This is to certify that the

dissertation entitled

QUALITY STUDIES ON OLIVE OIL

presented by

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has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Food Science

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Major professor

Date 23 Nov. 1982

MSU is an Affirmative Action/Equal Opportunity Institution O-12771

QUALITY STUDIES ON OLIVE OIL

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A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1982

ABSTRACT

QUALITY STUDIES ON OLIVE OIL

By

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The effect of harvesting processes, of extraction systems and of packaging and storage conditions on the quality of olive oil were studied. The photooxidation of the oil with fluorescent light and the effect that certain substances exert on it, were also investigated.

The harvesting of fruit directly from the tree had little effect on the quality of the oil. Neither hydrolytic nor oxidative deterioration were noticeable during the time that fruits remained on the trees. Collection of fruit from the nets, after natural falling, affected the quality of the oil when fruits remained there longer than a month.

The compared extraction systems (Pieralisi, Hiller and Rapanelli-Sinolea) did not affect significantly the initial quality of the oil. Only the Rapanelli-Decanter system had some effect on the color of the oil. Oil from all extraction processes except the Rapanelli-Decanter oil, exhibited similar resistance to oxidation during storage

in darkness. The degree of oxidation of Rapanelli-Decanter oil was higher than that of oil from other extraction processes and differed significantly ($P=0.05$). The oil obtained by the various systems was oxidized slowly in darkness, more readily in diffused light and even greater in direct sunlight.

Glass materials gave better protection against oxidation than polyethylene plastic bottles in olive oil stored in diffused light.

In the presence of fluorescent light, unbleached olive oil was oxidized to a greater degree than bleached oil. In the absence of light, however, the reverse was observed. The natural antioxidants in olive oil exhibited appreciable effect only in the absence of light.

The addition of chlorophyll a and pheophytin a + b promoted photooxidation of the bleached olive oil. Higher oxidation rates were stimulated by chlorophyll than by pheophytin in the first hours of illumination. From then on the effect of pheophytin was higher. Pheophytin degraded less than chlorophyll during exposure to light. The latter exhibited no antioxidant effect in darkness.

β-Carotene showed more protective effect against oxidation than α-carotene. Ni-chelate exhibited a greater effect than β-carotene in preventing the photooxidation of bleached olive oil containing chlorophyll. In the presence of chlorophyll, D-α-tocopherol did not prevent photooxidation in bleached oil. In the absence of chlorophyll however, α-tocopherol showed some protective effect after 48 hours of illumination.

The presence of conjugated and nonconjugated hydroperoxides indicated that singlet oxygen could have been involved in the photooxidation of olive oil when chlorophyll or pheophytin was present.

Light from a fluorescent light source or as diffused or direct sunlight, appears to be the primary factor bringing about oil oxidation and loss of quality.

DEDICATION

to my wife Ritsa

ACKNOWLEDGEMENTS

My sincere appreciation and thanks are extended to my advisor Dr. L.R. Dugan, Jr. for bringing out the very best in me throughout the period of my studies at Michigan State University.

I am most grateful to Dr. P. Markakis for his assistance, understanding and encouragement during the course of this work. I also express my sincere gratitude to Dr. A.M. Pearson, Dr. L.E. Dawson (acting Chairman) and Dr. D.R. Dilley for their time and effort in serving very effectively on my commitee. Their assistance is really appreciated.

Special appreciation and gratitude is expressed to Dr. I. Gray for providing me all the facilities to work in his lab, for his interest and his advice.

Acknowledgments are extended to the Greek Institute of Subtropical and Olive Trees for giving me the opportunity to do part of the experimental work there.

I am grateful to my friend Dr. Karim Nafisi for his help on the drawing of the figures.

Thanks are extended to Lou Anne Alderman for typing the manuscript and to my fellow students in Lab 311 for generating an amicable atmosphere, and particularly to Ms. S. Cuppett for her technical assistance.

Sincere appreciation is due to my brother Dimitris, my mother-in-law Androniki and my aunt Betty for moral support during the last months at M.S.U.

Finally my deepest gratitude is given to my children, Kostas and Ioanna for their patience while I was away completing my studies and to my wife, Ritsa, for her assistance, and sacrifices made during the course of this work.

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INTRODUCTION

Olive oil is the oil extracted from the fruits of the tree Olea europea. Cultivation of the olive tree probably commenced in the Eastern Mediterranean region approximately 6,000 years ago.

While most of the world supply of olive oil today is produced in the Mediterranean countries some olive oil is also produced in California. Olive oil is a traditional staple food for Mediterranean people. Greeks consume more calories from olive oil than any other nation.

Good quality olive oil is characterized by a fragrant and delicate flavor. It is almost unique among the vegetable oils in that it can be consumed in its natural form without any treatment, thus conserving the vitamins, essential fatty acids and other natural nutrients.

Unfortunately, not all olive oil produced in the world is of good quality. Thus, vast quantities must be refined because of high free fatty acid content and or other disagreeable characteristics. Refined olive oil has almost entirely lost the properties which differentiate it from other edible vegetable oils and which give it a superior value.

Olive oil represents a small percentage of the world production of vegetable oils and cannot compete with them in price. Therefore it has to compete in quality.

Like other vegetable oils, olive oil undergoes hydrolytic and oxidative deterioration which affect its quality and leads to the modification of its important organoleptic characteristics and affects its nutritional value as well.

Hydrolytic deterioration of olive oil is almost always the consequence of faulty handling of the fruit, while oxidative deterioration is due to oxygen as mitigated by light, temperature, and other factors.

In recent years, there has been renewed interest in the role of light in the oxidation of vegetable oils containing natural pigments, known as photosensitizers. There has been increasing evidence that singlet oxygen can react directly with the double bonds in unsaturated fatty acids to produce hydroperoxides. Since olive oil contains naturally occuring photosensitizers, it may be susceptible to increased oxidation upon exposure to light.

The present study was designed to gain knowledge of olive oil quality deterioration before processing, during processing and during storage. The major objectives were: to study the quality changes in olive oil before extraction, to study the effect of extraction systems on the quality of olive oil, and to study the effect of packaging and storage conditions on olive oil quality. Special emphasis was placed on the photooxidation of olive oil and the effect that several natural constituents or added substances exert on it.

LITERATURE REVIEW

Fatty Acid Composition of Olive Oil

Fatty acid composition of olive oil, as well as of other vegetable oils, varies widely depending on the subcultivar and climatic conditions of the area where the trees are grown (Amellotti *et al.*, 1973; and Vitagliano, *et al.*, 1961).

The major fatty acids in olive oil, as determined by gas chromotography, are oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and palmitic ($C_{16:0}$). Christakis *et al.* (1980) reported that analysis of 3,000 samples of Greek olive oil, nearly 1,000 samples of Italian olive oil and substantial numbers of Spanish, Argentine, Tunisian, Portuguese and American olive oil samples established the range of oleic acid between 54.0 to 93.5%. The range of linoleic acid varied from 1.0 - 23.6%, palmitoleic 0.2 - 5.5%, palmitic 7.1 - 21.1% and stearic 0.3 - 3.8% of the total fatty acids. In spite of the substantial variability, oleic acid remains the predominant fatty acid.

Two types of oils can be described based on fatty acid composition: one has low linoleic, low palmitic and high oleic acid content; the second has relatively high linoleic and palmitic, and a lower percentage of oleic acid (Iverson and Firestone, 1965).

In addition to the major fatty acids cited above, traces of myristic ($C_{14:0}$), lauric acid ($C_{12:0}$), arachidic ($C_{20:0}$), eicosenoic ($C_{20:1}$) and small amounts of linolenic acid ($C_{18:3}$) are also found in olive oil.

According to Amelotti (1973), elaidic ($C_{18:1}$) is also present, but in trace amounts. Tiscornia and Bertini (1972), however, stated that this acid is not present in olive oil.

Viola (1975) noted that linoleic acid is found in an ideal concentration in olive oil and in excellent ratio with Vitamin E (1 g linoleic acid/1.87 mg vitamin E), which gives a high nutritive value to olive oil.

Non-glyceride Components

Olive oil contains several chemical compounds in small amounts referred to as minor components. The most important of these compounds are the following:

Hydrocarbons:

Fedeli (1977) was able to identify several benzenoid hydrocarbons like naphthalene and naphthalene derivatives.

Squalene is the main component of the hydrocarbon fraction of olive oil. The content of squalene in olive oil is higher (2500-9250 μg/g oil) than that of other vegetable oils (soybean, cottonseed, coconut, avocado) (Gutfinger and Letan, 1974).

According to Boskou and Katsikas (1979), the hydrocarbon fraction of olive oil, added to cotton seed oil, increased the oxidative stability of the latter. The same was true for the triterpine alcohols which showed a more pronounced effect (Boskou and Katsikas, 1979).

Fatty Acid Esters:

Esters of n-aliphatic alcohols (C_{27}-C_{32}), of sterols (b-sitosterol, campesterol, stigmasterol) and of triterpenic alcohols (cycloartenol, 24 methylene-cycloartanol) have been identified in the non-glyceride fraction of olive oil. Methanol and ethanol esters and mono- and diglycerides have been found in small amounts (Fedeli, 1977).

4α-Methylsterols:

A small fraction, whose polarity in TLC is very similar to that of sterols, has been identified in olive oil, as in other vegetable oils (Fedeli, 1977). Some of these constituents are 4a-methyl, 24-methylene-Δ^7-cholestene-3β-ol, 4a-methyl-24-methyl-Δ^7-cholestene-3β-ol and 4a-methyl, 24-ethylidene-Δ^7-cholestene-3β-ol.

According to Boskou (1978), 4α-methyl-sterols are more susceptible to oxidation compared to the common sterols and triterpene alcohols.

Dihydroxy Triterpenes:

The presence of a dihydroxy pentacyclic triterpenic compound, named erythrodiol, has been reported in olive oil (Cucurachi, _et_ _al_., 1975; Fedeli, 1977; and Leone _et_ _al_., 1978). Gas chromatography analysis showed that erythrodiol is present in olive oil at an average of 7.2% (Cucurachi, _et_ _al_., 1975).

