinone

PG = Propyl gallate

CA = Citric Acid

dissolved in a mixture of ethanol and distilled water 1:1(v/v) and was then added to olive oil. All treated samples and the control were left at room temperature for 24 hours, being stirred occasionally for about 20 minutes.

Oven Test

For the oven test (usually referred to as the Schaal oven test) the oil was placed in petri dishes (5.5" in diameter and 3/4" in height) and kept in an electric oven, and tested periodically for peroxide formation. The petri dishes were arranged on two shelves, the bottom shelf being 4 1/4" from the bottom of the oven, and the top shelf 8 1/4" from the bottom. All the samples were rearranged shelfwise and positionwise after each testing to get more uniform results because, according to Eubank and Gould (1949), the arrangement of the samples inside the oven is a factor affecting the results. A total of four tests were run for the oven stability studies, using samples of the olive oil No. 1, No. 2 and No. 3. One of these tests was run at 100°C while the other three were run at 65°C. The sample size was uniform 100g in all tests, except for one where samples of both 50 g and 100 g were used in order to study the effect of the sample on the rate of oxidation.

The end point for the oven test was the day when the peroxide value of the oil reached 120 meq/kg oil.

Preparation of the Samples for the Storage Stability Studies

For the long term storage studies 100 g of olive oil No. 2 (with or without antioxidants) was placed in clear glass jars 2" in

diameter and 4.5" in height. All jars were loosely capped. The one hundred and five (105) jars used were separated into three groups. The first group of 50 jars was stored in an incubator maintained at 50°C. The second group of another 50 jars, containing samples identical to those in the first group, was stored at room temperature ranging from (20-28°C) in darkness, while the third group of 5 jars, containing no antioxidants and used as control, was stored at room temperature but was exposed to daylight. The latter experiment was intended to study the effect of light on the photooxidation of virgin olive oil. For each oil sample, five jars were stored and each time a sample was taken from a different jar until all five were used and then sampling was repeated following the same order. This sampling technique was employed to reduce the number of samplings taken from the same jar in a short time span.

At one week intervals during the first month, and two weeks for the rest of the period, a jar was removed and shaken in order to mix the oil well, and samples were taken and examined for peroxide value, ultraviolet absorption at 233 nm and TBA absorption values.

To evaluate the effect of antioxidants in virgin olive oil in the presence of light, another experiment was conducted at room temperature using olive oil No. 1. Five jars of 150 g of oil treated with antioxidants and one containing no antioxidants were stored under diffused light for a period of six months. This oil was analyzed every two months for peroxide formation.

Analytical Techniques

Free Fatty Acid (FFA) Determination

The official AOCS (1974) method was used for free fatty acid determination of olive oil No. 1, No. 2 and No. 3. The content in FFA of the samples used was found to be 0.5%, 2.1% and 1% respectively expressed as oleic acid.

Preparation of Methyl Esters for Gas Liquid Chromatography (GLC)

Methyl esters of olive oil were prepared by a modified Morisson and Smith (1964) method. Boron Trifluoride-methanol (Sigma Chemical Company) was the reagent used. Two hundred to three hundred mg of oil were dissolved with 1 ml benzene in a 30 ml test tube. A total of 2.5 ml of 14% (BF_3 - MeOH) was added and the tube was sealed with a screw cap and was placed for 40 minutes in a steam bath. After cooling the sample at room temperature, the esters were extracted by adding two volumes of hexane and one volume of water to the tube.

The tube was then shaken vigorously until two layers were formed. A portion from the upper layer was taken and dried with approximately 0.3 g of anhydrous sodium sulfate, and then different quantities of the dried samples were used for analysis by gas liquid chromatography.

Fatty Acid Composition of Olive Oil

The fatty acid of olive oil was identified using a Beckman-GC-4-Gas Chromatograph equipped with a hydrogen flame detector. The glass column (6 ft x 2 mm (i.d.)) was packed with 10% (w/w)

diethylene glycol succinate (DEGS) on 100/120 mesh Supelcoport (Supelco, Inc.). The column oven temperature was 100°C, the injection port was maintained at 210°C and the detector at 185°C. The helium carrier was adjusted to 40 ml/minute. The flow rate of hydrogen and oxygen was 30 ml/minute.

Esters were identified by a comparison of their retention time to those of standard mixtures of known fatty acid methyl esters. Peak areas were calculated by multiplying peak height by the width at half-height and from this it was possible to determine the relative percentage composition of the total fatty acids.

Measurement of Rancidity

Peroxide Value (PV). Peroxide values were determined by a modified Wheeler method (1932), and were reported as milliequivalents/kg oil.

A 4.4 g or 5 ml of oil taken by weight from the stored samples or by pipetting from the oven test samples was dissolved in 30 ml of glacial acetic acid-chloroform (3:2) solution, and 0.5 ml of saturated KI solution was added. The mixture was shaken and allowed to stand for one minute. It was then diluted with 100 ml of distilled water, and titrated with 0.1 N sodium thiosulfate. The reason for using 100 ml of distilled water is that the olive oil used, had a dark green color and because Stansby (1941) reported that the emulsion which is formed when the iodine in a colored oil is titrated with thiosulfate makes the starch end point very difficult to distinguish. This can be avoided by using a larger ratio of water-solvent for the titration.

Ultraviolet Absorption (UV). The absorption of the oil at 233 nm was determined during storage using the following procedure. After four weeks of storage and every two weeks thereafter ten (10) mg of oil were weighed accurately into small petticups and placed into 30 ml test tubes. Ten (10) ml of purified iso-octane (2,2, 4-trimethyl pentane) was added to the sample and shaken in a Fisher mini shaker to assure complete dilution of the oil. The mixture was then filtered through a Whatman No. 1 filter paper and the ultraviolet measurements were taken on a Beckman DU spectrophotometer using pure iso-octane as a blank.

Purification of Iso-octane. This solvent was purified using silica gel, according to AOCS (1974) method. Silica gel was activated before use by being dried in an oven at 130°C for four hours. About 3 1/2" of glass wool was put in the bottom of a (24" x 2") filter tube and then 12" of silica gel was added. The tube was fastened vertically to a ring stand and a 1-liter Erlenmeyer flask was put under the stopcock. Iso-octane was poured slowly into the tube, filling it almost to the top and the tube was then covered with aluminum foil. The iso-octane was filtered through the silica gel as many times as needed to get an absorbance lower than 0.070 at 233 nm wavelength.

TBA Test. The 2-thiobarbituric acid (TBA) test employed in this study was a modification of the Dunkley and Jennings (1951) method. The first test was run after six weeks of storage and then repeated every two weeks for both the oil samples stored at room temperature and at 50°C.

Reagents--The TBA reagent consisted of 0.025 M 2-thiobarbituric acid in M phosphoric acid. The TBA solution was prepared by dissolving 0.720 g of TBA with distilled water in a 100 ml volumetric flask and completing the volume to 100 ml. The solution was then poured into a 250 ml beaker where 19.6 g of phosphoric acid, diluted to exactly 100 ml of distilled water in a volumetric flask, was added. This TBA mixture was then heated for several minutes and was continuously stirred as to assure complete dilution. The TBA solution was prepared fresh each week that it was used.

Procedure--Four (4) ml of oil was pipetted into a 55 ml test tube and an equal volume of TBA solution was added. Mixing by shaking in a Fisher mini shaker followed and the tubes were put in a boiling water bath for 25 minutes. Two to three granules were added to each tube for smooth boiling. Tubes were cooled in cold water upon removal from the bath. For the color extraction, 8 ml of chloroform, distilled in glass, was added in each tube. After they were vigorously shaken in the Fisher mini shaker, the tubes were left for a few minutes to settle and the aqueous layers were transferred to tubes which were centrifuged for five minutes in a centrifuge at a minimum of 3,000 rpm.

Part of the clear layer was removed and its absorbance at 532 nm and 535 nm was determined with a Beckman DU spectrophotometer using TBA solution as a blank.

Removal of Interfering Pigments

Many researchers have reported the presence of yellow and orange colors with an absorption maxima at 450-460 in TBA reaction

solution. In this study, a separation of pigments by a chromatographic column was employed using the Yu and Sinnhuber method (1962). Pyrex chromatographic tubes 300 x 10 mm were packed with cellulose powder (Whatman standard grade) to a height of about 12 cm under a 9.0 psi nitrogen pressure. Five ml of the TBA reaction solution were added to the column. After the solution had passed through the cellulose column, five ml of phenol solution (phenol 5 g, ethanol 15 ml and water 25 ml) were added, to wash the sides of the tubes. The column was then washed with 1-3 ml of 0.1 N HCl in order to remove all the yellow pigments which were collected in 15 ml test tubes. Finally about 20 ml of 0.1 N NaOH were added to the column to elute the pink color. When the pink band was 2-3 cm above the bottom of the column, the eluate was collected in a total volume of 10 ml in a volumetric flask containing 1 ml of 1.2 N HCl and 2 ml 25% ethanol.

Using a Beckman DU spectrophotometer the absorbance was read at 532 nm and 535 nm for both yellow and pink color against a TBA solution used as blank.

RESULTS AND DISCUSSION

The olive oil used in this study was in its natural form, that is there had been no refining of the oil. Virtually all olive oil is consumed in this state and it seemed appropriate therefore to study the effect of antioxidants on such olive oil.

The antioxidants used were evaluated under long term storage and accelerated conditions (Schaal Oven Test).

The results presented in this study are the average of duplicate determination.

Gas Liquid Chromatography (GLC) Analysis

The fatty acid composition of olive oil was determined by gas liquid chromatography. The data in Table 2 reveal the fatty acid composition of olive oil No. 1 and No. 2. Both samples were found to contain traces of linolenic ($C_{18:3}$) and palmitoleic ($C_{16:1}$) acid and they both contained a high percentage of oleic acid ($C_{18:1}$).

The difference in quantitative composition between the two samples may be due to the difference in growth conditions, unsaturation of the fruits and parental trees, since these samples came from different areas and the fruits were harvested at different times. Swern (1964) reported that olive oil tends to become more unsaturated with advancing maturity of the fruits and this probably explains the higher percentage of unsaturation in olive oil No. 2 (Table 2).

Table 2.--Fatty Acid Composition of Greek Olive Oil.

	Percentage	
Sample No.	1	2
Year of Production	December 1975	March 1976
Palmitic, $C_{16:0}$	13.57	7.57
Palmitoleic, $C_{16:1}$	Tr.	Tr.
Stearic, $C_{18:0}$	3.03	3.25
Oleic, $C_{18:1}$	75.61	83.82
Linoleic, $C_{18:2}$	7.63	5.31
Linolenic, $C_{18:3}$	Tr.	Tr.

Instrument: Beckman GC-4 chromatograph

Detector: Hydrogen flame

Column: Glass (6 ft x 2 mm i.d.)

Carrier gas flow rate: 40 ml/minute

Column temperature: 100°C

Injection temperature: 210°C

Oven temperature: 185°C

Sensitivity (attenuation) of 5 x 10^3

Oven Stability Studies

As indicated in Tables 3, 4, 5, and 6, all antioxidants used (BHA, BHT, TBHQ, and PG) had inhibitory effects to various degrees on the peroxide formation in all three samples of olive oil used.

The oven test was terminated when the peroxide value had reached 120 meq/kg oil. The number of days required to reach this point varied between the control and the samples containing antioxidants and between the three samples of oil used.

Data in Table 3 demonstrate that samples containing 0.005% TBHQ exhibited better stability than those containing 0.01% BHT which in turn exhibited better stability than those containing 0.01% BHA. When the phenolic antioxidants BHA and BHT were used in combination with TBHQ, better stability of the samples was achieved (Figure 1).

It is interesting to note that the order of effectiveness of the antioxidants was reversed when olive oil No. 2, which had a higher initial peroxide value (35) was used. In other words, samples containing 0.005% TBHQ showed the lowest stability while those containing 0.01% BHA showed the highest (Table 4). When TBHQ was used in 0.01%, it caused the oxidative stability of the sample to be enhanced and it gave the same results as did 0.01% BHA. These data also show that the antioxidants BHA and BHT used in combination with TBHQ gave better results than when used alone. BHA performed better than BHT, regardless of whether they were used alone or in combination with 0.005% or 0.01% TBHQ. When 0.01% BHA was combined with 0.01% TBHQ it increased the protective factor from 1 to 2 and this was the best combination of those evaluated.

Table 3.--Stability Studies with Olive Oil No. 1 Treated with Antioxidants (Oven Test at 65°C).

Antioxidant (wt %)	Stability of Olive Oil[a]			
	100 g Sample		50 g Sample	
	Oven Days[b]	Protective Factor[c]	Oven Days[b]	Protective Factor[c]
None (control)	32	1.00	28	1.00
0.01 BHA	36	1.12	30	1.07
0.01 BHT	38	1.18	31	1.10
0.005 TBHQ	41	1.28	36	1.28
0.01 BHA + 0.01 TBHQ	51	1.59	41	1.46
0.01 BHT + 0.01 TBHQ	51	1.59	41	1.46

[a]Initial peroxide value 16

[b]Time in days required for olive oil to reach a peroxide value of 120.

[c]Protective Factor is expressed as:

$$\frac{\text{stability of the sample containing antioxidant}}{\text{stability of the control sample}}$$

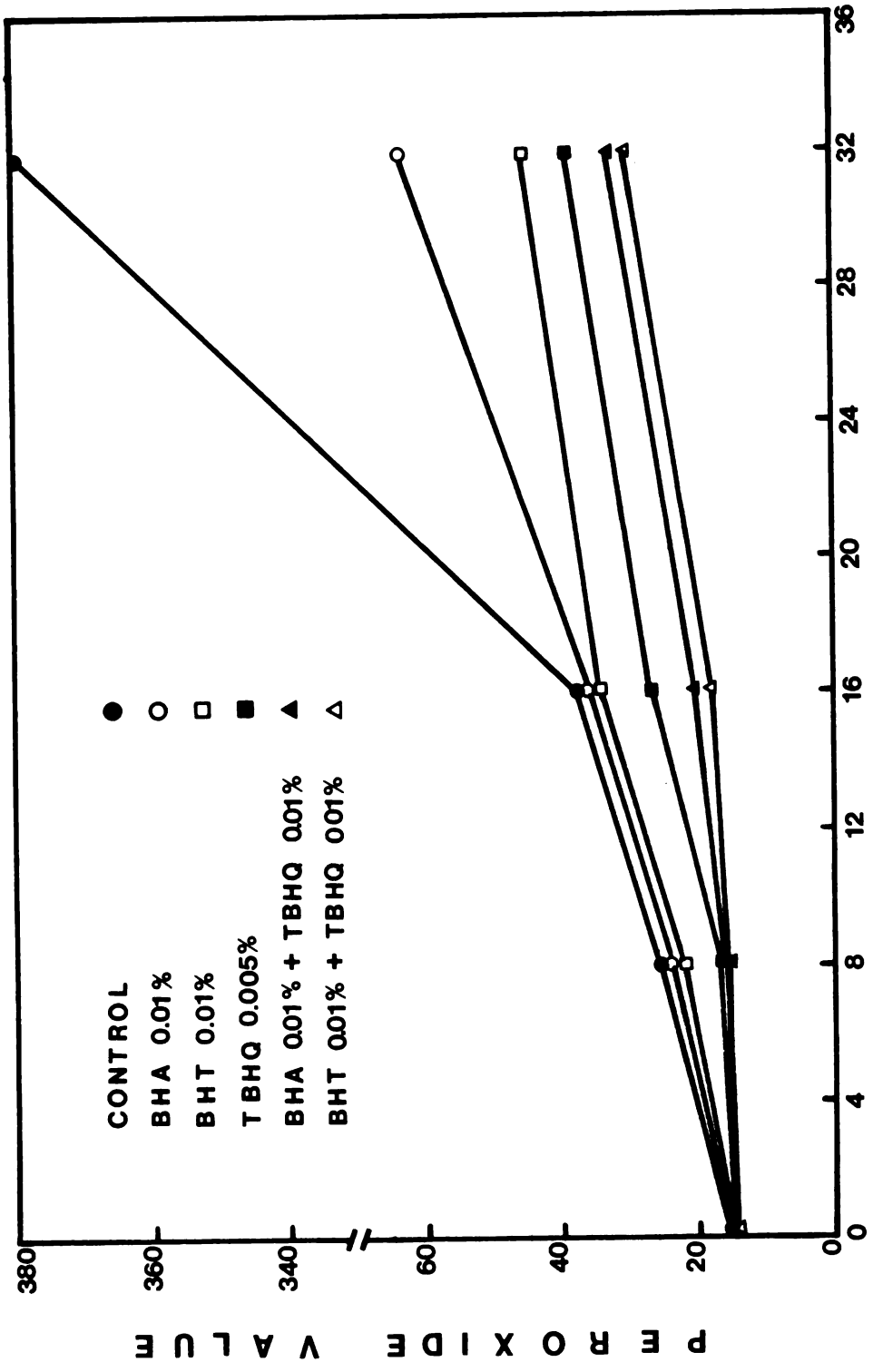

Figure 1. The Effect of Certain Antioxidants on Peroxide Formation in Olive Oil No. 1 (Oven Test at 65°C).

Table 4.--Stability Studies with Olive Oil No. 2 Treated with Antioxidants (Oven Test at 65°C).

Antioxidant (wt %)	Stability of Olive Oil[a]	
	Oven Days[b]	Protective Factor[c]
None (control)	20	1.00
0.01 BHA	31	1.55
0.01 BHT	25	1.25
0.005 TBHQ	24	1.20
0.01 TBHQ	31	1.55
0.01 BHA + 0.005 TBHQ	34	1.70
0.01 BHT + 0.005 TBHQ	28	1.40
0.01 BHA + 0.01 TBHQ	40	2.00
0.01 BHT + 0.01 TBHQ	33	1.65
0.02 PG	60	3.00
0.005 Citric Acid	20	1.00

[a]Initial Peroxide value 35.

[b]Time in days required for olive oil to reach a peroxide value of 120.

[c]Protective Factor is expressed as:

$$\frac{\text{stability of the sample containing antioxidant}}{\text{stability of the control sample}}$$

Good results were also obtained in the oven test when 0.02% PG was added to the sample. The addition of 0.005% citric acid had absolutely no effect on the results (Table 4).

Since olive oil No. 1 and No. 2 had relatively high initial peroxide value, (16) and (35) respectively, a third quantity of olive oil was obtained from Greece which had a lower peroxide value (12) than the other two, but still somewhat higher than anticipated. This olive oil (No. 3) was used only for oven studies. In the oven test which was run with this oil, antioxidants were used at the maximum permitted level (0.02%).

Results obtained from this test are shown in Table 5 and Figure 2. These results indicate that olive oil No. 3 which had the lowest degree of oxidative degradation (as determined by the initial peroxide value), showed lower stability than No. 1 and No. 2 (Tables 3, 4 and 5). It was found that BHA and PG were quite effective in increasing the stability of olive oil No. 3, while TBHQ and BHT were only slightly effective.

The difference in the oven stability of the three samples of olive oil might be due to the fact that the amount of natural antioxidants present in olive oil (tocopherols and phenols) differed from one sample of oil to another. It might also be due to the percentage variations of unsaturated fatty acid of the oil (Catalano, 1971), which variations in turn depend on the area where the trees are grown (Petruccioli, 1966) and or on the difference in the degree of maturation of the fruits (Swern, 1964).

Table 5.--Stability Studies with Olive Oil No. 3 Treated with Antioxidants (Oven Test at 65°C).

Antioxidant (wt %)	Stability of Olive Oil[a]	
	Oven Days[b]	Protective Factor[c]
None (control)	16	1.00
0.02 BHA	27	1.68
0.02 BHT	20	1.25
0.02 TBHQ	20	1.25
0.02 PG	26	1.62

[a]Initial Peroxide value 12.

[b]Time in days required for olive oil to reach a peroxide value of 120.

[c]Protective Factor is expressed as:

$$\frac{\text{stability of the sample containing antioxidant}}{\text{stability of the control sample}}$$

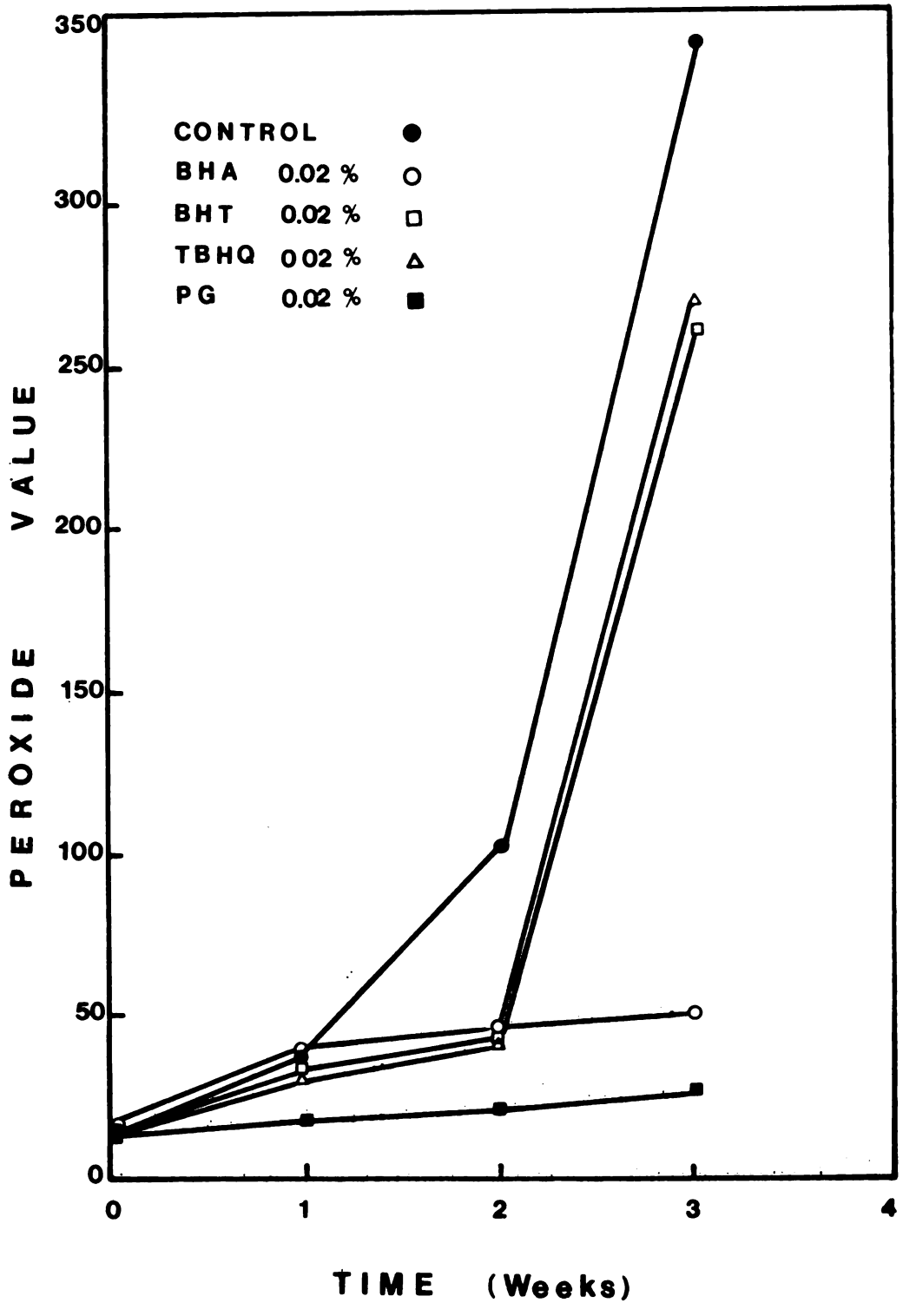

Figure 2. The Effect of Certain Antioxidants on Peroxide Formation in Olive Oil No. 3 (Oven Test at 65°C).

Advanced maturation of the fruits also affects the free fatty acid content which in turn, as Suarez (1975) reported, affects the sensory characteristics of the oil.

The Effect of Sample Size on the Rate of Oxidation

An experiment was run in order to determine the effect of sample size on the rate of oxidation. Two oil samples of different sizes, 50 g and 100 g, were used. Table 3 shows that at 65°C a decrease in the sample size from 100 g to 50 g resulted in apparent lower stability for the control samples and those containing anti-oxidants. This may be due to the difference in surface-volume ratio between the two samples. This observation is in agreement with the finding of Ewbank and Gould (1942). Studying the effect of the sample size under accelerating conditions, they found that a decrease of the size of the sample markedly affected the rate of oxidation.

The data in Table 6 also show that in the oven test run at 100°C, the 50 g samples reached higher peroxide value than the 100 g samples after one week. Further examination of peroxide formation indicated a decrease in peroxide value. This may be due to the fact that hydroperoxides were decomposed at a higher rate than they were fomred at 100°C.

Privett and Quackenbush (1954) studied the destruction of hydroperoxides of lard at 100°C under vacuum and found a 50% loss after about 14 hours. Cooney et al. (1958) demonstrated that hydroperoxides are considered unstable products, especially at temperatures higher than 100°C.

Table 6.--The Effect of Sample Size on the Stability of Olive Oil No. 1 Treated with Antioxidants (Oven Test at 100°C).

Antioxidant (wt %)	Peroxide Values After 1 Week[a] (Sample Size in g)	
	100	50
None (control)	214	263
0.01 BHA	211	275
0.01 BHT	216	287
0.005 TBHQ	204	257
0.01 BHA + 0.01 TBHQ	182	232
0.01 BHT + 0.01 TBHQ	190	241

[a]Initial peroxide value 16.

Results obtained from the oven studies indicate that the effect of antioxidants differed from one sample of oil to another and their effectiveness depended on the concentration. In other words, equal amounts (0.01%) of BHA and TBHQ used in the oil No. 2 resulted in equal stability while, whereas if double the quantity (0.02%) of the forementioned antioxidants was used in olive oil No. 3, different levels of stability were noted with BHA being superior (Tables 4 and 5).

The Effect of Antioxidants on the Storage Stability of Olive Oil

In this study the effect of antioxidants BHA, BHT and TBHQ used alone and in combinations in olive oil No. 2 was examined for two sets of samples, one stored at room temperature (20-28°C) and the other at 50°C.

The results were based on the measurements of peroxide value (PV), absorbance at 233 nm, and thiobarbituric (TBA) acid determinations at 532 nm, and 535 nm. Measurements of the peroxide value were taken once a week for the first month, but since the changes in this value were not considerable it was decided to make the analysis once every other week for the rest of the period. The ultraviolet absorption measurements and the thiobarbituric (TBA) acid test, were begun four and six weeks respectively after this study was initiated and were repeated every two weeks.

Oxidation of Olive Oil at Room Temperature in the Dark

The peroxide values obtained as a result of storage at room temperature are presented in Tables 7 and 8. The data indicate that all samples stored at room temperature ranging from (20-28°C) had almost the same peroxide value at the end of the storage period as they did at the beginning, even though they varied widely during the course of the study.

The reason that the final peroxide value of these samples was almost the same as that of the intial, might be due to the fact that the oil used was virgin containing chlorophyll, which according to Interesse et al. (1971) acts as an antioxidant in the dark. Gutfinger et al. (1975) found that olive oil stored in the dark at room temperature showed a reduction in peroxide value after a year of storage while a slight increase occurred in absorbance at 232 nm.

Results obtained from this study make it obvious that antioxidants used in samples stored at room temperature in the dark had no effect on the outcome since no difference in peroxide value

Table 7.--Peroxide Values Obtained from Olive Oil No. 2 Treated with Antioxidants and Stored at Room Temperature (20-28°C).

Storage Time (Weeks)	Antioxidant Treatment					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.01%	TBHQ 0.005%	CA 0.005%
0	35	35	35	35	35	35
1	41	42	41	41	40	40
2	56	66	66	64	67	42
3	41	40	39	39	39	40
4	40	39	39	39	38	38
5	52	45	52	45	51	51
6	41	42	41	40	40	40
8	51	51	48	50	53	51
10	51	51	51	50	49	51
12	44	45	44	41	42	41
14	47	46	46	43	43	45
16	41	42	40	38	39	41
18	37	36	35	34	34	36
20	38	38	36	34	34	36
22	37	37	36	34	34	36

Table 8.--Peroxide Values Obtained from Olive Oil No. 2 Treated with a Combination of Antioxidants and Stored at Room Temperature (20-28°C).