Due to the presence of the above cited components, and of some others, olive oil has important metabolic effects according to Christakis _et_ _al_. (1980). He noted that the following factors merit consideration:

1. The ratios of the individual fatty acids, ie. saturated/monounsaturated etc.
2. The ratio of Vitamin E/polyunsaturated fatty acid content, and the presence of other antioxidants in optimal concentration.
3. The content of essential fatty acids; approximately 10% linoleic is compatible with the requirement for essential fatty acids when olive oil is used as a sole source of seasoning fat.
4. The relatively high percentage of squalene may also be metabolically important

Flavor Components of Olive Oil

Olive oil is particularly palatable due to its organoleptic properties which are attributed to a number of pleasant flavoring substances (Fedeli, 1977). Fedeli _et_ _al_., (1974), Flath _et_ _al_., (1973) and Nawar (1970) isolated a large number of volatile constituents belonging to aliphatic and aromatic hydrocarbons, aliphatic and terpenic alcohols, aldehydes, ketones, ethers, esters, furan and thiophene derivatives. Particularly complex are the aromatic hydrocarbons and the aldehyde fractions most of which probably originate from the oxidative degradation of the oil (Fedeli, 1977).

An attempt to evaluate the organoleptic importance of the polyphenol and volatile compounds of olive oil, was made by some workers (Olias and Gutierrez, 1973 and Olias _et_ _al_., 1974).

In a study carried out in Italy (Lercker _et_ _al_., 1973), it was found that the concentration of volatile compounds was <lppm in extra virgin oils. The same worker observed that for different regions of Italy there were quantitative differences in the volatile constituents, which are responsible for the various organoleptic characteristics of the oils.

Doubtless there are quantitative differences among the volatile constituents of the different cultivars (Montedoro _et_ _al_., 1978). The formation of the volatile (flavor) compounds of olive oil occurs in connection with the destruction of the cell structure. The processes involved in these mechanisms are hydrolysis and oxidation which proceed with an amazingly high speed depending on the pH and on the temperature (Montedoro _et_ _al_., 1978).

According to Montedoro _et_ _al_. (1978), the concentration of the different constituents increases with the degree of pigmentation until a stage is reached beyond which an inversion of this relationship is observed.

Among the different aromatic constituents present in olive oil, the aldehydes are always found in higher quantities. The values for aldehydes are about 50% for green olives and 75% for black olives, of the total amount of the constituents (Montedoro *et al.*, 1978). The most significant constituents, as far as their quantity and their organoleptic role is concerned, are the 2-hexenal (85-90%) of the aldehydes, the trans-2-hexen-1-ol (16% of the alcohols) and the cis-3-hexenyl acetate (20% of the total esters) (Montedoro *et al.*, 1978). Certain of the volatile compounds identified in head space of stored olive oil are shown in Table 1.

Determination of Olive Oil Quality

The basic criteria for evaluation of olive oil quality are the free fatty acid content, the peroxide value, the ultraviolet absorption values and the organoleptic characteristics (taste and odor).

Ultraviolet absorption measurements are made at 232 and 270 nm where the initial hydroperoxides of linoleic acid and the secondary products (aldehydes and ketones) of oxidation absorb. Besides the oxidation products, conjugated trienes, formed during refining or bleaching, absorb at 270 nm.

Ultraviolet absorption measurements of olive oil are made also at 262, 268, and 274 nm for determination of the variation of the absorbance near the wavelength 270 nm using the formula:

Table 1 Certain volatile components identified in olive oil.*

Hydrocarbons	Aldehydes	Ketones
n-Hexane	Acetaldehyde	3-Pentanone
n-Octane	2-Methylbutanal	4-Methyl-3-penten-3-one
Ethylbenzene	Pentanal	Esters
Alcohols	Hexanal	Ethyl acetate
Ethanol	cis-2-Pentenal	Isobutyl acetate
1-Penten-3-ol	trans-2-Hexenal	3-Methyl-1-butyl acetate
3-Methylbutan-1-ol	Octanal	Hexyl acetate
1-Pentanol	Nonanal	cis-3-Hexenyl acetate
1-Hexanol	Benzaldehyde	
cis-3-Hexen-1-ol		
trans-2-Hexen-1-ol		
1-Octanol		

*Adapted from Montedoro *et al*. (1978).

$$\Delta K = K_{268} - \frac{K_{262} + K_{274}}{2}$$

Usually the specific absorbance ($E^{1\%}_{1cm}$) is determined in each of the cited wavelengths.

The International Olive Oil Council (1966) used the criteria of FFA, peroxide value, ultraviolet absorption and or organoleptic properties to characterize the qualities of virgin olive oil as shown in Table 2.

Changes in Quality Characteristics

It has been noticed (Martinez, 1975) that, during the time that oil remains in the fruit, the values of the above criteria are changed. Some become higher and some lower in organoleptic score.

The free fatty acid content is increased due to hydrolysis of glycerides. Hydrolysis is caused by microorganisms, enzymes or water present in the fruits (Martinez, 1975). The activity of some microorganisms is such that the time between the milling of the olives and the separation of the oil from the vegetable water is not sufficiently short to exclude the possibility of a certain amount of hydrolytic action on the components of the oil (Martinez, 1975). Enzyme lipases are present in the fruit and become active with the process of maturation (Martinez, 1975).

Infestation of fruits by dacus oleae fly or fungi or any other damage to the fruit results in an increase in the

Table 2 Quality criteria of olive oil.

Virgin olive oils	FFA (as oleic acid)	Peroxide value (meq O_2/Kg oil)	Specific Extinction in UV		Flavor
			K_{270}	ΔK	
Extra	≤1.0%	≤20	≤0.20	≤0.010	Absolutely perfect
Fine	≤1.5%	≤20	≤0.25	≤0.010	Absolutely perfect
Ordinary	≤3.3	≤20	≤0.25	≤0.010	Absolutely perfect

FFA content of the oil (Martinez, 1975; Newenshuanter and Michelakis, 1978).

Studies on the aromatic constituents of the oil and on the changes that these undergo have been made by Montedoro *et al.*, 1978). He noted that even during storage of fruits in the mill, there is a loss of olive oil constituents due to the hydrolytic enzymatic mechanism of the cell wall. He believes that this process begins during ripening of the fruits.

Storage of olive fruits for ten days caused a decrease in aldehyde content from 26.62% to 13.58% and in phenols from 104 mg/Kg oil to 89 mg/Kg oil (Montedoro *et al.*, 1978).

Tocopherols, Phenols and Sterols as Natural Antioxidants

Tocopherols are natural antioxidants which are primarily responsible for the stability of vegetable oils. There are four commonly occuring tocopherols designated α-(alpha), β-(beta), γ- (gamma), and δ-(delta) tocopherols. The relative effectiveness of these four tocopherols as antioxidants is $\delta>\gamma>\beta>\alpha$ (Daubert, 1950; Dugan, 1976). A similar order ($\delta>\gamma>\alpha$) was reported by Sherwin (1976). He noted, however, that this order of antioxidant potency in vegetable oils may be influenced significantly by temperature and light conditions. If the residual tocopherols are stripped completely from vegetable oil by distillation or some other efficient method, it will be observed that the oxidative stability of the oil will be reduced to an extremely low level (Sherwin, 1976).

Lea and Ward (1959) observed that tocopherols were much less effective as antioxidants in light than in dark.

Like other antioxidants, the tocopherols are themselves readily oxidizable. Oxidation of tocopherols lead to the formation of tocoquinone (Tappel, 1962; Sonntag, 1979), which is not an antioxidant (Sonntag, 1979). According to Gracian and Arevalo (1965), γ-tocopherol present in olive oil is a product of α-tocopherol oxidation.

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

Gutfinger and Letan (1974) found that lipids extracted from the olive seed kernel were higher in tocopherols than the lipids from the fleshy part of olives (291 μg/g oil vs 186 μg/g). In the former, the relative content of γ-tocopherol was higher (25% vs 7%). Neither olive seed kernel nor olive flesh contained δ-tocopherol.

Fedeli (1977) noted that the concentration of different tocopherols in olive oil was as follows: 88.5% α-tocopherol, 9.9 % $\beta+\gamma$ tocopherols and 1.6 % δ-tocopherol.

Using dimensional paper chromatography, Gracian and Arevalo (1965) identified only α-tocopherol in olive oil. Vitagliano (1960) and Boatella (1975) both agreed that olive oil contains α-tocopherol but they reported different quantities; the first 12-102 ppm and the other 70-150 ppm.

Gracian and Arevalo (1965) reported that the variations in the concentration of different tocopherols in olive oil may be explained by the progressive destruction of tocopherols.

Tocopherol content of olive oil can be used for detecting the adulteration of the oil with other oils (Ninnis _et_ _al_., 1969; Gutfinger and Letan, 1974). According to Gutfinger and Letan (1974), addition of soybean oil to the relatively expensive olive oil can be recognized by the presence of excessive amounts of γ or δ-tocopherol. On the other hand, addition of cottonseed oil to olive oil can be determined by the presence of excessive amounts of γ-tocopherol.

Simple as well as complex phenol structures are found in olives (Fedeli, 1977). Most of these constituents go into the aqueous phase as the oil is processed in the mill. However, a fraction remains in the oil and favors its stability to oxidation (Cantanelli and Montedoro, 1969, Notte and Romito, 1971; Vazquez _et_ _al_., 1976; Fedeli, 1977).

Vazquez _et_ _al_. (1976) demonstrated that the polyphenol content of olive oil varies according to cultivation procedures and environmental factors. Montedoro _et_ _al_. (1978), on the other hand, noted that the factors which can affect the phenolic constituents of olive oil are the harvesting period, the condition of fruit preservation and even the extraction system.

Montedoro _et_ _al_. (1978) noted that the decrease of phenolic constituents during the extraction process may be explained by the solubilization effect of the vegetation water and particularly by the dissolving of the colloidal substances (proteins and polysaccharides) which bind these components.

Vazquez _et_ _al_. (1976) found that the main polyphenols present in virgin olive oil were tyrosol and 3-hydroxytyrosol and observed some antioxidant effect in 3-hydroxytyrosol.

Phenolic acids like caffeic, vanillic, p-coumaric, p-hydroxybenzoic, and protocatechuic have been found in olive oil (Vazquez _et_ _al_., 1976). Cortesi _et_ _al_. (1981) determined (with HPLC) the phenolic acids present in virgin olive oil, refined, and solvent extracted oil and found that virgin oil had higher levels of protocatechuic and cinnamic than the other oils.