Storage Time (Weeks)	Combination of Antioxidants				
	Control	0.01% BHA +0.005% TBHQ	0.01% BHT +0.005% TBHQ	0.01% BHA +0.01% TBHQ	0.01% BHT +0.01% TBHQ
0	35	35	35	35	35
1	41	41	42	42	41
2	56	66	67	67	65
3	41	39	40	39	40
4	40	38	38	39	39
5	52	49	50	50	49
6	41	41	41	41	40
8	51	49	53	50	51
10	51	49	50	48	49
12	44	42	40	40	39
14	47	44	44	42	44
16	41	40	39	38	39
18	37	34	35	34	34
20	38	34	34	34	34
22	37	34	34	34	34

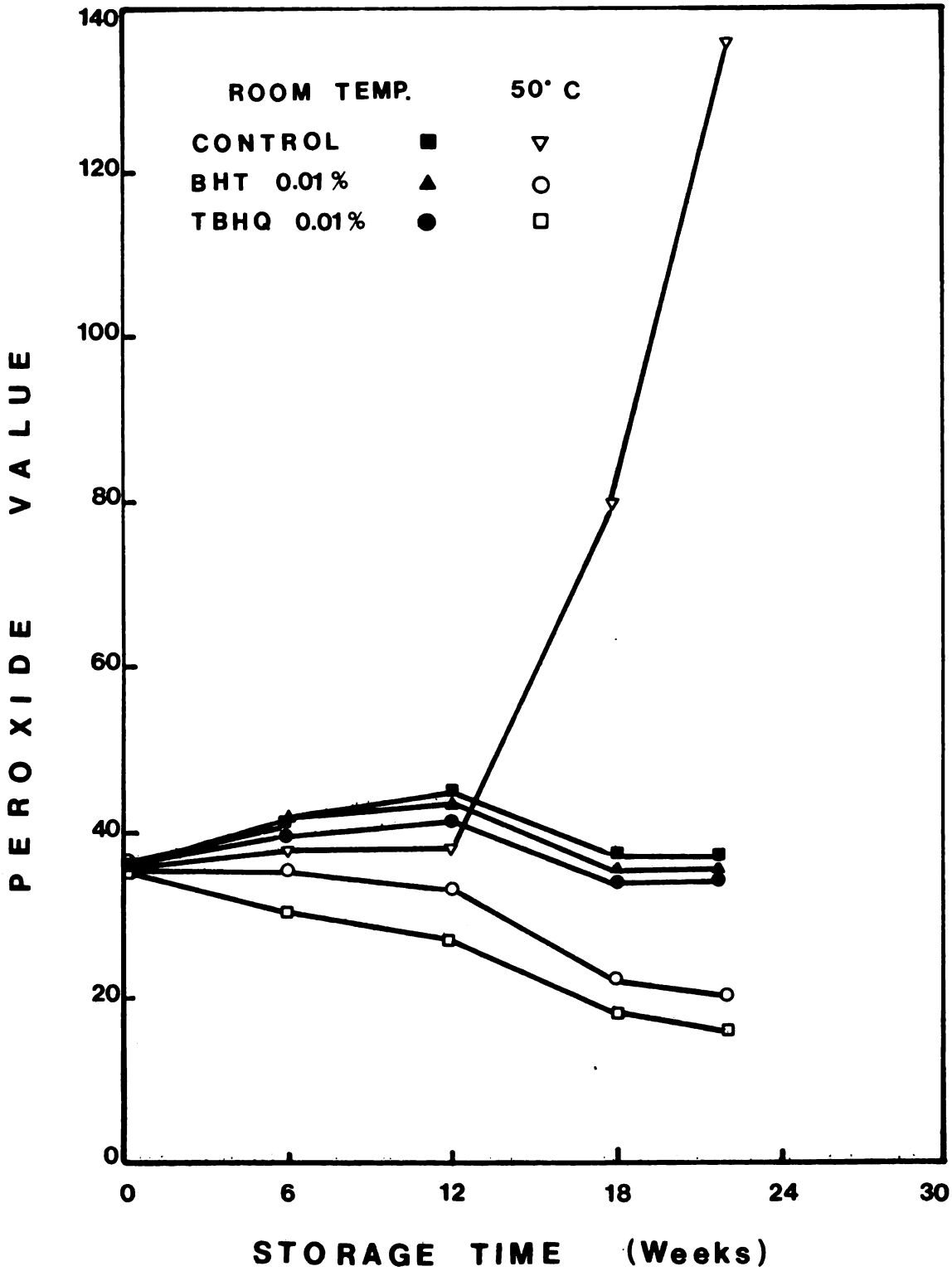

Figure 3. Peroxide Formation in Olive Oil No. 2 Containing Antioxidants.

between the control and the antioxidant treated samples was observed (Figure 3). The addition of 0.005% of citric acid alone brought about no changes.

Removal of Interference Pigments by a Chromatographic Column

Caldwell and Grogg (1955) observed the presence of yellow interfering pigments in the TBA reaction solution and Yu and Sinnhuber (1962) demonstrated two methods that could be used for the separation of these pigments. One of these methods, the chromatographic column separation, was used in this study in order to determine any interference due to the presence of yellow pigments. This method showed that although yellow pigments were present, no significant difference in the absorption values of these pigments was observed between the control and the samples containing antioxidants, during the first month. All other TBA determinations were therefore performed without passing the solution through the column.

The presence of the yellow pigments in the TBA reaction mixture, may be attributed to the fact that olive oil contains carotenoids and carotenes. Vitagliano (1960) reported that the olive oil content of the above pigments is 38-956 and 33-310 γ/100 g oil respectively. Figure 4 illustrates the absorption spectrum of yellow pigments present in olive oil No. 2.

Tables 9 and 10 present the TBA absorption values at 532 nm and 535 nm, obtained from samples stored at room temperature in the dark. In all cases the absorbance at 532 nm exceeded the absorbance at 535 nm and generally all TBA absorption values were lower than in the case of samples stored at 50°C (Tables 9, 10, 14, and 15).

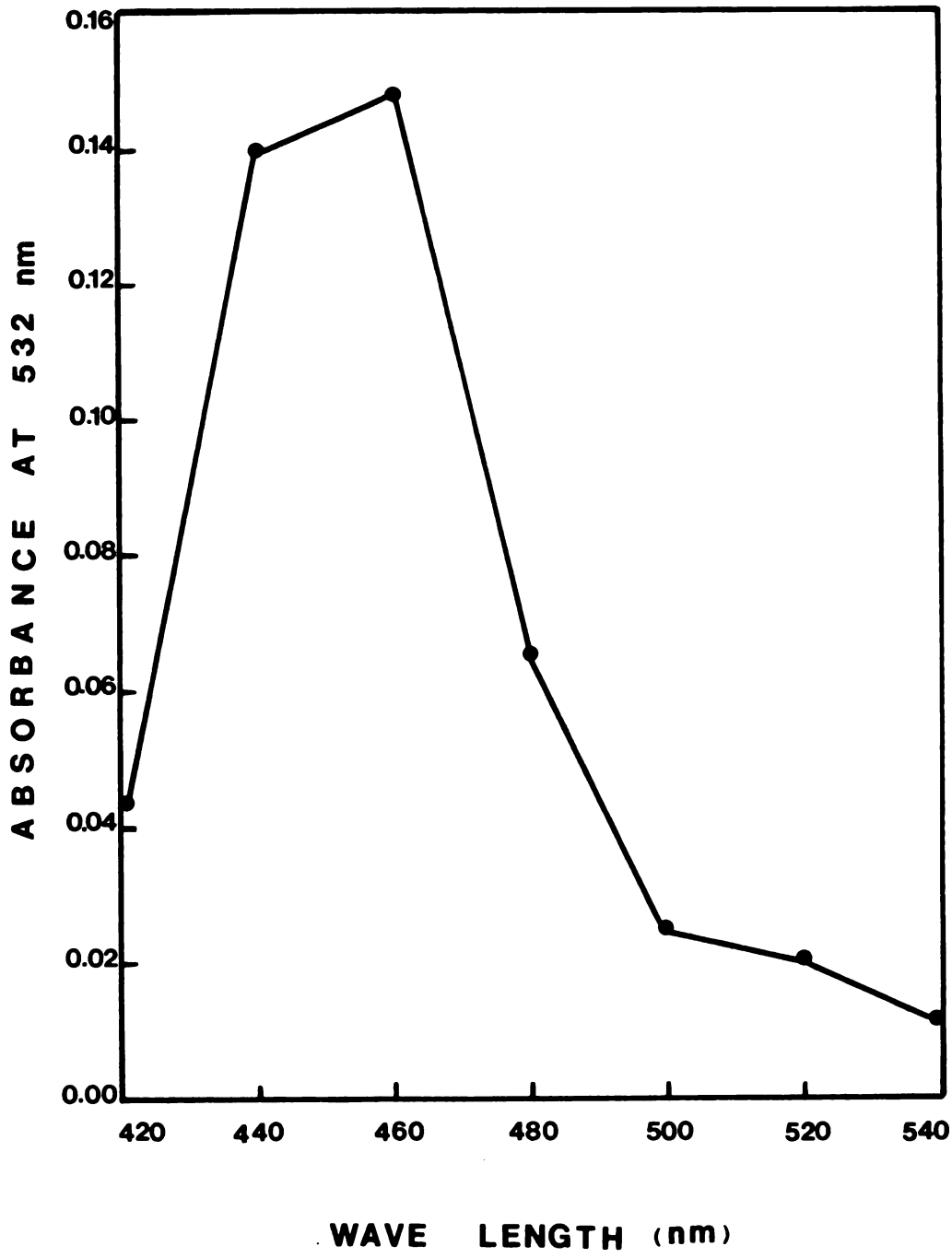

Figure 4. The Absorption Spectrum of Yellow Pigments Present in Olive Oil No. 2.

Table 9.--TBA Absorption Values Obtained from Olive Oil No. 2 Treated with Antioxidants and Stored at Room Temperature (20-28°C).

Storage Time (Weeks)	Absorbance at 532 nm					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.01%	TBHQ 0.005%	CA 0.005%
6	0.217	0.210	0.186	0.119	0.121	0.179
8	0.248	0.219	0.157	0.122	0.130	0.236
10	0.150	0.144	0.123	0.074	0.075	0.205
12	0.216	0.203	0.161	0.093	0.097	0.191
14	0.209	0.192	0.161	0.087	0.104	0.310
16	0.190	0.220	0.150	0.078	0.096	0.220
18	0.187	0.185	0.118	0.063	0.069	0.176
20	0.200	0.225	0.179	0.102	0.110	0.238
22	0.274	0.215	0.212	0.124	0.125	0.274
	Aborbance at 535 nm					
6	0.211	0.202	0.177	0.117	0.120	0.175
8	0.236	0.210	0.150	0.118	0.125	0.224
10	0.143	0.139	0.117	0.070	0.069	0.200
12	0.206	0.194	0.154	0.090	0.094	0.182
14	0.199	0.185	0.152	0.083	0.100	0.295
16	0.180	0.210	0.143	0.075	0.094	0.211
18	0.180	0.176	0.114	0.060	0.063	0.168
20	0.191	0.216	0.171	0.098	0.105	0.227
22	0.259	0.206	0.204	0.121	0.122	0.254

Table 10.--TBA Absorption Values Obtained from Olive Oil No. 2 Treated with a Combination of Antioxidants and Stored at Room Temperature (20-28°C).

Storage Time (Weeks)	Absorbance at 532 nm				
	Control	0.01% BHA +0.005% TBHQ	0.01% BHT +0.005% TBHQ	0.01% BHA +0.01% TBHQ	0.01% BHT +0.01% TBHQ
6	0.217	0.128	0.116	0.115	0.113
8	0.248	0.144	0.140	0.127	0.144
10	0.150	0.082	0.075	0.070	0.080
12	0.216	0.095	0.090	0.092	0.083
14	0.209	0.091	0.085	0.090	0.092
16	0.190	0.094	0.103	0.098	0.099
18	0.187	0.073	0.083	0.067	0.069
20	0.200	0.117	0.092	0.084	0.087
22	0.274	0.152	0.121	0.109	0.112
	Absorbance at 535 nm				
6	0.211	0.125	0.112	0.111	0.108
8	0.236	0.140	0.132	0.122	0.140
10	0.143	0.080	0.073	0.066	0.077
12	0.206	0.091	0.086	0.088	0.079
14	0.199	0.087	0.080	0.087	0.088
16	0.180	0.090	0.100	0.094	0.092
18	0.180	0.069	0.079	0.063	0.066
20	0.191	0.112	0.089	0.081	0.082
22	0.259	0.146	0.116	0.104	0.107

Although no significant difference in peroxide values was observed during the 22 weeks of storage period between the control and the samples containing antioxidants and stored at room temperature, TBA absorption values varied widely.

The difference in TBA absorption values between the control and the antioxidant treated samples might be due to the fact that small variation in the peroxide values between the samples was enough to cause considerable differences in TBA absorption values.

Samples containing 0.01% TBHQ exhibited lower TBA absorption values than those containing 0.005% TBHQ, while peroxide values obtained from these two samples throughout the storage period were almost identical.

The fluctuation of TBA absorption values throughout the storage period is probably due to the fact that the malonaldehyde precursor is not a stable product (Tarladgis and Watts, 1960). This also might be attributed to errors made during the analysis.

An attempt was made to measure the absorbance of the oil samples at 233 nm during the storage period, and to learn if there is any correlation between peroxide, TBA and ultraviolet absorption values. Absorption at 233 nm is due to the presence of conjugated double bonds. Angelo et al. (1975) noted that the mechanism causing the peroxidation of polyunsaturated fatty acids produces conjugated diene hydroperoxides (CDHP).

Table 11 shows the results from diene studies on samples stored at room temperature in the dark. Control samples (containing no antioxidant) gave slightly higher ultraviolet absorption values than the samples treated with antioxidants.

Table 11.--Ultraviolet Absorption Values Obtained from Olive Oil No. 2 Treated with Antioxidants and Stored at Room Temperature (20-28°C).

Storage Time (Weeks)	Absorbance at 233 nm					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.01%	TBHQ 0.005%	CA 0.005%
4	0.410	0.415	0.397	0.380	0.379	0.377
5	0.402	0.410	0.407	0.382	0.369	0.372
6	0.383	0.384	0.380	0.370	0.360	0.376
8	0.332	0.332	0.327	0.301	0.295	0.300
10	0.330	0.330	0.315	0.298	0.279	0.296
12	0.266	0.264	0.252	0.248	0.250	0.251
14	0.324	0.300	0.293	0.267	0.269	0.262
16	0.262	0.260	0.249	0.216	0.230	0.259
18	0.427	0.425	0.396	0.374	0.391	0.401
20	0.430	0.430	0.400	0.378	0.380	0.411
22	0.435	0.432	0.410	0.390	0.398	0.420

Storage Time (Weeks)	Control	0.01% BHA + 0.005% TBHQ	0.01% BHT + 0.005% THBQ	0.01 BHA + 0.01% TBHQ	0.01% BHT + 0.01% TBHQ
4	0.410	0.390	.0385	0.388	0.390
5	0.402	0.365	0.371	0.372	0.364
6	0.383	0.374	0.389	0.368	0.375
8	0.332	0.306	0.293	0.314	0.346
10	0.330	0.292	0.286	0.281	0.310
12	0.266	0.258	0.260	0.259	0.252
14	0.324	0.281	0.278	0.250	0.268
16	0.262	0.229	0.239	0.235	0.240
18	0.427	0.374	0.370	0.375	0.376
20	0.430	0.378	0.376	0.374	0.378
22	0.435	0.397	0.395	0.398	0.394

Somewhat unexpectedly in this case, UV absorption values showed a decrease at some points during the storage period while, for the last few weeks, they showed a continuous increase, an indication of the formation of conjugated linoleate hydroperoxides (Table 11).

Oxidation of Olive Oil at 50°C

The effect of elevated temperature on peroxide formation in olive oil No. 2 is shown in Table 12 and 13. Data in these tables indicate that all samples stored at 50°C developed essentially the same hydroperoxide values during the first two weeks of storage.

Up to the tenth week of storage, the samples containing no antioxidants (control) and those containing either 0.01% BHA or 0.01% BHT showed similar changes. From then on the control samples showed a continuous and rapid increase in peroxide formation while the samples with 0.01% BHA and those with 0.01% BHT showed a decrease in peroxide value, which continued until this study was terminated (Figure 5). The effect of 0.01% BHT was found to be slightly superior to that of 0.01% BHA in reducing the peroxide content of the oil.