Montedoro _et_ _al_. (1978) declared that the most interesting constituents from the organoleptic point of view are the polyphenols: β-3,4 dihydroxyphenylethanol (hydroxytyrosol) found only in very good quality oils, the 3-hydroxy-phenylethanol (tyrosol) and some phenolic acids, found in oils of poor quality.

It is believed that hydroxytyrosol and tyrosol are derived from the hydrolysis of oleuropein while others (benzoic and cinnamic acids) are derived from the hydrolysis of flavonoids (anthocyanins, flavones) (Montedoro and Cantarelli, 1969; Vazquez et al., 1976) which are found in considerable amounts especially at the ripening stage.

Notte and Romito (1971) demonstrated that polyphenols extracted from olive leaves acted as antioxidants in olive oil. These polyphenols were effective in a concentration of more than 20 mg/100 g of oil. Cantarelli and Montedoro (1969), on the other hand, observed that phenols extracted from olive oil with 80% MeOH acted as antioxidants in other oils while the extraction of phenols from olive oil caused its rapid oxidation. Gutfinger (1981) also reported that removal of polyphenols from olive oil markedly increased the oxidation rate.

Fedeli et al. (1966) used GLC to identify stigmasterol and campesterol in olive oil. Fedeli (1977) reported that Italian olive oil contains 95.6% β-sitosterol, 1.3% stigmasterol and 3.1% campesterol. Boskou and Morton (1975) on the other hand, stated that Greek olive oil contains traces of cholesterol, 2.0% campesterol, 0.5% sigmasterol, 89.5% β-sitosterol and 8% anemasterol.

Gutfinger and Letan (1974) found that the sterol compositions of olive, cottonseed and avocado oils were similar. In those oils, β-sitosterol was the dominant sterol (Ca 90%), and it was accompanied by only limited amounts of stigmasterol (0-2.3%). Different amounts of sterols were present in olive oils extracted from either the flesh or the seed kernel of olives but the composition of the oils' sterol fractions were almost the same (Gutfinger and Letan, 1974).

According to Leone *et al*. (1978), the β-sitosterol content of olive oil depends on the cultivar and oil acidity.

The determination of the sterol content of olive oil can be used for the detection of adulteration. Excessive amounts of stigmasterol indicate the presence of soybean oil in olive oil (Gutfinger and Letan, 1974).

The antioxidant properties of some sterols present in olive oil were studied by Boskou and Morton (1976). They found that Δ^5-anemasterol added to heated cottonseed oil eliminated the oxidative deterioration of the oil, while a sterol mixture consisting of 94% β-sitosterol showed some prooxidant effect.

Leone *et al*. (1976) demonstrated that any increase in the peroxide value of olive oil during storage is associated with a decrease in sterols.

Effect of Harvesting on Olive Oil Quality

A variety of factors influence the quality of oil present in olive fruits such as: cultivar of olives, variability of soils (physical and nutritive elements), and pests (which either facilitate or impede the development of micro-organisms) (Martinez, 1975). Methods used for harvesting olives influence the characteristics of the oil to a degree.

There are two important harvesting factors to consider: the season and the method of harvesting.

As to season, according to Martinez (1975), olives should be harvested at the moment of optimum maturity which is when the fruits have the maximum quantity of the oil with the best characteristics. This moment can be established visually when the fruit begins to darken.

According to Martinez (1975), the fruits yield a different type of oil during each stage of maturity. The time of maturity, however, is not always the same even when the conditions are identical and the trees are within the same olive grove. Therefore it is difficult for all of the olives to be harvested at the optimum stage because maturity is not the same from zone to zone, even with olives on the same tree.

Martinez (1975) stated that fruits harvested early give a lower yield of oil, with low free fatty acid content, greener in color and with a fruity savor, whereas, when the harvest is delayed, it produces a greater yield, with slightly higher acidity, a yellow color and oils that are generally less aromatic.

Montedoro _et_ _al_., (1978) demonstrated that the concentration of different constituents of olive fruits increases with the degree of pigmentation until a stage is reached beyond which an inversion of this relationship is observed. They (1978) noticed that the highest concentrations of both (total volatile and total phenol) constituents are found during the phenological period between the semiblack and completely black olives. This period corresponds to the one during which the oil concentration nearly reaches its maximum and the aromatic characteristics of "fruity" are the highest.

According to Martinez (1975), collection methods which harm the olive fruits result in losses in oil quantity and quality. He reported that olive fruits collected from the ground produce oil of higher acidity and of different organoleptic characteristics than those gathered directly from the trees.

New Systems for Olive Oil Extraction

The main systems used today for olive oil extraction are the traditional, the centrifugal and the mixed. The traditional systems, used for thousands of years in the olive oil industry, are based on the application of hydraulic pressure.

Centrifugal Systems

Centrifugal systems in industrial base, were first made at the beginning of the decade of 1960 despite the fact that centrifugal extractors were known since the end of the 19th centruy.

Experiments recorded were not only those carried out by Boulier in 1903 and the Agricultural Experimental Station of California in 1904, but also those of Brani and Merer-Revoil in 1908, Degli Alti in 1906, of the California Packing Corporation in 1927, of Ortiz and Rouseau in 1929, of Ferraris, Pantanelli and Brandonisic in 1937, of Perogio in 1941 and of Tostorelli in 1950 (Petruccioli, 1975).

Systems based on the centrifugation of olive paste have progressed considerably in the last few years. Different companies such as ALFA-LAVAL, PIERALISI, HILLER, THEOCHARIS and others make centrifugal systems.

According to Petruccioli (1975), these systems will replace traditional (presses) in the future. Advantages claimed over the traditional systems include, among others, improvement of the quality of the oil obtained. The operation of the centrifugal systems is based on the different specific gravity of the constituents of olive paste (oil, water, cake). The separation of the three phases is accomplished in a decanter (centrifugal), the main component of the processing system.

The velocity of separation during the centrifugation is given by the formula:

$$V = \frac{2R^2(S_2-S_1)\cdot g}{g_e}$$

where: V = Velocity of separation
R = Radius of heavier phase
S_1 = Specific gravity of the light phase
S_2 = Specific gravity of the heavier phase
g = Gravity
e = Viscocity coefficient of light phase

The processing steps in a centrifugal system are shown in Appendix 1.

Mixed Systems

The operation of the mixed system is based on selective filtration and on centrifugation or pressing. At the beginning of the present century, the Spaniard Acapulco discovered that if a thin layer of the paste of de-stoned olives was placed on a filter medium (this could be cotton cloth) the filtered liquid collected is oil and is practically free of the vegetable water which accompanies the fruit. This observation was the basis for making a system of extraction, which, although often modified and varied, is employed today to a certain degree in combination with other systems (principally with centrifugal).

The first complete installation using the new method was constructed with the aid of the agronomist Quintanilla and thus the system is known throughout the world as the Acapulco-Quintanilla system.

According to Petruccioli (1975), the greatest improvement on the original Acapulco system was achieved by Buendia in 1951 with his ALFIN extractor, better known in Italy and Greece under the name SINOLEA. Since then, the ALFIN extractor has received a variety of modifications.

In 1972 a complete system based on selective filtration and on centrifugation was constructed by the company RAPANELLI. This is known as a mixed system since it gives about 80% of the oil of paste with selective filtration through the unit of SINOLEA, while the rest is accomplished with centrifugation through a DECANTER unit.

The basic steps of processing in the RAPANELLI system, giving SINOLEA and DECANTER oils, are shown in detail in Appendix 2.

Effect of Extraction System on Olive Oil Quality

Olive oil quality may undergo deterioration during the processing of olives for oil extraction. Mendoza (1975) noted that the milling operation plays a very important role in the extraction of olive oil because the way it is carried out and the type of machine used have a direct influence on the processes of later operation (malaxation, decanting, centrifuging) and particularly on the yield and quality of olive oil. It mainly affects its organoleptic characteristics.

According to Mendoza (1975), the exposure of olive past to the air during the milling operation results in loss of aroma. He noted that the type of materials used affect considerably the quality of the oil. Traces of metals carried away from the metallic surfaces of mills cause changes in the organoleptic characteristics of the oil particularly in color and taste. Moreover, these metals can act as catalysts in the oxidation of olive oil (Mendoza, 1975).

He further demonstrated that heating (>25°C) of the oils during processing (milling, malaxation etc) leads to changes which significantly alter olive oil quality, because the volatile components which contribute to the aroma of good oils are lost rapidly. High temperatures can also cause changes of color to a reddish hue, increase the FFA content, and increase consuption of energy.

Montedoro _et_ _al_. (1978) studied the effect of paste mashing (during the extraction) on the phenolic compounds in the oil. They found that, by direct pressing, oil obtained finally had 616 mg/Kg total phenols, by milling and pressing 450 mg/Kg, and by milling, mashing and pressing 260 mg/Kg.

Martinez (1975) also noted that the different systems used for processing of olive oil may affect its quality.

Felice _et_ _al_. (1979), in their studies with centrifugal and traditional (with pressure) systems, found that oil obtained from centrifugal systems had lower total polyphenol content (expressed as gallic acid) and iron content than oil from traditional systems. According to them centrifugation oil had a reduced shelf-life in spite of the lower iron content.

Cakmak (1978) studied the factors influencing yield and quality of olive oil obtained by pressing in industrial-scale trials. He found that oil yield increased with increasing grinding time, the peroxide value increased after grinding for 15 min but the FFA content remained unaffected. He further noticed that the FFA content, the peroxide value and the spectrophotometric absorption values (at 232 and 270 nm) of the oil increased with increasing pressing time. Organoleptic properties of the oil also deteriorated with increasing pressing time.

Leone _et_ _al_. (1979) compared the qualitative characteristics of oil obtained by single and double pressure extraction with an automatic plant (Belgioni system) that squeezes olive pastes (at 600 Kg/cm^2) without using vegetable or synthetic fibre filters. He found that the FFA content of oil from the Belgioni system was not different from traditionally single pressure extracted oil, the spectrophotometric indices and the peroxide values were lower, the polyphenol and tocopherol content higher, the sterol and dialcohol terpene contents similar and the resistance to autoxidation higher than that of traditionally extracted oil. He demonstrated that the organoleptic quality of this oil corresponded to extra quality oil.