TBHQ did not prevent peroxide formation during the first two weeks, but it reduced the peroxide content of the oil for the rest of the storage period. The beneficial effect of this antioxidant increased when the quantity used was doubled from 0.005% to 0.01% (Table 12, Figure 5).

When BHA and BHT were used in combination with TBHQ, better results were obtained than when BHA and BHT were used alone. Samples containing 0.01% BHT + 0.005% TBHQ and those containing 0.01% BHA + 0.005% TBHQ showed a decrease in peroxide value which started the

Table 12.--Peroxide Values Obtained from Olive Oil No. 2 Treated with Antioxidants and Stored at 50°C.

Storage Time (Weeks)	Antioxidant Treatment					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.01%	TBHQ 0.005%	CA 0.005%
0	35	35	35	35	35	35
1	42	42	42	41	42	43
2	46	46	45	42	42	46
3	41	42	41	35	39	41
4	40	41	36	34	34	39
6	38	38	35	30	32	36
8	45	45	44	35	39	44
10	40	40	38	31	33	40
12	38	38	33	27	32	39
14	42	31	30	22	26	44
16	48	24	23	18	24	84
18	80	25	22	18	22	99
20	99	24	22	17	20	121
22	136	24	20	16	18	172
24	164	22	18	13	16	190

Table 13.--Peroxide Values Obtained from Olive Oil No. 2 Treated with a Combination of Antioxidants and Stored at 50°C.

Storage Time (Weeks)	Combinations of Antioxidants				
	Control	0.01% BHA +0.005% TBHQ	0.01% BHT +0.005% TBHQ	0.01% BHA +0.01% TBHQ	0.01% BHT +0.01% TBHQ
0	35	35	35	35	35
1	42	41	41	41	41
2	46	43	44	43	44
3	41	38	38	39	38
4	40	36	35	35	34
6	38	34	32	32	31
8	45	40	40	39	37
10	40	40	35	32	32
12	38	32	30	27	25
14	41	28	24	21	22
16	48	25	23	20	21
18	80	23	20	19	18
20	99	22	19	17	17
22	136	19	18	16	16
24	164	17	15	14	14

eighth and tenth week respectively and continued to the end of the storage period (Table 13, Figure 6). When the quantity of TBHQ combined with BHT or BHA was increased from 0.005% to 0.01% the antioxidant effect was even more pronounced. In other words, samples containing 0.01% BHA + 0.005% TBHQ showed always a higher peroxide value than those containing 0.01% BHA + 0.01% TBHQ and at the end of this study the two samples were found to have a peroxide value of 17 and 14 respectively.

When 0.01% TBHQ was used, the results were almost the same throughout the storage period whether it was combined with BHA or BHT. When TBHQ was used at 0.005%, however, the combination of TBHQ with BHT was more effective than the combination of TBHQ with BHA (Table 13); therefore BHT was more effective than BHA whether it was used alone or combined with 0.005% TBHQ. It is interesting to note that the best results were obtained when 0.01% TBHQ was used alone (Figures 5 and 6).

This superiority of TBHQ over BHT or BHA in various fats and oils agrees with the findings of Cort et al. (1975), and also with the findings of Sherwin and Luckadoo (1970) and Chahine and MacNeill (1974) who observed a higher antioxidant effect with TBHQ than with BHA.

The results obtained when citric acid was used alone were unexpected. This acidic compound seemed to be without significance in the system until the twelfth week of storage; after which, samples treated with 0.005% citric acid developed a rapid peroxide formation at a much faster rate than the control samples (Figure 6). Our results are similar to those obtained by Lea (1944) who observed

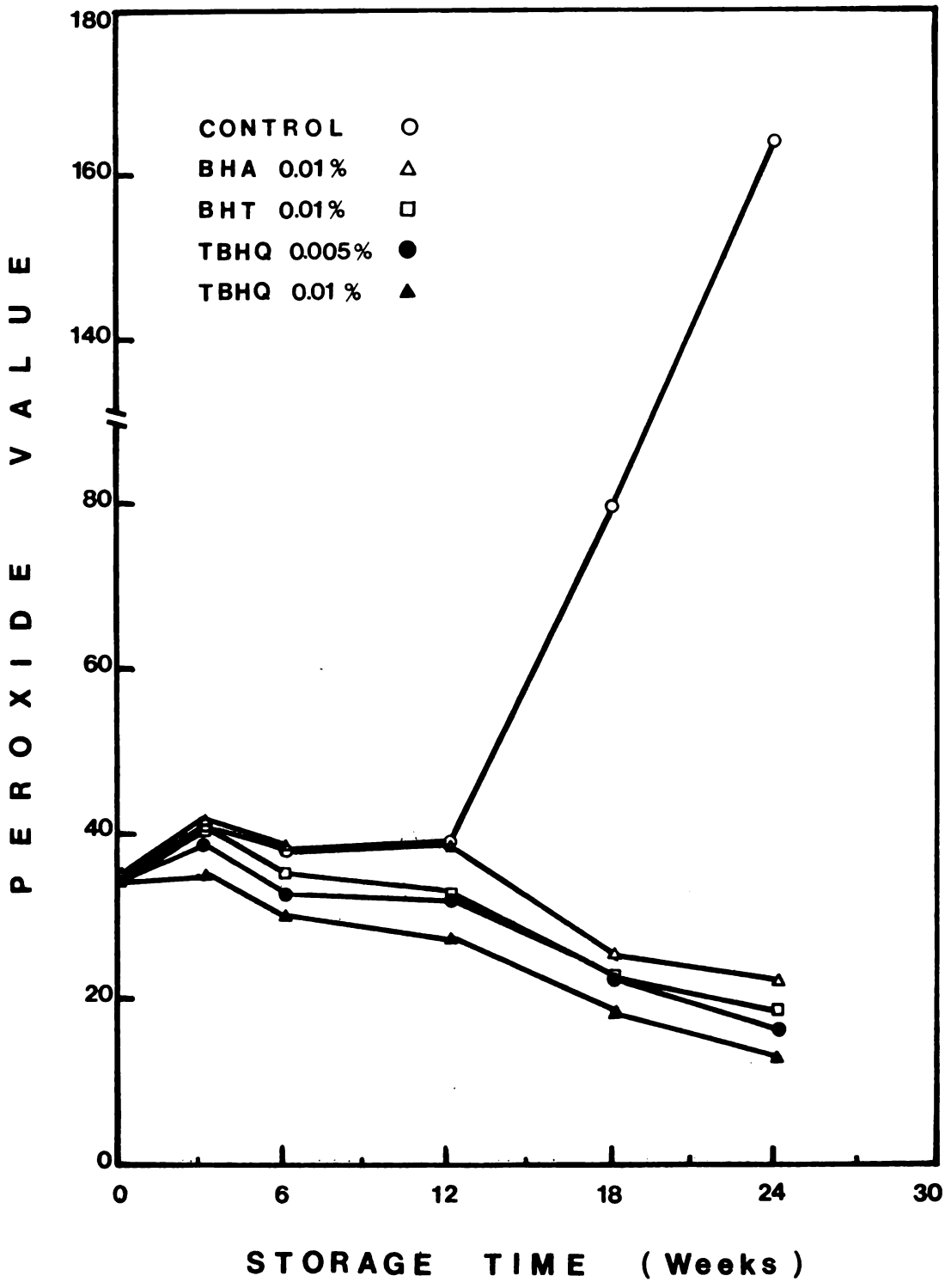

Figure 5. Peroxide Formation in Olive Oil No. 2 Containing Antioxidants and Stored at 50°C.

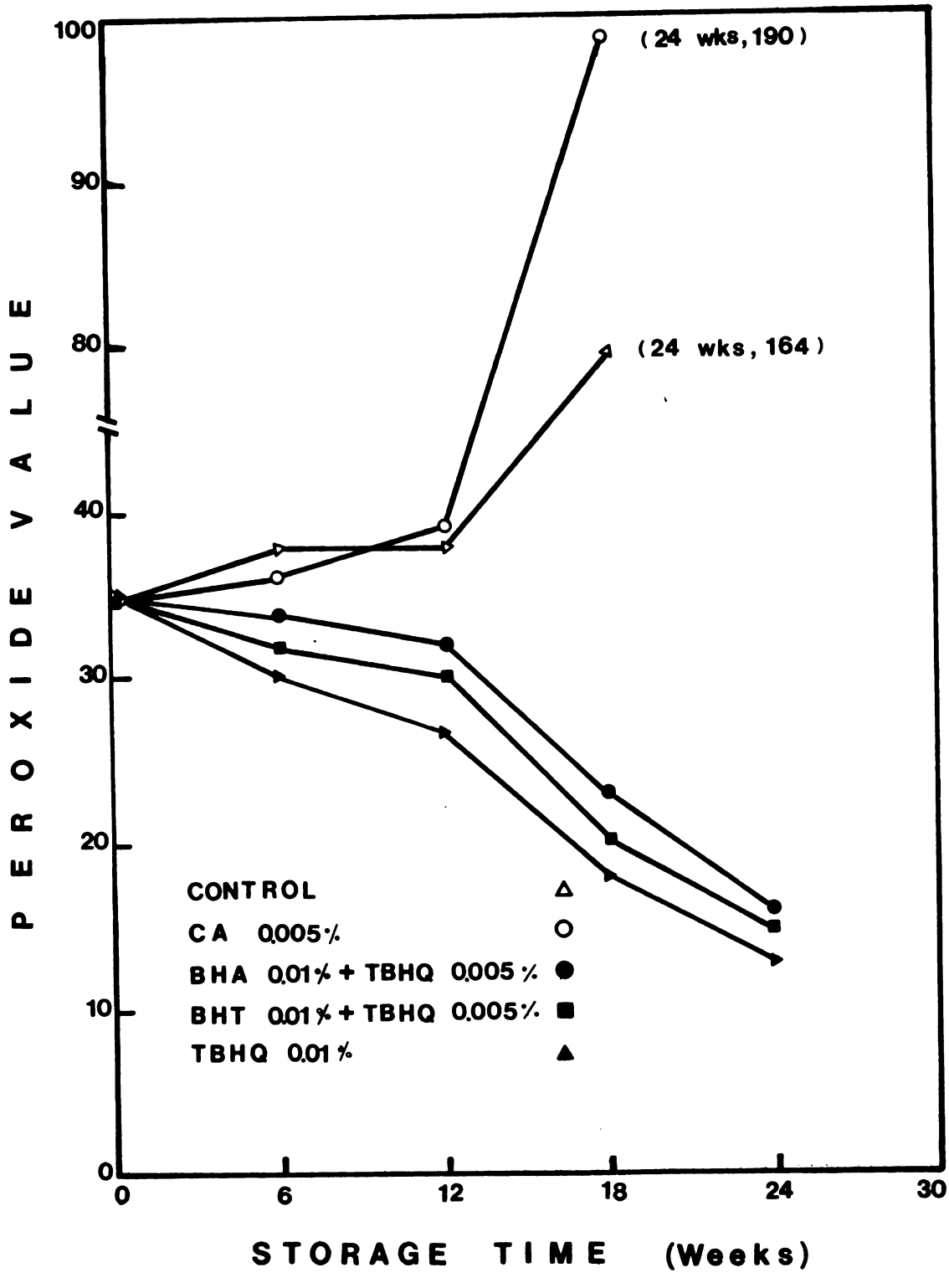

Figure 6. Peroxide Formation in Olive Oil No. 2 Containing Antioxidants and Stored at 50°C.

a prooxidant effect of citric acid when used alone in milk fat. Lemon et al. (1950) also pointed out that the citric acid in substrates free of primary antioxidants does not prevent the oxidation of fatty acids.

In view of the findings in the present study, one may consider that peroxide value is a useful tool for measuring the extent of lipid oxidation and for evaluating the effect of different antioxidants. It is obvious that at room temperature (20-28°C) the use of antioxidants was not found to be of benefit during the time of the tests, while at 50°C the effectiveness of antioxidants was very clear. The peroxide value of all samples containing antioxidants and stored at 50°C was decreased during the storage period and this indicated that the antioxidants probably acted as peroxide decomposers (Dugan, 1961).

The results obtained when the thiobarbituric acid (TBA) test was employed for the samples stored at 50°C, are presented in Tables 14 and 15.

The data show that citric acid added to the oil resulted in TBA absorption values higher than the control samples and those containing antioxidants (Figure 7). This was also true for the peroxide values (Table 12). Citric acid resulted in a gradual increase in TBA values during the first 14 weeks of storage while a rapid increase in these values appeared at the sixteenth week. In the case of the control samples a gradual increase was observed until the sixteenth week with the exception that on the eighth week there was a decrease, while a rapid increase in TBA absorption values started on the eighteenth week of storage. A similar rapid increase was observed in peroxide values at the corresponding weeks (Table 12).

Table 14.--TBA Absorption Values Obtained from Olive Oil No. 2 Treated with Antioxidants and Stored at 50°C.

Storage Time (Weeks)	Absorbance at 532 nm					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.01%	TBHQ 0.005%	CA 0.005%
6	0.339	0.291	0.250	0.150	0.160	0.390
8	0.257	0.281	0.206	0.115	0.230	0.410
10	0.312	0.310	0.220	0.144	0.204	0.500
12	0.333	0.279	0.250	0.118	0.224	0.546
14	0.402	0.194	0.174	0.102	0.171	0.738
16	0.622	0.204	0.162	0.085	0.170	1.080
18	1.214	0.280	0.243	0.125	0.208	1.260
20	1.290	0.299	0.250	0.159	0.214	1.420
22	0.690	0.327	0.271	0.148	0.229	0.895
24	0.723	0.268	0.238	0.122	0.212	0.792
	Absorbance at 535 nm					
6	0.327	0.270	0.237	0.171	0.148	0.372
8	0.243	0.265	0.195	0.109	0.218	0.386
10	0.305	0.297	0.210	0.138	0.197	0.475
12	0.314	0.262	0.237	0.113	0.214	0.506
14	0.378	0.184	0.166	0.097	0.162	0.682
16	0.575	0.196	0.154	0.074	0.156	0.890
18	1.123	0.266	0.231	0.120	0.201	1.236
20	1.210	0.273	0.240	0.151	0.203	1.360
22	0.634	0.310	0.259	0.141	0.219	0.809
24	0.658	0.251	0.225	0.117	0.203	0.725

Table 15.--TBA Absorption Values Obtained from Olive Oil No. 2 Treated with a Combination of Antioxidants and Stored at 50°C.