Lipid Oxidation

Autoxidation

Several studies on autoxidation of fatty acids have been reported previously (Ross *et al.*, 1949; Boland and Ten Have, 1947; Dugan, 1961). A mechanism which is now generally accepted is that the autoxidation of lipid involves a free radical mechanism. The oxidation is initiated by H_2 abstraction followed by addition of O_2 to the carbon radical generated.

In the entire mechanism, which involves the steps of initiation, propagation, and termination, the following reactions are involved (Boland and Ten Have, 1947).

1. Initiation

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

2. Propagation

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

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

3. Termination

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

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

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

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.

According to Logani and Davies (1979) the final distribution of the products of oxidation depends on the secondary reactions such as rearrangements of the intermediate radicals or of the final products and of further oxidation.

Despite any secondary reactions involved in oxidation of lipids in the dark, this can be inhibited by free radical quenchers such as antioxidants (Carlsson et al., 1976; Logani and Davies, 1979).

Various extraneous influences may be present that affect the rate of oxidation. These factors are temperature, light, ionizing radiation, enzymes, prooxidant metals and metallic compounds, presence of oxygen, and use of antioxidants (Lea, 1962).

Mechanism of Photooxidation

Satter and DeMan (1975) reviewed the fact that chemical compounds absorb light energy in different wavelength bands depending on molecular structure. The absorption of radiant energy is known as the primary photochemical process and results in an activated molecule:

$$A + h\nu \longrightarrow A^* \qquad (1)$$

They pointed out that this is followed by secondary reactions in which the excited molecule can use the activation energy to emit light (Eq. 2) or heat (Eq. 3), form a new activated species (Eq. 4), form a bond (Eq. 5), or dissociate into ions (Eq. 6) or free radicals (Eq. 7).

$$A^* \longrightarrow A + h\nu \qquad (2)$$

$$A^* + A \longrightarrow A + A + \text{heat} \qquad (3)$$

$$A^* + B \longrightarrow A + B^* \qquad (4)$$

$$A^* + A \longrightarrow A\text{-}A \qquad (5)$$

$$A^* \longrightarrow A^+, + e^- \qquad (6)$$

$$AB^* \longrightarrow A\cdot + B\cdot \qquad (7)$$

The two most important types of reactions concerning light induced lipid oxidation are 1) a photosensitized reaction in which the light absorbing species does not undergo permanent chemical change:

$$S + h\nu \longrightarrow S^* \qquad (8)$$

$$S^* + A \longrightarrow A^* + S \qquad (9)$$

$$A^* + B \longrightarrow AB \qquad (10)$$

and 2) a photoinduced reaction in which the reactive species produced by the radiant energy initiates another reaction (such as a free radical reaction):

$$I \longrightarrow I^* \qquad (11)$$

$$I^* \longrightarrow I\cdot \qquad (12)$$

$$I\cdot + A \longrightarrow A\cdot + I \qquad (13)$$

Schenck (1963) has categorized photosensitized oxidation reactions according to the intermediates formed. A "type I" sensitized reaction, which can proceed in the absence of oxygen, is one in which free radicals and electronically excited molecules are involved. Compounds which are readily oxidized or reduced favor this type of reaction. Substances such as olefins, dienes, or aromatic compounds, that are not easily oxidized or reduced by a sensitizer favor a "type II" reaction in which oxygen participates and which occurs only through electronically excited molecules as intermediates.

Oxygen may occur in a singlet or triplet state depending on the arrangement of electrons in the outer orbitals. Ground state triplet oxygen has two electrons in separate orbitals with opposed angular momentum and parallel spins. The first excited singlet state ($^1\Delta$) has both electrons in the same orbital with the same angular momentum and opposed spins. A second excited singlet state ($^1\Sigma$) has a very short lifetime and decays to the $^1\Delta$ state after 1 X 10^{-11} sec.

The relationship between the oxygen states is shown in Table 3.

Table 3 Relationship between the three states of oxygen.*

State of O_2 Molecule	Symbol	Relative Energy	Occupancy of Highest Orbitals
Second excited	$^1\Sigma$	+37 kcal	↑ ↓
First excited	$^1\Delta$	+22 kcal	↑↓ —
Ground	$^3\Sigma$	0 kcal	↑ ↑

*Adapted from Foote (1968).

The $^1\Delta$ state has a lifetime of several μsec. and is highly electrophilic in nature since it seeks electrons to fill its vacant molecular orbital (Korycka-Dahl and Richardson, 1978). Therefore $^1O_2$ reacts readily with moieties containing high electron densities, such as double bonds. There are several reactions of $^1O_2$ which may be of importance in biological systems.

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

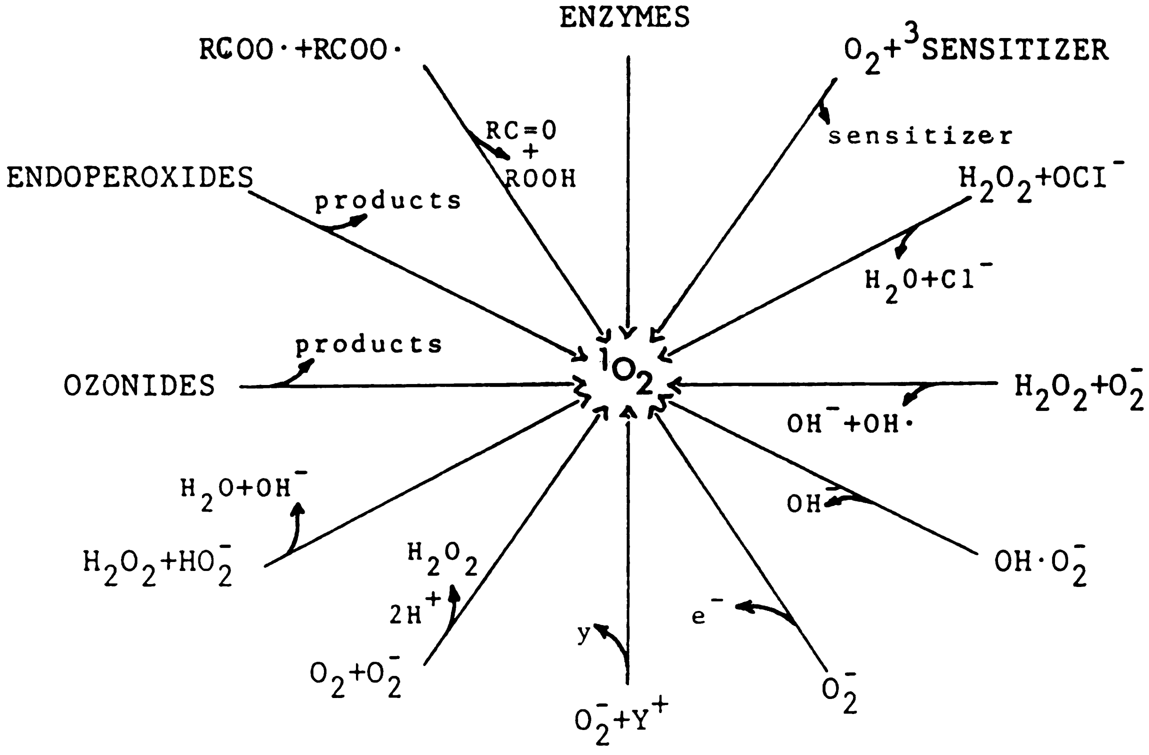


Fig. 1 Production of $^1O_2$ by photochemical, chemical and biological systems (adapted from Krinsky, 1977).

Photooxidation of Lipids

Photooxidation of lipids caused by chromophore impurities present such as chlorophyll, porphyrins, myoglobins, pheophytins etc., has been studied by several workers (Coe, 1937; French and Lundberg, 1944; Rawls and Van Santen, 1970; Koch, 1973; Carlsson *et al.*, 1976; Chan, 1977; Krinsky, 1977; Terao and Matsushita, 1977; Malgorzata and Richardson, 1978; and Vianni, 1980).

Rawls and Van Santen (1970) have identified $^1O_2$ as a likely source of hydroperoxides which initiate autoxidation in oils originally free of all hydroperoxides. They (1970) proposed the following mechanism by which singlet oxygen is produced during photooxidation.

$$\begin{aligned}
&{}^1S + h\nu \longrightarrow {}^1S^* \longrightarrow {}^3S^* \\
&{}^3S^* + {}^3O_2 \longrightarrow {}^1O_2^* + {}^1S \\
&{}^1O_2^* + RH \longrightarrow ROOH \\
&ROOH \longrightarrow \text{Free radical products}
\end{aligned}$$

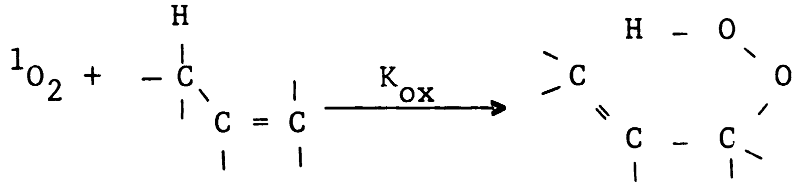
(S is sensitizer, superscripts refer to the spin multiplicity and the asterisk indicates electronic excitation).

According to Rawls and Van Santen (1970), singlet oxygen ($^1O_2$), generated externally, was shown to react directly with extremely pure samples of methyl linolate at a rate at least 1450 times that of oxygen in the ground state.

Carlsson et al. (1976) noted that during exposure of chlorophyll in light the following reaction is involved:

$$\mathrm{Chl} \xrightarrow{h\nu} [\mathrm{Chl}]^{*} \xrightarrow{{}^{3}O_2} \mathrm{Chl} + {}^{1}O_2$$

The singlet oxygen (${}^{1}O_2$) formed then reacts rapidly with C-C unsaturation to give hydroperoxides (Kaplan, 1971).



These hydroperoxides can then cleave thermally even at room temperature and so initiate a conventional free radical autoxidation to produce more hydroperoxides (Carlsson et al., 1976).