Storage Time (Weeks)	Absorbance at 532 nm				
	Control	0.01% BHA +0.005% TBHQ	0.01% BHT +0.005% TBHQ	0.01% BHA +0.01% TBHQ	0.01% BHT +0.01% TBHQ
6	0.339	0.201	0.168	0.162	0.144
8	0.257	0.190	0.193	0.119	0.114
10	0.312	0.246	0.214	0.134	0.139
12	0.333	0.205	0.166	0.117	0.123
14	0.402	0.165	0.148	0.107	0.114
16	0.622	0.174	0.149	0.093	0.107
18	1.214	0.218	0.153	0.126	0.112
20	1.290	0.206	0.158	0.136	0.134
22	0.690	0.220	0.194	0.138	0.135
24	0.723	0.222	0.186	0.130	0.131
	Absorbance at 535 nm				
6	0.327	0.184	0.150	0.152	0.141
8	0.243	0.181	0.185	0.114	0.110
10	0.305	0.235	0.187	0.125	0.131
12	0.314	0.195	0.158	0.112	0.116
14	0.378	0.157	0.141	0.101	0.112
16	0.575	0.162	0.140	0.089	0.098
18	1.123	0.209	0.147	0.122	0.103
20	1.210	0.195	0.149	0.128	0.127
22	0.634	0.209	0.186	0.131	0.128
24	0.658	0.211	0.177	0.124	0.129

Both the control and the citric acid treated samples reached their maximum level after 20 weeks of storage and then began declining until the end of this study, as evidenced by the data plotted in Figure 7. This may be due to the fact that malonaldehyde production declines after reaching a peak as Tarladgis and Watts (1960) concluded that the malonaldehyde precursor is not a stable end product.

The variations in TBA absorption values in samples containing antioxidants were probably due to the fact that when the decrease in these values was observed, the destruction of malonaldehyde was greater than its formation. Data in Table 14 show, as was the case with samples stored at room temperature, TBHQ at 0.005% resulted in higher TBA absorption values than when it was used in 0.01%.

As can be observed from Figure 7, BHA resulted in higher TBA absorption values than did BHT, which in turn gave higher values than TBHQ, when those three antioxidants were used in the same concentrations. The results obtained when the combination 0.01% BHA + 0.01% TBHQ was used were very similar to these obtained when 0.01% BHT + 0.01% TBHQ was used (Table 15). This also held true in the case of peroxide value.

The pink color formation in olive oil, despite the fact that Gas Liquid Chromatography (GLC) analysis showed that there were only traces of linolenic acid (Table 2), can be explained if it is considered that olive oil contains linoleic acid and, according to Pryor et al. (1976), malonaldehyde arises from the decomposition of endoperoxides which can be formed in a diene system. Wilbur et al. (1949) and Kenaston et al. (1955) observed formation of TBA color in oxidized linoleate.

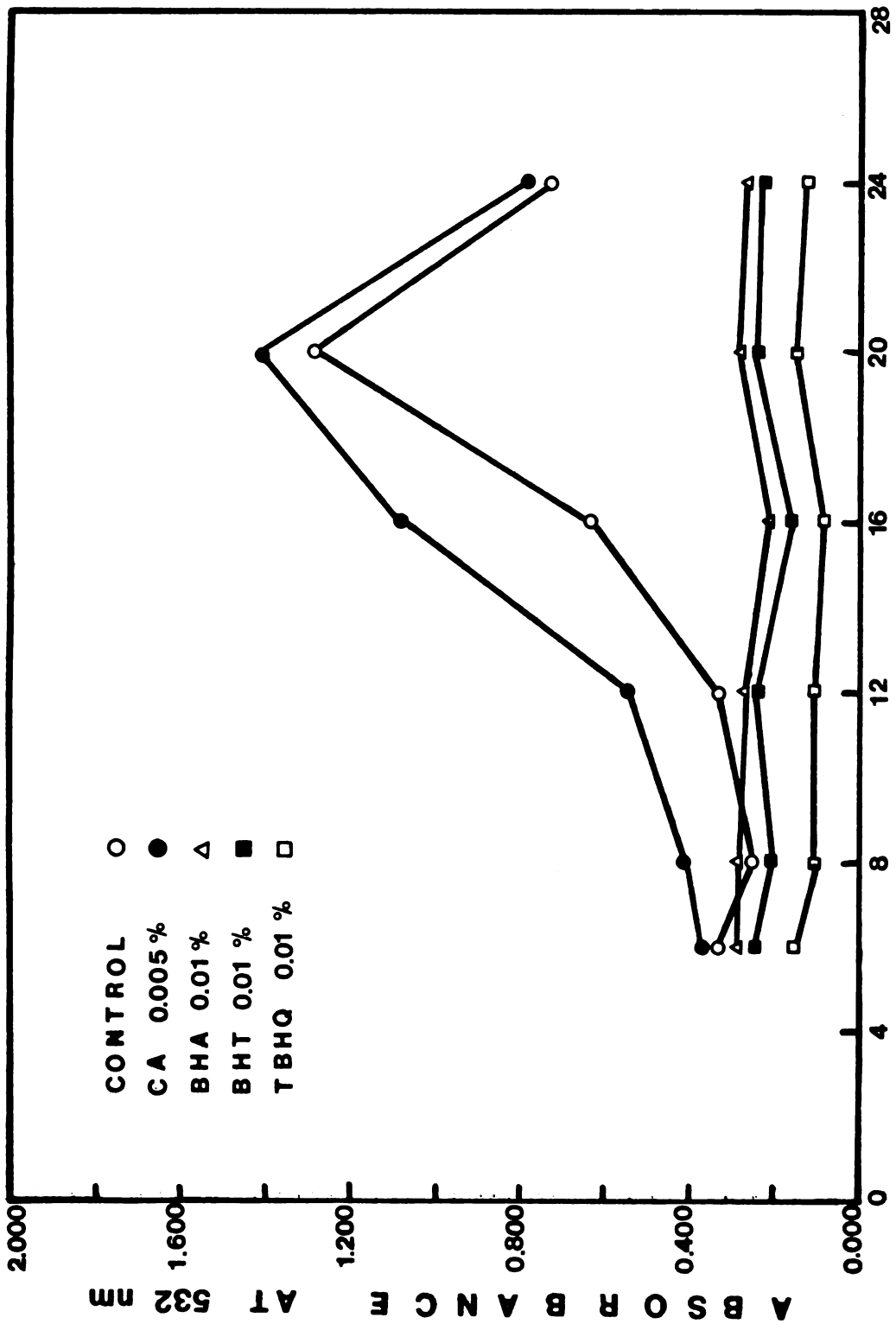

Figure 7. TBA Absorption Values of Olive Oil No. 2 Containing Antioxidants and Stored at 50°C.

TBA absorption values were more meaningful for changes occurring in the samples stored at 50°C than those stored at room temperature (20-28°C). When the TBA is the only test used to determine the level of oxidation, its significance is variable because the products it measures do not increase at all stages in proportion to the degree of oxidation. This is shown in Table 12 and Figure 6 where, after the twentieth week, the degree of oxidation of the control and the citric acid treated samples appeared to increase by the peroxide test while the TBA test showed a decrease in values (Table 14, Figure 7). It seems that TBA color reactants are oxidized further and produce more unstable products or disappear by reacting with themselves or with other components of the system.

Ultraviolet absorption values at 233 nm for the samples stored at 50°C, are presented in Table 16. These samples exhibited better results than those stored at room temperature.

Samples without antioxidant and those containing 0.005% citric acid followed an identical pattern. These samples showed a rapid increase in absorbance at 233 nm from the twelfth week on, due to diene conjugation (Figure 8). A continuous increase in peroxide value was also observed after the twelfth week and this is in accordance with the findings of Ninnis and Ninni (1966, 1968), Montefredine and Luciano (1968) and Bartolomeo and Sergio (1969), who reported that the increase in ultraviolet absorption in olive oil is well correlated with the change in peroxide value. It is evidenced from Figure 8 that the addition of 0.005% citric acid resulted in higher ultraviolet absorption values and this correlated with the PV and TBA absorption values to indicate that citric acid had a prooxidant effect.

Table 16.--Ultraviolet Absorption Values Obtained from Olive Oil No. 2 Treated with Antioxidants and Stored at 50°C.

Storage Time (Weeks)	Absorbance at 233 nm					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.01%	TBHQ 0.005%	CA 0.005%
4	0.425	0.425	0.420	0.405	0.406	0.434
6	0.450	0.450	0.445	0.434	0.441	0.492
8	0.479	0.480	0.440	0.387	0.420	0.485
10	0.425	0.420	0.375	0.278	0.325	0.362
12	0.420	0.402	0.381	0.301	0.340	0.394
14	0.505	0.452	0.396	0.304	0.368	0.550
16	0.624	0.466	0.407	0.350	0.435	0.945
18	1.120	0.624	0.506	0.382	0.471	1.200
20	1.400	0.659	0.560	0.470	0.550	1.500
22	1.700	0.666	0.590	0.486	0.569	1.900
24	1.950	0.670	0.600	0.490	0.572	2.200

Storage Time (Weeks)	Control	0.01% BHA + 0.005% TBHQ	0.01% BHT + 0.005% TBHQ	0.01% BHA + 0.01% TBHQ	0.01% BHT + 0.01% TBHQ
4	0.425	0.416	0.403	0.369	0.385
6	0.450	0.440	0.443	0.440	0.420
8	0.479	0.450	0.448	0.424	0.436
10	0.425	0.348	0.314	0.281	0.290
12	0.420	0.355	0.330	0.290	0.280
14	0.505	0.448	0.382	0.306	0.323
16	0.624	0.412	0.390	0.316	0.328
18	1.120	0.545	0.445	0.413	0.420
20	1.400	0.554	0.460	0.433	0.435
22	1.720	0.579	0.507	0.485	0.489
24	1.950	0.585	0.510	0.488	0.492

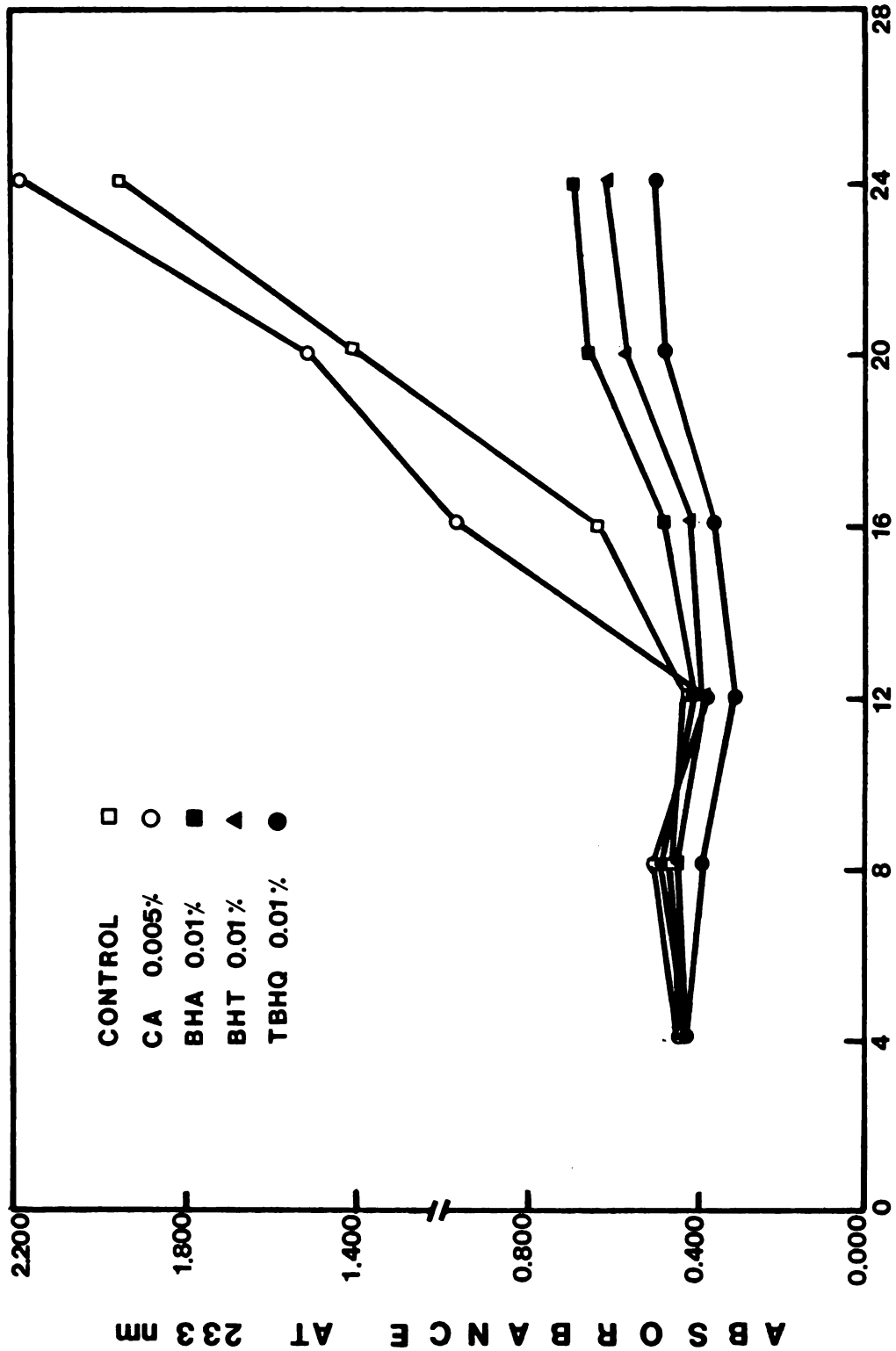

Figure 8. Ultraviolet Absorption in Olive Oil No. 2 Containing Antioxidants and Stored at 50°C.

Although it is not possible to explain the phenomenon, this may be due to the fact that naturally occurring trace metals in olive oil are not readily available for chelation prior to deodorization or heat treatment (Cooney et al., 1958).

This test, just as in the previous two (PV and TBA), revealed that when 0.01% TBHQ was used, lower values were obtained than when 0.005% TBHQ was used. The addition of 0.01% BHA, which exhibited the least effect in the decomposition of hydroperoxides, resulted in higher ultraviolet absorption values. The combination of 0.01% TBHQ with 0.01% of BHA or with 0.01% of BHT which had shown a similarity in peroxide values exhibited almost the same ultraviolet absorption values at 233 nm (Table 16).

Samples containing antioxidants, whether alone or in combination, showed some fluctuation in ultraviolet absorption values during the storage period. These values were in reverse relation to the peroxide values from the tenth week on (Tables 12, 13 and 16) when a continuous decrease in the peroxide values started, probably due to the decomposition effect of the antioxidants (Dugan, 1961).

It is possible that if the oil used did not have such a high initial peroxide value (35), the results might have been different and instead of a reverse relation we might have had a correlation of peroxide value and diene conjugation as was the case with the control and the citric acid treated samples (Figure 9).

Photooxidation of Olive Oil

In this study, some effort was made to determine the effect of light on the oxidation of virgin olive oil and to evaluate the

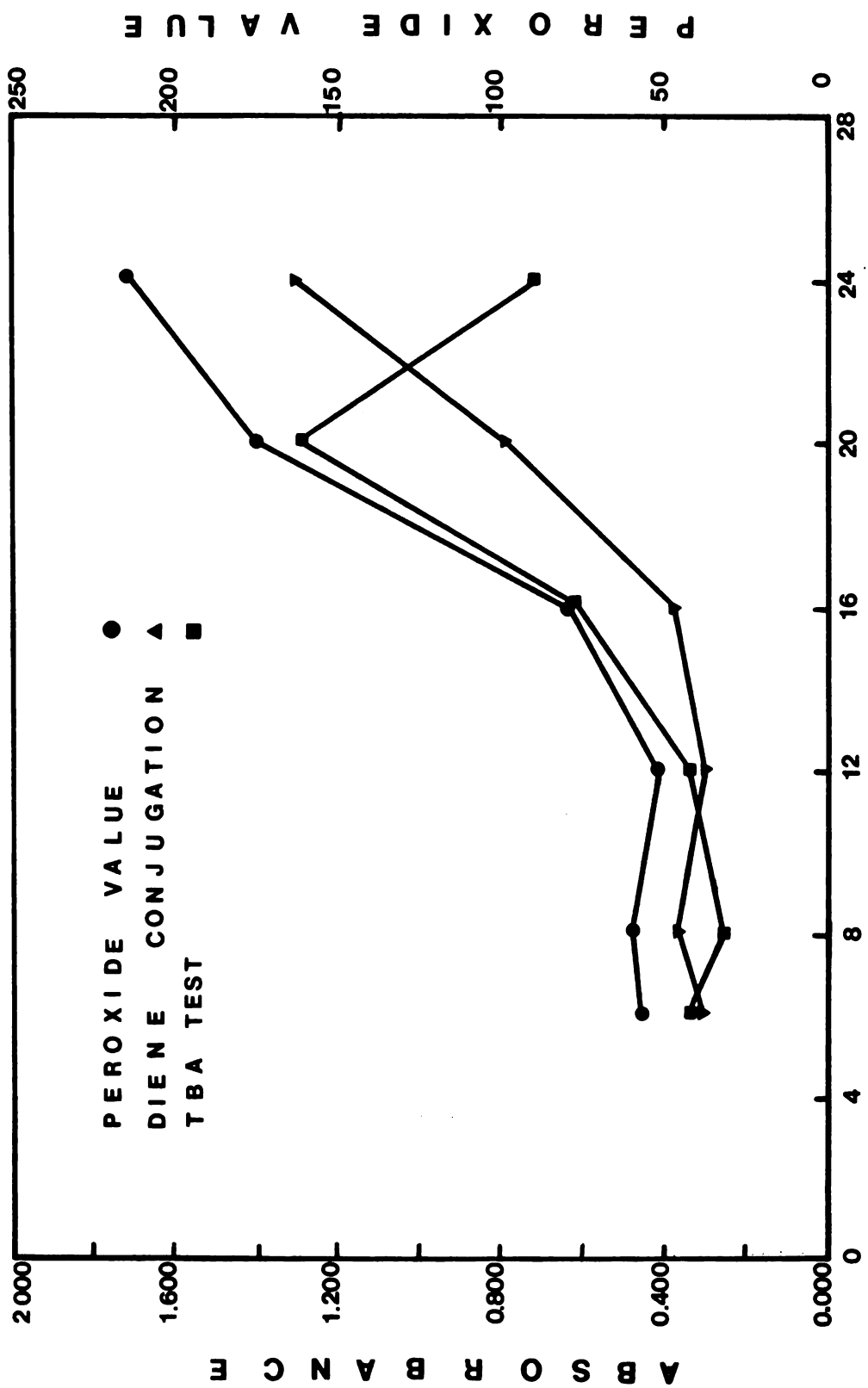

Figure 9. Changes in Olive Oil No. 2 during Storage at 50°C.

usefulness of antioxidants in this case. Lundberg (1962) denoted that the most harmful radiation for accelerating rancidity is ultraviolet and visible radiation which causes decomposition of hydroperoxides.

The results in Tables 17 and 18 demonstrate clearly the effect of light on the oxidative degradation of virgin olive oil. Figure 10 illustrates a significant difference in peroxide formation between samples containing no antioxidants and being exposed to the light and those stored in the dark. These results are in agreement with the findings reported by Pretzch (1970) and Gutierrez (1970).

A major difference in the degree of oxidation of the olive oil exposed to the light and that kept in the dark is probably related to the photocatalytic oxidation of the oil due to the presence of chlorophyll. Fedeli and Brillo (1975) reported that there is no numerical relation between chlorophyll concentration and the rate of autoxidation in virgin olive oil, while Saffar and Deman (1976) found that there is no correlation between the stability of the oil and the degree of unsaturation in photooxidation.

Studying the photooxidation in vegetable oils Coe (1938) reported liberation of nascent hydrogen from the photosensitizer (chlorophyll) which unites with molecular oxygen to form unstable hydroperoxides. Rawls and Van Santen (1970) and Clements et al. (1973) demonstrated that the oxidation of unsaturated oils in the presence of diffused or artificial light is sensitized by chromophoric impurities in the oil (chlorophyll, pheophytin etc.). According to them, photooxidation does not involve a free radical mechanism but the action of singlet oxygen ($^1\Delta_g$, $^1O_2$) which is produced by the

Table 17.--The Effect of Light on the Oxidation of Olive Oil No. 2 Stored at Room Temperature (20-28°C).

Storage Time (Weeks)	Stability of Olive Oil					
	Stored in the Dark			Exposed to the Daylight		
	Peroxide Value	Absorbance		Peroxide Value	Absorbance	
		at 233 nm[a]	at 532 nm[b]		at 233 nm[a]	at 532 nm[b]
6	41	0.383	0.217	118	0.465	0.384
8	51	0.332	0.248	153	0.475	0.434
10	51	0.330	0.150	152	0.494	0.321
12	44	0.266	0.216	167	0.505	0.454
14	47	0.324	0.209	170	0.472	0.630
16	41	0.262	0.190	209	0.558	0.640
18	37	0.427	0.187	214	0.710	0.678
20	38	0.430	0.200	233	0.750	0.705
22	37	0.435	0.274	239	0.792	0.810

[a]Diene Conjugation

[b]TBA Test

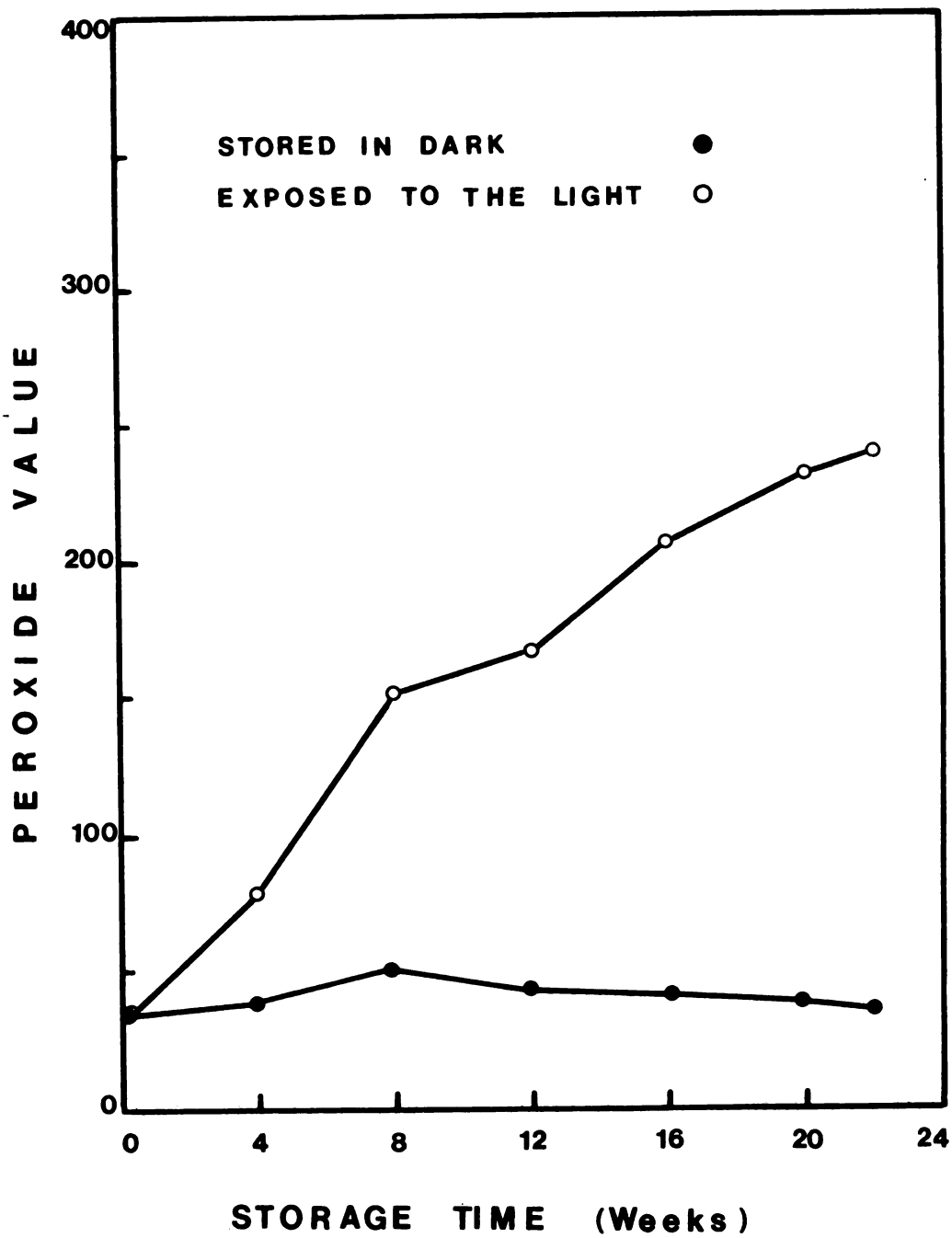

Figure 10. The Effect of Light on Peroxide Formation in Olive Oil No. 2 (Room Temperature Storage).

Table 18.--The Effect of Light on Peroxide Formation in Olive Oil No. 1 Treated with Antioxidants and Stored at Room Temperature (20-28°C).

Storage Time (Months)	Antioxidant Treatment					
	Control	BHA 0.01%	BHT 0.01%	TBHQ 0.005%	0.01% BHA +0.01% TBHQ	0.01% BHT +0.01% TBHQ
0	16	16	16	16	16	16
2	103	101	99	98	99	102
4	165	160	116	163	164	157
6	191	189	123	190	163	161

transfer of excitation energy from chromophoric impurities to oxygen, as is shown by the reaction:

$$Ch \xrightarrow{h\nu} |Ch|^* \xrightarrow{^3O_2} Ch + {}^1O_2$$

This singlet oxygen, which is more active than triplet, reacts rapidly with unsaturated fatty acids to give hydroperoxides which can decompose at room temperature, initiating the free radical mechanism of autoxidation (Foote, 1968 and Kaplan, 1971). Skinner (1976) reported that singlet oxygen reacts in an electrophilic rather than in a free radical fashion.

Figure 11 illustrates that samples containing no antioxidants and exposed to the daylight followed a similar pattern in all three tests employed. The continuous increase in absorbance at 233 nm for the samples exposed to light indicates that conjugated linoleate hydroperoxides were formed during the photooxidation of olive oil.

According to Rawls and Van Santen (1970) conjugated and non-conjugated hydroperoxides are formed as primary oxidation products

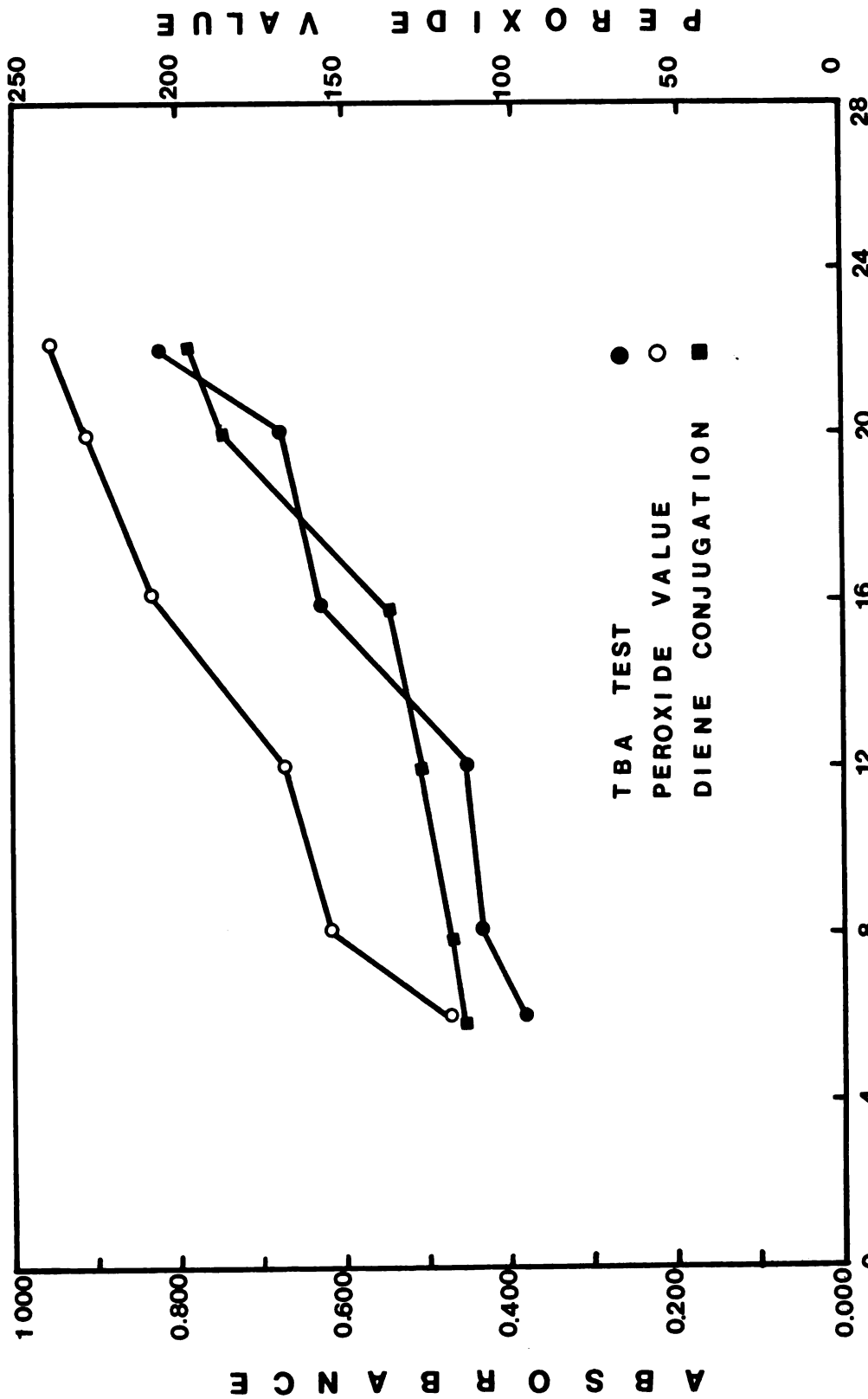

Figure 11. Changes in Olive Oil No. 2 during Storage at Room Temperature in the Presence of Light.

during chlorophyll photooxidation. Since nonconjugated hydroperoxides do not absorb at 233 nm it seems logical that there was an accumulation of more hydroperoxides which remained undetected.