Although it is generally believed today (Rawls and Van Santen, 1970; Carlsson et al., 1976; Terao and Matsushita, 1977; Frankel et al., 1979) that singlet O_2 is involved during photooxidation, some photosensitized oxidation of lipids may involve the triplet oxygen stage (Chan, 1977). In this case the sensitizer (riboflavin) reacts after light absorption with the substrate (A) to form intermediates which in turn react with ground state (triplet) oxygen to yield the oxidation products. This is known as a Type I pathway for photo-sensitized oxidation.

$$\text{Sens} + \text{A} + h\nu \longrightarrow \text{(Intermediates-I)}$$
$$\text{(Intermediates-I)} + O_2 \longrightarrow \text{products} + \text{Sens}$$

Besides Type I, there is a Type 2 pathway for photosensitized oxidation (Chan, 1977). In this case, molecular oxygen rather than the substrate, is the species which reacts with the sensitizer after light absorption.

$$\text{Sens} + O_2 + hv \longrightarrow \text{(Intermediates-II)}$$
$$\text{(Intermediates-II)} + \text{A} \longrightarrow \text{products} + \text{Sens}$$

In the Type 2 photo-sensitized oxidation, singlet molecular oxygen is generally regarded as the reactive species responsible for oxygenation of the substrate. This excited molecular species is formed by reaction of ground state oxygen with an excited triplet state of the sensitizer.

Unlike autoxidation, photooxidation involving triplet oxygen doesn't involve chain reactions. In addition photooxidation doesn't require an induction period whereas the "dark" oxidation involves a long induction period (Logani and Davies, 1979).

During autoxidation of poly-unsaturated fatty acids via a free radical mechanism, all hydroperoxides formed are conjugated (Khan *et al*., 1954). However, when methyl oleate and methyl linoleate were illuminated, in the presence of chlorophyll, conjugated and nonconjugated hydroperoxides were formed (Khan *et al*., 1954). Similar observations were made by Rawls and Van Santen (1970). Nonconjugated hydroperoxides which were absent in autoxidation reactions were formed from methyl linoleate during photosensitized oxidation and on exposure to externally generated singlet oxygen.

Role of Chlorophyll in Photooxidation of Oils

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, Tollen and Green (1960) showed that very strong prooxidants are necessary.

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

Clements *et al*. (1973) used sodium chlorophillin to study the participation of singlet oxygen in photosensitized oxidation of soybean oil. They reported that chlorophyll-like sensitizers are probably unimportant in well-refined soybean oil.

Rawls and Van Santen (1970) in their studies of a possible role of singlet oxygen using chlorophyll, observed that singlet oxygen accounts for approximately 80%

of the chlorophyll induced photooxidation. They noted that the proton abstraction by the photoactivated carbonyl group of chlorophyll could account for the remaining 20% of the observed photooxidation.

Vazquez _et_ _al_. (1960) found that olive oils, because of their chlorophyll content are very sensitive to radiation between 320 and 720 nm whether in the presence or the absence of antioxidants. According to Francesco (1961) virgin olive oils have a chlorophyll absorption peak at 665 nm.

Due to its chlorophyll content, virgin olive oil is easily oxidized and is very sensitive to light (Vitagliano, 1960).

Interesse _et_ _al_. (1971) demonstrated that under the action of light, the four pigments (chlorophyll a and b and pheophytin a and b) present in olive oil develop an oxidizing effect, while in the dark they act as antioxidants.

Singlet Oxygen Quenchers

Photosensitized oxidation is not prevented by the antioxidants commonly used to inhibit dark oxidation (Carlsson _et_ _al_., 1976; Logani and Davies, 1979). Photooxidation can be prevented by singlet oxygen quenchers (Carlsson _et_ _al_., 1976) which can have the opposite effect of sensitizers and decrease the rate of photosensitized oxidation either by physically or chemically reacting with the $^1O_2$. Thus they deactivate it to the ground state.

$$^{1}O_2 + \text{quencher} \xrightarrow{Kq} {}^{3}O_2 + \text{quencher}$$

β-Carotene has been shown to quench $^{1}O_2$ efficiently and thereby inhibit oxidation by $^{1}O_2$ (Foote and Denny, 1968; Carlsson et al., 1976; Matsushita and Terao, 1980). This quenching is believed to be the major mode of protection against photodymamic action in living organisms (Foote et al., 1970a). In methylene blue photosensitized oxygenations, one molecule of β-carotene was shown to quench up to 1000 molecules of $^{1}O_2$ before being consumed (Foote et al., 1970b). Naturally occuring α-tocopherols also quench singlet oxygen efficiently, but are themselves oxidized in the process (Carlsson et al., 1976). Addition of δ-tocopherol was found (Matsushita and Terao, 1980) to increase the life of β-carotene in photosensitized systems.

To assess $^{1}O_2$ participation in oxidation reactions, $^{1}O_2$ quencher inhibition and presence of nonconjugated hydroperoxides are now commonly used.

Effect of Storage Conditions on the Oxidation of Olive Oil

Pretzch (1970) reported that olive oil exposed to light and air undergoes greater oxidation than that stored in the dark. Interesse et al. (1971) also reported that exposure of olive oil to light accelerates oxidation. Valentinis and Romani (1960), however, noted that in the absence of air, direct sunlight causes a decrease of peroxide and Kreis value during the storage of olive oil.

Gutierrez and Jimenez (1970), in their studies with virgin olive oil in polyethylene bottles, found that oil exposed to light undergoes greater oxidation than that stored in the dark.

In experiments carried out in open and closed tins, the increase in peroxide value of oil in open containers (exposed to unlimited air) was high. On the other hand the formation of peroxides in closed tins, due to the limited amount of oxygen present in the headspace, was generally insufficient to produce the typical rancid odor (Cucurachi, 1975). According to Cucurachi (1975), samples of oils with the same peroxide value have dissimilar organoleptical scores if they are packaged in different ways.

In storage studies with different oils (olive, peanut, almond), Wiegand (1978) realized greater resistance to oxidation by olive oil than other oils.

Cerezal *et al*. (1975) found that olive oil stored up to 325 days in a tank coated with epoxy resin was much more stable than olive oil stored in an iron tank with respect to FFA content, peroxide value, metal contents and organoleptic evaluation and was similar in these characteristics to olive oil stored at 4°C in glass bottles.

In a study of olive oil with different types of containers, the following peroxide values were recorded after 3 months of storage under light conditions: Samples in glass and PVC containers had PV lower than 20; samples in polypropylene had PV 20-40; samples in high-density polyethylene had PV 30-80; and finally samples in low-density polyethylene had PV 15-45 (Gutierrez, 1975). Gutierrez (1975) reported that storage of olive oil in containers impermeable to atmospheric oxygen and light conditions results in protection of the oil from oxidation. The elimination of these factors is not always sufficient to conserve unaffected the organoleptical characteristics of the oil.

Sanelli (1981a) studied the quality changes of different oils (olive, soybean, linseed and mixture of olive with others) in relation to the presence or absence of chlorophyll during their storage in closed bottles in darkness or in open bottles in light or darkness. He found that chlorophyll decreased the formation of free fatty acid and changed the unsaturated/saturated fatty acid ratios during storage.

In other studies, Sanelli (1981b) tried to determine the oxidative deterioration of virgin olive oil and other oils (refined olive, soybean oil and linseed) as pure and as mixtures in relation to the chlorophyll content and to degree of unsaturation. Samples were stored in closed glass bottles in light or darkness and in open bottles in light. Results demonstrated the great sensitivity of virgin olive oil to oxidation and the improved stability conferred by refining.

Unal (1978), in his storage studies with virgin olive oil in cans, colorless glass bottles or PVC bottles and stored in light (ambient temperature) or in dark (at 28±1°C), found that free fatty acid content of the oil increased during the storage period of 24 months. He reported that peroxide values of samples in cans or glass bottles decreased during storage, whereas those of samples in PVC bottles increased, probably because of the O_2 permeability of the PVC. He further demonstrated that the decreases in tocopherol, β-carotene and chlorophyll were greater in illuminated samples than in those stored in darkness. Samples stored in glass or PVC bottles in the light underwent greater changes in organoleptic properties than those stored in darkness.

Gutfinger _et_ _al_. (1975) studied the changes in peroxide value, TBA and acid values in samples of olive oil stored at 30°C in the dark in glass and PVC bottles and tin-coated cans. They found no significant differences between peroxide value of glass or PVC stored oil but lower peroxide values for canned oil in a 160 day storage period without shaking. Continous shaking accelerated oxidation of glass and PVC packaged oils but slightly reduced peroxide value of some canned samples. According to them, only minor changes occurred in TBA and acid values.

Cucurachi (1975) noted that the main factors concerning deterioration of olive oil quality during storage are: light, air, temperature and presence of metals.

During the storage time, the presence of metals such as iron and copper coming from the metallic surfaces of processing systems or storage media promote oxidation. The metals act as catalysts of oxidation by changing their valence.

$$\begin{array}{lll} & \mathrm{RH} + \mathrm{M}^{+n} \longrightarrow & \mathrm{R}\cdot + \cdot\mathrm{H} + \mathrm{M}^{+(n-1)} \\ \text{or} & \mathrm{ROOH} + \mathrm{M}^{+n} \longrightarrow & \mathrm{ROO}\cdot + \mathrm{H}^{+} + \mathrm{M}^{+(n-1)} \\ & \mathrm{ROOH} + \mathrm{M}^{+(n-1)} \longrightarrow & \mathrm{RO}\cdot + \cdot\mathrm{OH}^{-} + \mathrm{M}^{+n} \end{array}$$

(where RH:unsaturated fatty acid, M:metals)

Underground cisterns or tanks are recommended for storage of oil in large quantities. Thus the oil is protected from excessive changes of temperatures permitting the maintenance of an average temperature of 15°C which is ideal for good storage (Cucurachi, 1975).

Material used for construction of tanks, according to Cucurachi (1975), should be physically and chemically inert to the oil in order to avoid absorption of defective odors and flavor and mixing of the oil with the substances which can cause deterioration. He noted that glass is the material with the greatest inertness and is practically unattracted by the oil. This is used widely for packaging and particularly in the commercialization of small quantities of oil.