Table 18 and Figure 12 show the results obtained from olive oil No. 1 treated with antioxidants and stored at room temperature in the presence of light. The control samples and those containing antioxidants underwent almost the same oxidative degradation (measured by the peroxide value), throughout the storage period with the exception of the samples containing 0.01% BHT which showed a lower rate of peroxide formation from the second month to the end of the study.

The results of this research are similar to those obtained by Vazquez et al. (1960), Borbola (1963) and Carlsson et al. (1976) in their studies in olive oil. The latter reported that the photooxidation of unsaturated oils is retarded by chelaters which are able to quench singlet oxygen ($^1O_2$). He also reported that alpha-tocopherol present in oil exposed to the light undergoes rapid peroxidation and thus this natural antioxidant has no effect.

Discoloration of the Oil

Changes in the color characteristics of the oil parallel to the extent of oxidative deterioration were realized in our study and they were attributed to the bleaching of chlorophyll as a result of photooxidation.

Ramunni (1964) demonstrated that olive oil stored in colorless glass flasks in the presence of light became rancid very quickly and complete decomposition of chlorophyll and a loss of about 70% of the

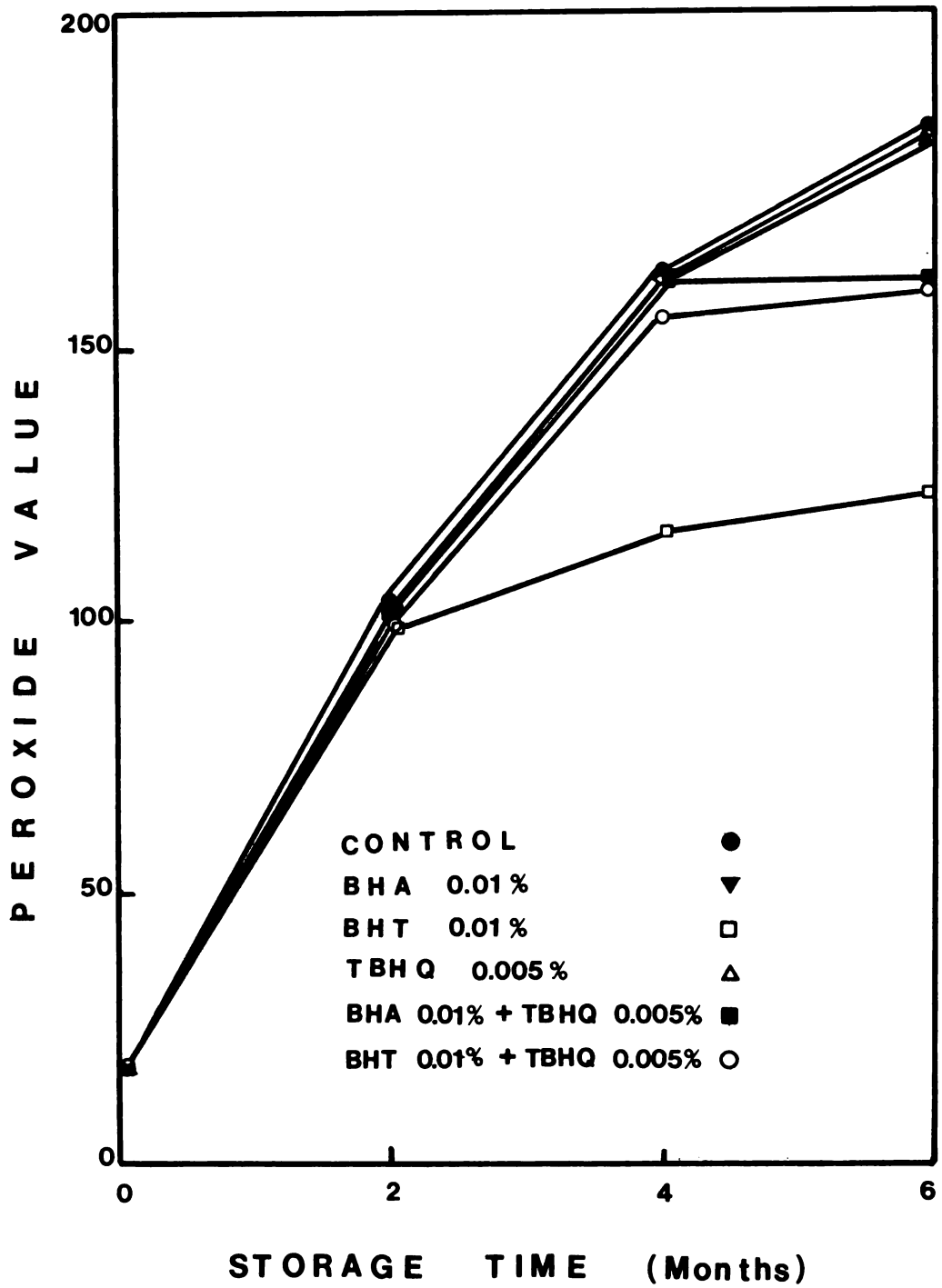

Figure 12. The Effect of Light on Peroxide Formation in Olive Oil No. 1 Containing Antioxidants (Room Temperature Storage).

carotene present, occurred. Sastry et al. (1973) postulated a bleaching mechanism of chlorophyll during oxidation in visible light.

Pictures of the samples used in this study, taken at the end of the storage period are shown in Figures 13, 14 and 15. The sample containing 0.01% BHT maintained its color more than other samples (Figure 13). This sample had the lowest peroxide value when this study was terminated. The sample containing 0.005% TBHQ was able to maintain its color even though it showed almost the same degree of oxidation with the control sample. The presence of 0.01% BHA, however, did not prevent the loss of the oil color.

Figure 14 illustrates the difference in color between untreated samples, one stored in the dark and the other in the presence of light.

The color difference between five of the samples which were stored at 50°C and underwent different degree of oxidation can be observed from Figure 15. It is shown in this figure that TBHQ, which provided the best stability, was able to maintain the original color of the oil. The control sample and the one containing citric acid, however, underwent the highest degree of oxidation and lost most of their original color (Figure 15).

Figure 13. Changes in the Color of Olive Oil Containing Antioxidants and Stored at Room Temperature in the Presence of Light.

Figure 14. Changes in the Color of Olive Oil During Storage at Room Temperature in the Presence of Light.

Figure 15. Changes in the Color of Olive Oil Containing Antioxidants and Stored at 50°C.

SUMMARY AND CONCLUSIONS

Three different samples of virgin olive oil (No. 1, No. 2, and No. 3) with initial peroxide values of 16, 35, and 12, respectively, were used in this study. The effectiveness of the antioxidants BHA, BHT, TBHQ, and PG was evaluated under accelerated conditions (Schaal Oven Test) and other storage conditions.

The development of rancidity in the olive oil under accelerated conditions was measured by the method of peroxide value only, while in the case of other storage conditions, results obtained from peroxide value, diene conjugation and TBA test were compared. The best results were obtained from peroxide values.

The usefulness of the TBA test in olive oil is questionable, since the TBA absorption values of the control and the citric acid treated samples, after reaching a peak, fell to lower levels, while the same samples showed a continuous increase of oxidation when the other two tests were used.

Gas Liquid Chromatography (GLC) analysis for oil No. 1 and No. 2 showed that each had a high percentage of oleic ($C_{18:1}$), low percentage of linoleic ($C_{18:2}$) and traces of linolenic ($C_{18:3}$) acid.

Sample size was found to affect the rate of oxidation; the smaller the size, the higher the rate of oxidation.

The use of antioxidants had a considerable effect on the oven stability of the oil. The degree of effectiveness, however, was found to be different in the three samples of olive oil used. When the antioxidants were used at 0.02% in the oven test the effectiveness was in the following order: BHA > PG > TBHQ and BHT. The most effective combination in the oven stability studies was found to be 0.01% BHA + 0.01% TBHQ.

The use of citric acid alone had no effect on the stability of the oil in the oven test.

Results related to the oil stored at room temperature in the dark indicated that the use of antioxidants under these conditions had practically no significance over the period of the test. The peroxide values, ultraviolet absorption at 233 nm and TBA absorption values, were poorly related in this case.

Samples containing antioxidants and stored at 50°C showed a considerable decrease in peroxide value during the storage period, in contrast with the control and samples containing citric acid which showed a continuous increase. A good correlation between the peroxide value and diene conjugation was obtained in the case of the control and the samples containing citric acid, especially after the fourteenth week of storage.

In the storage conditions at 50°C, in contrast with the oven test, TBHQ was found to be the most effective antioxidant with the order of effectiveness being TBHQ > BHT > BHA. This held true even when the amount of TBHQ used was half as much as that of BHT or BHA. BHA and BHT were more potent when they were used in combination with TBHQ, but still not as effective as TBHQ used alone.

Citric acid did not affect the oxidative stability of the oil stored at room temperature but it did exhibit a negative effect at 50°C.

Exposure of the samples to daylight resulted in rapid oxidation, probably due to the catalytic action of chlorophyll. The usefulness of antioxidants in this case was not apparent. The resulting peroxide, ultraviolent absorption and TBA absorption values were in good agreement in these samples.

BIBLIOGRAPHY

BIBLIOGRAPHY

Angelo, A. J. St., R. L. Ory and L. E. Brown. 1975. Comparison of methods for determining peroxidation in processed whole peanut products. J. Am. Oil Chem. Soc. 59, 34.

Astudillo, M. D., E. Funes and F. Sanz. 1968. Effects of radiation on olive oil. An. Quim. 64, 787.

Badings, H. T. 1970. Cold storage defects in butter and their relation to the autoxidation of unsaturated fatty acids. Thesis, Wageningen.

Bartolomeo, D. and V. Sadini. 1959. Ultraviolet spectrophotometry of olive oil. Boll. Lab. Chim. Provinciali. 10, 124.

Bartolomeo, D. and R. Sergio. 1969. Physicochemical features and acidic composition of some meridional virgin olive oils. Riv. Ital. Sost. Grasse. 46, 467.

Bernheim, F., M. L. C. Bernheim and K. M. Wilbur. 1947. The reaction between thiobarbituric acid and the oxidation products of certain lipids. J. Biol. Chem. 174, 257.

Boatella, R. 1975. Analysis of the tocopherols of vegetable oils by gas-phase chromatography. J. An. Bromatol. 27, 287.

Borbolla, J. M. R., Alcala and S. R. V. Larbon. 1963. The influence of light on the alterations on olive oils. III. Virgin oils. Grasas y Aceites. 14, 12.

Caldwell, E. F. and B. Grogg. 1955. Application of the thiobarbituric acid test to cereal and baked products. Food Technol. 9, 185.

Cantarelli, C. and Montedoro. 1969. Phenolic substances present in olive oil. Riv. Ital. Sost. Grasse. 46, 115.

Cantarelli, C. and Montedoro. 1972. Extraction of natural olive antioxidants. Nat. Synth. Zusatzstoffe Nahr. Menschen. Int. Symp. 14th, 84.

Carlsson, D. J., T. Suprunchuk and D. M. Wiles. 1976. Photooxidation of unsaturated oils. Effects of singlet oxygen quenchers. J. Am. Oil Chem. Soc. 53, 656.

Casillo, R. 1968. Quality evaluation of virgin olive oils: Thiobarbituric acid test. Riv. Ital. Sost. Grasse. 45, 753.

Catalano, M. 1971. Stability of olive oil. Effect of fatty acid composition. Ind. Agrar. 9, 388.

Catalano, M. and M. Felice. 1970. Autoxidation of fats. Influence of free fatty acids. Riv. Ital. Sost. Grasse. 47, 484.

Cerutti, G. 1956. Antioxidant action of ascorbyl palmitate in some vegetable oils. Olearia. 10, 130.

Chahine, M. H. and R. F. MacNeill. 1974. Effect of stabilization of crude whale oil with TBHQ and other antioxidants upon keeping quality of resultant deodorized oil. J. Am. Oil Chem. Soc. 51, 37.

Chahine, M. H. and F. A. El-Shobaki. 1966. The stability of oils and fatty foods. IV. The effect of added oleic acid and antioxidants on the oxidative stability of shark liver oil and methyl esters. Grasas y Aceites. 17, 195.

Chipault, J. R. 1962. Autoxidation and antioxidants. Lundberg, W. O., ed. Interscience Publishers, Wiley & Sons, Inc., New York, Vol. II, p. 477.

Clements, A. H., R. H. Van Den Engh, D. J. Frost, K. Hoogenhout and J. R. Nooi. 1973. Participation of singlet oxygen in photosensitized oxidation of 1, 4-Dienoic systems and photooxidation of soybean oil. J. Am. Oil. Chem. Soc. 50, 325.

Coe, M. R. 1938. Photochemical studies of rancidity: The mechanism of rancidification. Oil and Soap. 15, 230.

Cooney, M. P., C. D. Evans, A. W. Schwab and J. C. Cowan. 1958. Influence of heat on oxidative stability and on effectiveness of metal-inactivating agents in vegetable oils. J. Am. Oil Chem. Soc. 35, 152.

Corrao, A. 1966. Autoxidation of virgin and crude oils produced in Sicily. Olearia. 20, 5.

Cort, W. M., J. M. Scott, M. Araufo, W. J. Mergens, M. A. Cannalonga, M. Osadca and H. Harley, D. R. Parrish, W. R. Pool. 1975. Antioxidant activity and stability of 6- Hydroxy-2, 5, 7, 8-tetramethylchroman-2 carboxylic acid. J. Am. Oil Chem. Soc. 6, 174.

Dahle, L. K., E. G. Hill and R. T. Holman. 1962. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch. Biochem. Biophys. 98, 253.

Didenko, N. V., S. G. Dyrikova and A. K. Plisov. 1968. p-Aminosalicylic acid as an antioxidant for fats. Maslozhir Prom. 34, 7.

Dugan, L. R., Jr. 1955. Stability and rancidity. J. Am. Oil Chem. Soc. 32, 605.

Dugan, L. R., Jr. 1957. Antioxidants. "Encyclopedia of Chemical Technology." Inters. Encycl. Inc., New York, Vol. II, p. 588.

Dugan, L. R., Jr. 1961. Development and inhibition of oxidative rancidity of foods. Food Technol. 15, 10.

Dugan, L. R., Jr., L. Marx, C. E. Weir and H. R. Kraybill. 1954. Butylated hydroxytolyene. A new antioxidant for food use. Am. Meat. Inst. Foundation Bull. No. 18.

Dunkley, W. L. and W. G. Jennings. 1951. A procedure for application of the thiobarbituric acid test to milk. J. Dairy Sci. 34, 1064.

Evans, C. D. 1961. "Proceedings of the flavor chemistry symposium." Campbell Soup Co., Camden, N.J.

Ewbank, F. C. and I. A. Gould. 1942. The oven and aeration methods as means of accelerating fat oxidation. Oil and Soap. 19, 205.

Fahmy M. and H. El Said. 1962. Effect of vitamin E on the stability of olive oil. Agr. Res. Rev. (Cairo). 40, 154.