It has been pointed out (Cucurachi, 1975) that high acidity olive oils become transparent very quickly and deposit their impurities at the bottom of the tanks but good quality oils retain the vegetable water and impurities over a long period of time and those are deposited slowly. It is important therefore that the oil be free from vegetable water (free or emulsified with the oil). Due to content of lipolytic enzymes or of fermentable substances, the water becomes dangerous to quality during olive oil storage. It may cause an increase in free fatty acid content and deterioration of the organoleptic characteristics of the oil due to fermentation of substances (Cucurachi, 1975).

Gutierrez (1975) summarized the requirements of material used for olive oil packaging as follows:

1) Be impermeable to oil.
2) Neither toxic nor foreign materials should contaminate the oil.
3) Guarantee quality and avoid fraud which implies the use of an inviolable closure or seal.
4) To have a protective action against oxidation changes, avoiding as far as possible the action of atmospheric oxygen, light, heat and metals, particularly those that are the most active catalysts.
5) Make its commercialization and employment easy. In other words, be resistant to impact and presure, easily manipulated (opened, closed) and
6) Be economical.

Measurement of Lipid Oxidation

Many methods have been developed for measuring lipid oxidation. The most commonly used methods are: peroxide value (PV), thiobarbituric acid (TBA) test, diene conjugation method and carbonyl test. A review of methods measuring lipid oxidation was presented by Gray (1978).

Peroxide Value (PV)

The peroxide value involves measurement of the primary oxidation products (hydroperoxides). The results are expressed as moles of peroxides or milliequivalents of oxygen per 1000 gr of fat.

For peroxide determination, the iodometric methods of Lea (1931), Wheeler (1932), AOCS (1973) are widely used. The International Olive Oil Council (COI) proposed (1966) a method for measuring peroxide value in olive oil which is very similar to the Wheeler's method (1932). All the above methods are based on the measurements of the iodine liberated from potassium iodide by the peroxides present in the oil. According to Mehlenbacher (1960), the two principal sources of error in the iodometric methods (Lea, 1931; Wheeler, 1932) are: a) the absorption of iodine at unsaturated bonds of the fatty material and b) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated. The latter is often referred to as the oxygen error and leads to high results in the peroxide determination. Lea

(1931) attempted to eliminate this error by filling the sample tube with nitrogen at the beginning of the test and assuming that the evolution of chloroform thereafter would prevent the reentry of oxygen into the tube.

Wheeler (1932) used a homogeneous solution in an attempt to eliminate the need for shaking, thereby minimizing the effect of oxygen. It has also been established that other possible sources in the iodometric methods include variation in weight of sample, the type and grade of solvent used, the reducing agent employed, variation in the reaction conditions such as time and temperature and the constitution and reactivity of the peroxides being titrated (Gray, 1978).

Terao and Matsushita (1977) proposed a method for measuring hydroperoxides in photooxidized oil. Cadmium acetate was used and the absorbance of the color formed was measured at 350 nm. Hydroperoxide concentrations were calibrated using a standard solution of benzoyl peroxide in ethanol.

Eskin and Frenkel (1976) developed a colorimetric method based on complex formation between titanium and hydroperoxides resulting in a colored complex that can be measured in a spectrophotometer at 415 nm. Another spectrophotometric method for determining hydroperoxides has been developed by Takagi et al. (1978). After oxidation of iodide to iodine with the sample for 5 minutes under an inert atmosphere, the excess of iodide ion is immediately converted to a cadmium complex for protection from atmospheric oxygen. The iodine is then measured at 358 or 410 nm and the peroxide value is calculated from the absorbance.

Tabasago *et al*. (1979) proposed a new IR spectroscopic method for evaluating the oxidative stability of fats and oils. IR spectra were determined and were calculated as ratio of a varying and a constant absorbance, ie ratio of absorbance at 3450 to those at 2850, 1740 or 1465 cm^{-1} and ratio of absorbance at 2850, 1740 or 1465 to that 3030 cm^{-1}. They demonstrated that data with this method were identical with those obtained by a weighing method. Maurikos *et al*. (1972) used a new polarographic method for determining the peroxide value in virgin olive oil. The supporting electrolyte was LiCl in methanol-benzene with a dropping mercury electrode.

Conjugated Diene Absorption Method

Mehlenbacher (1960) indicated that the oxidation of polyunsaturated fatty acids produces peroxides and the position of the double bond shifts to a conjugated form. Conjugated linkages give rise to characteristic and intense absorption bonds within the spectral range of 200-400 nm, while the absorption of isolated double bonds within the same region is very weak.

He noted that this characteristic is the basis for the ultraviolet absorption method for determining the state of oxidation.

Farmer and Sutton (1943) observed that the ultraviolet absorption in oxidized samples, increased proportionally to the uptake of oxygen and to the formation of peroxides in the early stages of oxidation.

Swern (1964) reported that the absorption is so weak in case of nonconjugated and saturated materials that it cannot be used for analytical purposes.

When ultraviolet absorption methods indicate the presence of small quantities of conjugated compounds in natural fats, the results must be carefully interpreted because nonconjugated polyunsaturated components may have undergone conjugation as a result of autoxidation or other mishandling (Swern, 1964).

In a conjugated system, dienes absorb at 233 nm while trienes absorb at 208 nm. Thus, oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption. The magnitude of change is not readily related, however, to the degree of oxidation because the effects upon the various unsaturated fatty acids vary in quality and magnitude. Therefore the changes in the ultraviolet spectrum of a given substance can be used as relative measurement of oxidation, rather than its measurement *per se*. (Swern, 1961; Gray, 1978).

In a study of the shelf-life stability of peanut butter during long and short-term storage, Angelo et al. (1975) found good correlation between the spectrophotometric determination of conjugated diene hydroperoxides and the peroxide value determinations over four and twelve week periods of storage. They (Angelo et al., 1975) stated that the conjugated diene hydroperoxides (CDHP) can be used as an index of progressive staling in place of, or in addition to, the peroxide value. According to them, the CDHP method is faster than the peroxide value method, is much simpler, requires no chemical reagent, does not depend upon chemical reaction or color development and can be conducted on smaller samples.

This method has been used fairly extensively in studying the autoxidation of drying oils since the conjugation of polyunsaturated components parallels oxygen absorption (Swern, 1964). It is applicable to the analysis of peroxides in vegetable oil products containing polyunsaturated fatty acids (Gray, 1978).

Golumbic et al. (1946) determined absorption curves of refined and deodorized soybean oil after exposure to visible radiation in air and in nitrogen. Samples of oil exposed to visible radiation in an atmosphere of nitrogen did not develop the maximum at 234 nm characteristics of the samples exposed to air.

In a study with olive oil, Bartolomeo and Sergio (1969) observed that the primary oxidation products of this oil show an absorption peak at 232 nm. For the secondary products - aldehydes, ketones, etc. - they found an absorption peak at 270 nm. The ultraviolet ratio A_{232}/A_{270} remains almost constant in virgin olive oils stored in the dark and decreases rapidly in virgin oils exposed to sunlight due to the rapid increase of oxidation (Jiminez and Gutierrez, 1970).

Montefredine and Luciano (1968) found a quasilinear relation between the absorbance at 232 nm and the peroxide value. According to Ninis and Ninni (1968), olive oil, like all oils free of conjugated double bonds, shows a slow increase in absorbtivity at 232 and 268 nm during the induction period which is followed by a sharp, sudden increase.

Ultraviolet spectrophotometric analysis can also be used to predict the thermal stability (Ninnis and Ninni, 1968 and Ninnis _et al_., 1968) and even to detect the adulteration of olive oil (Ninnis and Ninni, 1966). The better region of ultraviolet spectrum for the detection of olive oil adulterations is found at 208-210 nm where the vegetable oils show a specific absorption (A_{210} = 56-78), which is three times higher than that of olive oils (A_{210} = 13.8-21.6).

Thiobarbituric Acid Test (TBA)

The thiobarbituric acid (TBA) test is one of the more commonly used methods for the detection of lipid oxidation.

However the popularity of a method is not in itself ample proof that the method fulfills all the requirements of a reproducible technique (Gray, 1978).

Early investigation by Sinnhuber et al. (1958) helped to clarify the nature of the colorimetric reaction that occurs during the TBA test. They proposed that the chromagen was formed through the condensation of two molecules of TBA with one molecule of malonaldehyde. However no evidence was presented that malonaldehyde could be found in all oxidizing systems.

Dahle et al. (1962) postulated a mechanism for the formation of malonaldehyde as a secondary product in the oxidation of polyunsaturated fatty acids. This mechanism was based on investigations which showed that no color developed for linoleate even at peroxide values of 2000 or greater, but that for fatty acids with three or more double bonds the molar yield of the TBA color increased with the degree of unsaturation. They noted that only peroxides which possessed unsaturation β, γ to the peroxide group were capable of undergoing cyclization with the ultimate formation of malonaldehyde. Such peroxides could only be produced from fatty acids containing three or more double bonds.

Pryor et al. (1976) proposed a mechanism in which malonaldehyde arises at least in part from the acid catalyzed, or thermal decomposition of endoperoxides (2,3-dioxanorbornane compounds). They applied the theory of

Dahle et al. (1962) 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.

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

Tarladgis and his co-workers (1962) considered the effect of acid, heat, and oxidizing agents on the TBA reagent. They suggested steam distillation of the product to remove the volatile constituents that were assumed to be responsible for sensorial rancidity. These workers concluded that the structure of TBA was altered by acid and heat treatment as well as by the presence of peroxides and recommended that blank determinations be carried out in conjunction with the test.

The TBA test may be performed in two ways, either directly on a food product followed by extraction of the colored pigment, or on a portion of a steam distillate of the food. Both methods have in common the use of acid and heat. Dekoning and Silk (1963) reported that they were unable to successfully apply the TBA test, in either of its forms, to determine rancidity in fish oils.

The TBA test has been used by some workers (Gutierrez and Romero, 1960; Casillo, 1968) to measure oxidative rancidity in olive oil. Casillo (1968) reported that this test detects the rancidity of olive oil at a lower level than other tests (peroxide value and Kreis test).

MATERIALS AND METHODS

The materials and methods used will be described separately for each major component of the study.