Fedeli, E. and A. Brillo. 1975. Mechanism of autoxidation phenomena in fats. II olive oil. Riv. Ital. Sost. Crasse. 52, 109.

Fedeli, E., A. Brillo and G. Jacini. 1973. Metals affecting the autoxidation of vegetable oils. Riv. Ital. Sost. Grasse. 50, 102.

Foote, C. S. 1968. Photosensitized oxygenations and the role of singlet oxygen. Acc. Chem. Res. 1, 104.

Foresti, B. 1964. Spectrophotometric study of olive oil rancidity. Riv. Ital. Sost. Grasse. 41, 367.

Francesco, F. 1961. Instrumental analysis of oils and fats. III. Visible spectrophotometry. Olearia. 15, 153.

Franjo, M. 1963. Value of the TBA acid number in the investigation of vegetable oils and lard. Kem. Ind. 12, 147.

Gracian, J. and G. Arevalo. 1965. Tocopherols in vegetable oils with special reference to olive oil. Grasas y Aceites. 16, 278.

Gutfinger, T., O. Vatariu, M. Alter, and A. Letan. 1975. Chemical characteristics of Israel olive oils. Grasas y Aceites. 26, 8.

Gutierrez, R. G. Q. 1961. Estudios sobre el enranciamiento del aceite de oliva. X Experiencies sobre la concervation de aceite de oliva refinado envasado en latas. Grasas y Aceites. 12, 234.

Gutierrez, R. G. Q. 1962. Studies on olive oil rancidity. XI Experiments with antioxidants in oils stored in open glass containers at room temperature. Grasas y Aceites. 13, 69.

Gutierrez, R. G. Q. 1963. Ensayos sobre el empleo de "antioxidantes" en la conservacion de aceite de oliva "fino" y envasado en latas. Grasas y Aceites. 14, 50.

Gutierrez, R. G. Q. 1963. On the conservation with "antioxidants" of a blend of olive oil and soya oil 50% filled in open tins. Grasas y Aceites. 14, 249.

Gutierrez, R. G. Q. and J. M. O. Jimenez. 1970. Packing of olive oil in commercial type containers. II Packaging in glass, tin, polyethylene and poly (vinyl chloride) containers. Peroxide number evolution. Grasas y Aceites. 21, 217.

Gutierrez, R. G. Q. and A. V. Romero. 1960. Estudios sobre el enranciamiento del aceite de oliva. XI Comparacion entre las diferentes pruebas para la determinacion del grado de rancidez. Grasas y Aceites. 11, 67.

Hammond, F. G. and F. D. Hill. 1964. The oxidized-metallic and grassy flavor components of autoxidized milk fat. J. Am. Oil Chem. Soc. 41, 180.

Higgins, J. W. and H. C. Black. 1944. Preliminary comparison of the stabilizing effect of several recently proposed anti-oxidants for edible fats and oils. Oil and Soap. 21, 277.

Hill, L. M., E. G. Hammond and R. G. Seals. 1969. Effect of anti-oxidants and synergists on peroxide decomposition in milk fat. J. Dairy Sci. 52, 1914.

Hirahara, F., V. Takai, H. Iwao, 1974. Antioxidative activity of various spices on oils and fats. I Antioxidative activity towards oxidation on storage and heating. Jap. Jour. of Nutr. 32, 1.

Hoffman, G. 1962. 1-Octen-3-ol and its relation to other oxidative cleavage products from esters of linoleic acid. J. Am. Oil Chem. Soc. 39, 439.

Holman, R. T. 1954. Autoxidation of fats and related substances. The chemistry of fats and other lipids. Pargamon Press, London 2, 51.

Holman, R. T. and G. O. Burr. 1946. Spectrophotometric studies of the oxidation of fats. VI Oxygen absorption and chromophore production in fatty esters. J. Am. Oil Chem. Soc. 68, 562.

Hocvat, R. J., W. H. McFadden, N. Hawkins, D. R. Black, W. G. Lane and R. M. Teeter. 1965. Volatile products from mild oxidation of methyl linoleate. Analysis by combined mass spectrometry--gas chromatography. J. Am. Oil Chem. Soc. 42, 1112.

Hove, E. L. and Z. Hove. 1944. A method for estimating total fat-soluble antioxidants based on the relation between fat peroxides and carotene destruction. J. Biol. Chem. 156, 611.

Ingold, K. U. 1967. Principles of metal catalyzed lipid oxidation. A symposium on metal catalyzed lipid oxidation. Marcuse, R., ed. SIK-Rapport Nr. 240, p. 12.

Interesse, F. S., P. Ruggiero and M. Vitagliano. 1971. Autoxidation of olive oil. Effects of chlorophyll pigments. Ind. Agr. 9, 318.

Jimenez, O. J. M. and R. G. Q. Gutierrez. 1970. Packaging of olive oil in commercial type containers. III Preservation in glass, tin, polyethylene, and poly (vinyl chloride) containers. Changes in absorptivity at 232 and 270 nm. Grasas y Aceites. 21, 329.

Kaplan, M. L. 1971. "Singlet" oxygen. Chem. Technol. 1, 621.

Kenaston, C. B., K. M. Wilbur, A. Ottolenghi and F. Bernheim. 1955. Comparison of methods for determining fatty acid oxidation produced by ultraviolet irradiation. J. Am. Oil Chem. Soc. 32, 33.

Kohn, H. I. and M. Liversedge. 1944. On a new aerobic metabolite whose production by brain is inhibited by a pomorphine, emetine, erogotamine, and menadione. J. Pharmacol. 82, 292.

Lea, C. H. 1939. Rancidity in edible fats. (Special Report, Food Investigation Department of Scientific and Industrial Research). Chem. Publishing, New York. No. 46, p. 106.

Lea, C. H. 1944. Experiments on the use of antioxidants in dry edible fats. J. Soc. Chem. Ind., London Trans. 63, 107.

Lea, C. H. 1962. The oxidative deterioration of food lipids. In: Symposium on Foods: Lipids and their Oxidation. Schultz, H. W., E. A. Day and R. O. Sinnhuber, ed. Avi Publi. Co., Westport, Conn., p. 3.

Lemon, H. W., R. M. Knapp and A. Allman. 1950. The effect of citric acid upon the oxidation of peanut oil and of the methyl esters derived from peanut oil. Can. Res. Sec. F. 28, 453.

Lundberg, W. O. 1962. Autoxidation and Antioxidants. Lundberg, W. O., ed. Interscience Publishers, Wiley & Sons, Inc., New York. Vol. II, p. 453.

Lundberg, W. O. 1962. Mechanisms. In: Symposium on Foods: Lipids and their Oxidation. Schultz, H. W., E. A. Day and R. O. Sinnhuber, ed. Avi Publi. Co., Westport, Conn., p. 40.

Mahon, J. H. and R. A. Chapman. 1953. The relative rates of destruction of propyl gallate and butylated hydroxyanisole in oxidizing lard. J. Am. Oil Chem. Soc. 30, 34.

Mavrikos, P., D. Gegiou and G. Iliopoulos. 1972. Polarographic study of peroxides in olive oil. "Prakt. Panelleniou Chem. Syned. 4th Athens." Chem. Chron. 1, 118.

Miric, M. O., Z. V. Cupric and A. F. Damanski. 1964. Cong. Expertise chim, Vol. spec. conf. Commun. Athens 4th, 273.

Montedoto, G. and G. Petruccioli. 1972. Enzymic treatments in the mechanical extractions of olive oil. Acta Vitaminal Enzymol. 26, 171.

Montefredine, A. and L. Luciano. 1968. Quality characteristics of virgin Italian olive oils. Proposal of a new classification of virgin olive oils. Boll. Lab. Chim. Provinciali. 19, 784.

Morrison, R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride-methanol. J. Lipid Res. 5, 600.

Ninnis, L. N. and M. L. Ninni. 1966. Importance of UV absorption (190-220 nm) for the olive oil analysis. Rev. Fr. Corps Gras. 11, 1.

Ninnis, L. N. and M. L. Ninni. 1968. Heat stability of olive oil and its predictability by UV absorption of 232 and 268 nm. Rev. Fr. Corps Gras. 15, 441.

Ninnis, L., M. Ninnis, A. Chatoupis and E. Voudouris. 1962. Tocopherol content of Greek olive oil and its significance for detection of adulteration. Pract. Acad. Athenon. 37, 210.

Notte, E. and N. Romito. 1971. Autoxidation of olive oil. Effects of polyphenols. Ind. Agr. 9, 325.

Official and Tentative Methods of the Am. Oil Chem. Soc. 1974. Free fatty acid determination. Third ed. Vol. I. Ca 5a-40.

Official and Tentative Methods of the Am. Oil Chem. Soc. 1974. Poly-unsaturated acids. Ultraviolet spectrophotometric method. Third ed. Vol. II. cd 7-58.

Olcott, S. H. 1958. The role of free fatty acids on antioxidant effectiveness in unsaturated oils. J. Am. Oil Chem. Soc. 35, 597.

Oliver, G. D., W. S. Singleton and A. E. Bailey. 1944. Molecularly distilled peanut oil antioxidants and pure alpha-tocopherol as stabilizing agents for fats of poor keeping quality. Oil and Soap. 21, 188.

Petruccioli, G. 1966. Physicochemical characteristics of pure olive oil produced in Umbria. Olearia. 20, 24.

Pretzch, G. 1970. Peroxide number. Olive oil studies. Praktikantenbriefe. 16, 86.

Privett, O. S. and F. W. Quackenbush. 1954. Effect of antioxidants on the thermal decomposition of fat peroxide in vacuo. J. Am. Oil Chem. Soc. 31, 281.

Pryor, W. A., J. P. Stanley and E. Blair. 1976. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. Lipids 11, 370.

Ramunni, A. 1964. Storage of olive oil. Ann. Foc. Sci. 30, 1.

Rawls, H. R., P. Van Santen. 1968. Singlet oxygen and the initiation of fatty acid autoxidation. Tet. Letters, 14, 1675.

Rawls, H. R. and P. J. Van Santen. 1970. A possible role for singlet oxygen in the initiation of fatty acid autoxidation. J. Am. Oil. Chem. Soc. 47, 121.

Saffar A. and J. M. Deman. 1976. Stability of edible oils and fats to fluorescent light irradiation. J. Am. Oil Chem. Soc. 53, 473.

Sastry, Y. S. R., P. V. Rao and G. Lakshminarayana. 1973. Bleaching behaviour of chlorophyll substrates under vacuum on exposure to visible light. Oleagineux. 28, 467.

Schorderet, M. 1969. Prevention of autoxidation in galenical preparations by ascorbic acid esters. IV. Comparative study of the efficacy of ascorbic acid esters incorporated into fatty materials and in galenical preparations. Pharm. Acta Helv. 44, 13.

Seely, G. R. 1966. The chlorophylls. Vernon, L. R. and G. R. Seely, ed. Academic Press. New York and London, p. 558.

Sherwin, E. R. and B. M. Luckadoo. 1970. Studies on antioxidant treatments of crude vegetable oils. J. Am. Chem. Soc. 47, 19.

Sinnhuber, R. O., T. C. Yu and T. Chang Yu. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23, 626.

Skinner, K. 1976. Singlet oxygen may form thermally. Chem. and Engin. News 54, 25.

Spannuth, H. T. 1949. Stabilization and antioxidants. J. Am. Oil Chem. Soc. 26, 618.

Stansby, E. M. 1941. Determination of peroxide values for rancidity in fish oils. Ind. and Eng. Chem. 13, 627.

Stine, C. M., H. A. Harland, S. T. Coulter and R. Jenness. 1954. A. modified peroxide test for detection of lipid oxidation in dairy products. J. Dairy Sci. 37, 302.

Suarez, M. M. J. 1975. Manual of Olive Oil Technology. Moreno, M. M. J., ed. Food and Agr. Organ. Unit. Nat. (FAO) Rome, p. 9.

Swern, D. 1964. Bailey's Industrial Oil and Fat Products. Swern, D., ed. Interscience Publishers, Wiley & Sons, Inc., New York, p. 81, 202.

Tadaaki, B. and A. Kazuhito. 1967. Metal blockade and fat oxidation activities effected by hydroxycarboxylic acids. Yukagaku 16, 423.

Tarladgis, B. G. and B. M. Watts. 1960. Malonaldehyde production during the controlled oxidation of pure, unsaturated fatty acids. J. Am. Oil Chem. Soc. 37, 403.

Taufel, K., C. Franzke and G. Heder. 1959. The role of chlorophyll in the oxidation of olefinic fats. Fette Seifen Anstrich. 61, 1225.

Tollin, G. and G. Green. 1960. Light induced single electron transfer reactions between chlorophyll a and quinones in solution. I. Some general features of kinetics and mechanism. Biochem. Biophys. Acta. 60, 524.

Urakami, C., S. Kiyohara, H. Kameyama and H. Tanaka. 1961. Antioxidants for edible oils. The behaviour of egg yolk cephalins in the autoxidation of the methyl oleate fraction of olive oil. Osaka Shir. Daiaga Ku Kas. Kiyo. 9, 1.

Uri, N. 1961. Metal catalysis. Autoxidation and Antioxidants. Lundberg, W. O., ed. Interscience Publishers, Wiley & Sons, Inc., New York. Vol. I, p. 33.

Valentinis, G. and B. Romani. 1960. Changes of rancidity and peroxide number in edible oil. Boll. Lab. Chim. Provinciali. 11, 351.

Vazquez, R., J. M. R. Borbolla and Alcala. 1960. The influence of light on olive oil alterations. Grasas y Aceites. 11, 163.

Vazquez, R. A., J. C. Del Valle and J. L. M. Del Valle. 1973. Determination of total polyphenols in olive oils. Grasas y Aceites. 24, 350.

Vazquez, R. A., J. C. Del Valle and J. L. M. Del Valle. 1975. Polyphenol content and stability of olive oils. Grasas y Aceites. 26, 14.

Vega, M. N., J. M. R. Borbolla and Alcala. 1960. Studies on olive oil refining. II. The influence of the process on the stability of the oil. Grasas y Aceites. 11, 139.

Vioque, A. 1967. Demetalization of edible vegetable oils. A symposium on metal catalyzed lipid oxidation. Marcuse, R., ed. SIK-Rapport. Nr. 240, p. 231.

Vioque, A., M. Pilar Villagram and R. G. Q. Gutierrez. 1959. Stabilizing olive oil by demetalization with ion-exchange resins. Grasas y Aceites. 10, 119.

Vitagliano, M. 1960. Minor constituents of olive oil. Olii Minerali Grassi e Saponi, Coloni e Vernici. 37, 136.

Vitagliano, M. and A. Vodret. 1960. Possible improvements in olive oil production. Olearia. 14, 137.

Wheeler, D. H. 1932. Peroxide formation as a measure of autoxidative deterioration. Oil and Soap 9, 89.

Wilbur, K. M., F. Bernheim and O. W. Shapiro, 1949. The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. Arch. Biochem. 24, 305.

Yu, T. C. and R. O. Sinnhuber. 1962. Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid reaction. Food Technol. 16, 115.