I. Effect of Harvesting Processes on Quality in Olive Oil

Collection of Samples

Olive fruits of the cultivar "Tsounati" one of the main cultivars of Greece were used. Commercial plastic nets were used for collection of fruits from 12 olive trees. The olive trees were numbered and divided into three groups. Trees numbered from one to four formed the first group, from five to eight the second and from nine to twelve the third group. The area under the trees was covered with a layer of plastic nets and the trees were shaken so a considerable amount of olives fell on them. A second layer of nets was placed on the fallen olives to separate them from others falling later naturally.

Every two weeks, a quantity of about 0.5 Kg of olives was taken from the upper net layer, a similar quantity from the lower net layer, and a third one from the tree. The fruits were placed in plastic bags which were not tied for a better aeration. Samples upon arriving at

Institute of Subtropical and Olive Trees, at Chanea, where they were stored in the refrigerator (5°C) to avoid hydrolysis.

Every four samples coming from a certain group (3 groups) of trees and being of a certain type (ie directly from the tree, from the upper layer of nets, or from the lower one) were mixed thoroughly. Thus nine samples were gathered for analysis; three from each group of trees.

Extraction of the Oil

The olives were processed for oil extraction in a small experimental "mill" of the centrifugal type. The olive fruits were ground in a mill and the resulting mixture was allowed to undergo malaxation for about 15 min. Then a quantity of warm water (35-40°C) was added for easier release. After malaxation, the mixture was centrifuged for about 5 min for the solid and liquid substances to be separated. The liquids were then put in a separatory funnel, where most of the water was removed. The remaining oil-water mixture was centrifuged in a LABOFUGE type centrifuge, at 5000 rpm, for 10 min. Finally, clear samples of olive oil were obtained. The samples were kept in the refrigerator at 5°C until they were analyzed (3-5 days after extraction).

Analysis of the Fruit

The oil content of olive fruit was determined by using a oxhlet extractor and the method described by Mehlenbacher (1960) for cottonseed oil with some modifications. 50 g of fruits were used in a solvent extraction which lasted 12 hrs. The solvent was driven off from the oil by heating the mixture in an oven at 85°C until constant weight was obtained.

The moisture content was determined by drying the samples in an oven at 100°C to a constant weight.

Analysis of the Oil

Free fatty acids (FFA) were determined by the official AOCS (1974) method.

Twenty eight g of oil were weighed in an Erlenmeyer flask. Fifty ml of neutralized alcohol, and 2 ml of phenolphthalein indicator (1% in 95% alcohol) were added. The sample was titrated with 0.1 NaOH until the appearance of pink color persisted for 30 seconds. The percentage of free fatty acid was calculated as oleic acid.

The degree of oxidation of the oil was followed by determining the peroxide value (PV) according to the method recommended by the International Olive Oil Council (1966). Two g of oil were taken by weight from the stored samples and dissolved in 25 ml glacial acetic acid-chloroform (3:2) solution and 1 ml saturated KI solution was added. The mixture was shaken and allowed to stay for one minute in the dark. It was then diluted with 75 ml distilled water and titrated with sodium thiosulfate (0.005 or 0.01N) using 2 ml of starch indicator. Results were reported as milliequivalents O_2/Kg oil.

II. Comparison of Extraction Systems

The following extraction systems were compared: a) Pieralisi, b) Hiller and c) Rapanelli (Sinolea-Decanter).

The processing steps for oil extraction for the systems Pieralisi and Hiller (centrifugal) are shown in Appendix 1. Appendix 2 shows steps of the Rapanelli system.

As it has been pointed out in the literature review the Rapanelli system gives two types of oil, the "Sinolea" and the "Decanter". These two types of oil were studied separately.

Fruit Collection-Oil Extraction

Olive fruits from the cultivar "Koroneiki" were used for this study. The fruits were collected from the same area, put in cloth sacks and transferred by truck to the institute at Chanea. As soon as the fruits were brought in, they were mixed well and the entire mass, about 4 tons, was divided into three parts, not equal, since each system requires a different quantity of fruits for normal operation.

Soon after the separation, fruits were processed for oil extraction in the three systems. The experiment was repeated six times during the harvesting season.

Oil-Analysis

The oil was first analyzed immediately after the extraction and then periodically during storage in glass containers. Three different conditions of storage were explored:

a) dark, b) diffused natural daylight and c) direct sunlight irradiating the samples for 4 hrs a day with the remainder of the time being stored as samples (b).

In another experiment, the oil extracted by each system was stored in tin cans and subjected to analysis before and during storage.

The initial analysis included: a) moisture determination, b) foreign materials, c) free fatty acids (FFA) and peroxide value (PV). After storage only PV was determined.

Moisture Determination

The moisture determination was made by the AOCS method as described by Mehlenbacher (1960). In a 500 ml distillation flask, 175 g oil were weighed and an equal volume of toluene was added. After connecting the flask with the apparatus, specified by the AOCS, the receiver was filled with toluene by pouring it through the condenser until it began to overflow into the distillation flask. A piece of cotton was inserted loosely into the top of the condenser. The distillation lasted until the level of the water in the receiver remained unchanged for 30 min. Then the condenser was washed with 5 ml of toluene and the receiver was immersed in water of 25°C for about 15 min. The moisture content was determined by using the formula:

$$\%\ \text{moisture} = \frac{\text{Volume of water} \times 99.7}{\text{wt of sample (g)}}$$

Foreign Materials

The foreign materials of olive oil were determined by centrifugation in a Labofuge centrifuge, at 5000 rpm, for 30 min.

Chlorophyll Determination

The chlorophyll content of Rapanelli oil (Sinolea-Decanter) was determined at the end of the experiment by the official AOCS (1978) method. The method is described in experimental part IV.

Peroxide Value (PV)

The COI (1966) method described previously (Part I) was employed for the measurement of PV (initially and during storage).

Free Fatty Acid (FFA)

The free fatty acid content of the oil was determined using the official AOCS (1974) method as described previously (Part I).

III. Effect of Packaging and Storage Conditions on Olive Oil Quality

Packaging Material

Plastic and glass bottles were used for this study. Colorless glass bottles were obtained from a Sprite company while polyethylene plastic bottles were obtained from an olive oil company.

Storage Conditions

The samples of olive oil were put in the glass or in the polyethylene plastic bottles, 100 g and 900 g, respectively, and stored in diffused light and in direct sunlight. One-half of the samples were covered with aluminum foil to avoid passage of light through the transparent bottles.

The degree of oxidation of the samples was followed by measuring peroxide value (COI, 1960).

IV. Photooxidation of Olive Oil

Olive Oil

Virgin* olive oil obtained from the island of Crete was used for this study. The oil was extracted from fruits of the cultivar "Koroneiki", with the centrifugal Pieralisi system, and brought to the Food Science laboratory at Michigan State University in tin cans.

Chlorophyll, Carotene, Tocopherol and Pheophytin Standards

Chlorophyll a, α and β-carotene and d-α-tocopherol were obtained from the Sigma Chemical Company. A mixture of pheophytin a and b was purchased from ICN Pharmaceuticals Inc. (Plainview, N.Y.).

Other Chemicals

Nickel-Dibutyl di Thiocarbamate was obtained from Pealtzs' Bauer Inc. (Stanford, Conn). Caffeic acid (assay 63% by titration) was purchased from Mann Research

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*Extraction from healthy, mature olives by mechanical means without any chemical treatment.

Laboratories Inc. N.Y. Boron fluoride methanol and Folin Ciocalteau reagent were acquired from the Sigma Chemical Company. All other reagents and chemicals used were reagent grade.

Bleaching Agents

Activated Charcoal Darco G-60 (MCB Manufacturing Chemists Inc., Cincinati, Ohio). Tonsil optimum Extra (L.A. Salomon & Bro. Inc., Post Washington N.Y.). Florisil (60-100 Mesh)(Fisher Scientific Company). Absorptive Magnesia Sea Sorb 43 (Fisher Scientific Company). Hyflo -supercel and infusorial earth (Fisher Scientific Company).

Light Source

Two Sears, 20 watt (each), cool white fluorescent tubes were used in the photooxidation studies.

Analytical Techniques

Total Fatty Acids

Methyl esters of olive oil were prepared according to the method of Morrisson and Smith (1964). Boron fluoride methanol was used as reagent. The oil was put in centrifuge tubes and the reagent was added under nitrogen in a proportion of 1 ml reagent/4-16 g of oil. Tubes were closed with screw caps and heated in a heating source (PIERCE Reacti-Therm HEATING MODULE) for 30 minutes, cooled and opened. The esters were extracted by adding 2 volumes of pentane then 1 volume of water, shaking briefly and centrifuging until two layers were formed. The upper layer contained the esters.

The methyl esters obtained as mentioned above were injected into a Hewlett Packard Gas Cromatograph 5840 A equipped with a hydrogen flame detector. A coiled stainless steel column, 180 cm long and 2 mm i.d. packed with 15% (W/W) DEGS on 80/100 mesh chromosorb-W was used for methyl ester separation. The column oven temperature was 100°C, the injector temperature was maintained at 210°C and the detector temperature at 350°C. The nitrogen carrier gas was adjusted to 31.3 ml/min. The flow rate of hydrogen was 18.5 ml/min. The emerging components were identified by comparing the retention time of each to those of standard mixtures of known fatty acid methyl esters. Peak areas were calculated by an electronic integrator (5840 GC Terminal Hewlett-Packard).

<u>Chlorophyll</u>

The level of chlorophyll was determined by the official AOCS method (1973) in a Beckman DU Spectrophotometer using a 1 cm cell. Redistilled carbon tetrachloride, was used as blank. The absorbance values were measured at 630, 670 and 710 nm and the calculation was made using the formula:

$$\text{Chlorophyll (ppm)} = \frac{A_{670} - \dfrac{A_{630} + A_{710}}{2}}{0.1016\ L}$$

Where: A=Absorbance, L=Cell length in cm.

Color

The official AOCS method (1973) was utilized for color determination in the unbleached* and bleached olive oil. Samples without any treatment were filtered through a No. 4 Genuine Whatman filter paper and the transmittance was measured at 460, 550, 620 and 670 nm in a Beckman DU spectrophotometer using a 1 cm cell and redistilled CCl_4 as a blank.

β-Carotene

Two g samples of oil were weighed into 70 ml test tubes with screw caps. Twenty ml of 0.7 N alcoholic KOH solution were added, and the oil was allowed to saponify for 5 minutes in a beaker containing boiling water. The test tube was cooled at room temperature. Twenty ml of distilled water were added and the tube was shaken for 1 minute and fifteen ml of hexane were added and the tube was shaken vigorously for two minutes. The layers were allowed to separate. The upper layer was removed with a 20 ml pipette bulb. This extraction was repeated four times with the same amount of solvent. The hexane layers were collected in a round distilling flask and the solvent was evaporated in a rotary evaporator, under vacuum, at a temperature of 40°C.

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*The term unbleached olive oil will refer to initial olive oil (virgin) in the rest of this dissertation.

A glass column, 40x2 cm, was prepared according to the procedure described in the A.O.A.C. (1975). The packing material consisted of 2 cm of glass wool at the bottom and 12 cm of a pre-prepared mixture of 1:1 Seasorb 43 (MgO) and diatomaceous earth. About 2 cm of sodium hydroxide pellets were added and finally a 1 cm layer of anhydrous sodium sulfate placed on the top, completed the packing material of the column. The packing was carried out under reduced pressure produced by a water aspirator. Thirty ml of 9:1 hexane-acetone mixture was added to wet the column. The extract was transferred to the column with 20 ml of hexane-acetone mixture. Fifty ml of the same mixture of solvents were used to elute the beta-carotene fraction contained in the sample. The solvent mixture contained in the eluate was evaporated, as described before, in order to reduce the volume to 15-20 ml. This was transferred to a 25 ml volumetric flask and brought to volume with the same mixture of solvents.

To prepare the beta-carotene standard curve, 5.0 mg of beta-carotene were dissolved in 9:1 hexane-acetone in a 250 ml volumetric flask. From this solution, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were taken and placed separately into 50 ml volumetric flasks and brought to volume using the same mixture of solvents.

The absorbance of these solutions was read at 450 nm in a Beckman DU Spectrophotometer in 1 cm cell against a hexane-acetone (9:1) mixture as blank.

α-Tocopherol

The method described by Carpenter (1978) was used to determine α-tocopherol present in olive oil. Five g of unbleached olive oil was weighed into a 50 ml volumetric flask. The sample was brought to volume with a mixture of hexane-acetone 85:15 (HPLC grade). Smaller samples were used for the oxidized oil. The solution was filtered just before injection. The injection volumes were 50 μl.

The chromatographic separation was performed by a Waters Associates Liquid chromatograph on a μ-Porasil column. The UV detector was set at 280 nm.

α-Tocopherol solution (10.37 ppm) was used as a standard for peak identification and quantitation (by peak height). Separate curves were prepared for the non-oxidized and for oxidized samples. A straight line relationship between peak height and concentration was obtained.

Phenols

The total phenols were determined according to the Vazquez *et al*. (1976) method as described by T. Gutfinger (1981). Ten g of oil was put in 250 ml Erlenmeyer flask and dissolved with 50 ml hexane. The solution was extracted successively with three 20 ml portions of 60% aqueous methanol.

The mixture was shaken each time for 2 minutes in a BURRELL WRIST ACTION SHAKER. The combined extracts were dried in a vacuum rotary evaporator at 70°C. The residue was dissolved in 1 ml methanol. The concentration of total polyphenols in the methanolic extract was estimated with Folin Ciocalteau reagent. The procedure consisted of dilution of 0.1 ml methanolic extract with 5 ml of distilled water in a 10 ml volumetric flask and addition of 0.25 ml of Folin-Ciocalteau reagent (2N). After 3 minutes, 1 ml of saturated (Ca 35%) Na_2CO_3 was added. The content was diluted to volume with water. The absorbance was measured after 1 hour at 725 nm in a Beckman DU Spectrophotometer using reagent solution as blank. Caffeic acid served as a standard for preparing the calibration curve ranging from 0-100 μg/10 ml assay solution.

Trace Metal

About 15 g of sample was charred under 250°C for 4-5 hours and then ashed at 600°C overnight. The ash was dissolved in about 1 ml 6N HCl after adding a few drops of distilled deionized water. The measurements for Fe and Mg were taken in an IL951 Atomic Absorption Spectrophotometer according to the manufacturer's guide.

Ultraviolet Absorption

One g of unbleached oil was accurately weighed into a 100 ml volumetric flask and brought to volume with Spectra Grade iso-octane.

Measurements were taken at 232, 262, 268, 270 and 274 nm in a Beckman DU Spectrophotometer using iso-octane as a blank.

Peroxide Value (PV)

Peroxide values were determined by the official AOCS method (1973) and were reported as milliequivalents O_2/Kg oil. One to two g of oil, accurately weighed, were taken from the beakers, after thoroughly mixing the contents, at regular hourly intervals during irradiation. The samples were dissolved with 30 ml of glacial acetic acid-chloroform 3:2 (V/V). One ml 50% (W/V) KI solution was added. The mixture in the flask was shaken and allowed to stand for exactly 1 minute. Fifty ml of distilled water were added and titration followed with sodium thiosulfate solution (0.01 or 0.1 N). During titration the solution was mixed constantly with a Sargent magnetic stirrer. One ml of starch solution (Fisher Scientific Company) was used as an indicator.

Conjugated Dienoic Acid (CDA)

The official AOCS method (1973) was used for this determination. Ninety to 130 mg of oil were weighed into a 100 ml volumetric flask. About 75 ml of Spectra Grade iso-octane was added. The flasks were allowed to stand for a few minutes at room temperature and then iso-octane was added to make up to volume.

The absorbance of the solutions was measured at 233 nm in a Beckman DU Spectophotometer against an iso-octane blank.

To calculate the percentage conjugated dienoic acid the following formula was used:

$$\text{Conjugated dienoic acid \%} = 0.84(\frac{As}{bc} - K_o)$$

Where: K_o=0.07 (absorptivity for esters)

As= observed absorbancy at 233 nm

b= cell lengh in centimeters

c= concentration in g/liter

Thiobarbituric Acid (TBA) Test

The method of Sidwell et al. (1974) was used for this test. The TBA solution was prepared by dissolving 0.67 g of thiobarbituric acid in distilled water with the aid of heat from a steam bath. The solution was transferred to a 100 ml volumetric flask cooled and brought to volume.

The TBA reagent consisted of an equal volume of TBA solution and glacial acetic acid. Three g of oil were accurately weighed into a 125 ml Erlenmeyer flask and dissolved with 10 ml of CCl_4. Ten ml of reagent was added.

The flasks were stoppered and shaken for 4 minutes in a BURRELL WRIST ACTION SHAKER. The contents of the flask were transferred to a separatory funnel and the aqueous layer was withdrawn into a test tube. The tubes were immersed in a boiling water bath for 30 minutes, then cooled and centrifuged for 4 min. in a centrifuge (E.H. SARGENT & Co). Finally the clear portion of the contents was transfered to a 1 cm cuvette. The absorbance was measured at 530 nm in a Beckman DU Spectrophotometer against distilled water as a blank.

Bleaching Technique

Glass columns with 4.5 cm diameter were packed in the following way: About 2 cm of glass wool was placed on the bottom and then a prepared mixture consisting of 35 g of carbon, 50 g of extra Tonsil, 15 g of florisil and 25 g of hyflo supercell was added. A layer of about one cm of infusorial earth was placed on the top.

The column was packed under reduced pressure, produced by a water aspirator and pressed with a glass rod. About 150 ml of hexane was percolated through the column before the oil addition. After all hexane had passed through, a mixture of 100 g oil and 150 ml hexane was added to the column. The column was washed with 200 ml of hexane to elute all the remaining oil.

The eluate was collected in 500 ml filtering flasks. This was transferred into 500 ml round flasks and the solvent evaporated in a rotary evaporator under vacuum at 40°C. The oil yield was more than 90%.

During bleaching, the columns and the collecting flasks were covered with aluminum foil and a stream of nitrogen was applied to the top. The bleached oil obtained by this technique was kept under a nitrogen atmosphere, in the refrigerator, for later use.

Sample Preparation for Illumination

An appropriate quantity of sample (25-50 g) of unbleached and bleached olive oil was put in 250 ml beakers.

The additives chlorophyll a, pheophytin (a + b), α-tocopherol, and Ni chelate (Nickel-Dibutyl Di Thiocarbonate), were dissolved in a mixture of 9 ml hexane and 1 ml acetone. Stock solutions were prepared for all additives except Ni chelate. Appropriate quantities of these solutions were pipetted for each usage.

Illumination of Samples with Fluorescent Light

Beakers containing the different samples were placed in a stainless steel (50x29x5 cm) water bath apparatus. The interior surface of the apparatus was lined with aluminum foil. Two 20 watt fluorescent daylight tubes were suspended approximately 6 cm above the samples. The remaining open part of the top was covered with aluminum foil to avoid any effect of outsource light.

The amount (7500 Lux) of fluorescent radiation was measured with a light meter (Luna Pro by Gossen W. Germany).

The apparatus was kept in a room where the temperature stayed almost constant (24±2°C). The temperature inside the apparatus due to the action of light was higher (30±2°C).

In all the experiments the radiation source was turned off for sampling at fixed time intervals. In each sampling, the position of samples was changed to obtain illumination as uniform as possible for all samples.

RESULTS AND DISCUSSION

I. Effect of Harvesting Processes on Quality in Olive Oil

Results concerning the hydrolytic and oxidative deterioration of olive oil before processing, mainly during the time that olive fruits remain on the collection nets, are discussed here.

Statistical analysis was made for some of the data and the results are presented either in Tables or Figures.

Hydrolytic Deterioration of Olive Oil

Figure 2 shows the degree of hydrolysis (increase in free fatty acid content) that olive oil undergoes during the time the fruit remained either on the tree or on the nets. In this figure, line 1 represents the change in free fatty acid content of oil extracted from fruit from the lower net layer (fruit had fallen by shaking the trees). Line 2, from the same figure, shows the change in free fatty acid content of olive oil extracted from fruit collected from the upper net layer. Line 3 demonstrates changes in free fatty acid content in the oil from fruits collected from the tree.

Results of this figure clearly demonstrate that the increase in free fatty acid content was very high for the samples on the lower net layer, less for the samples of the

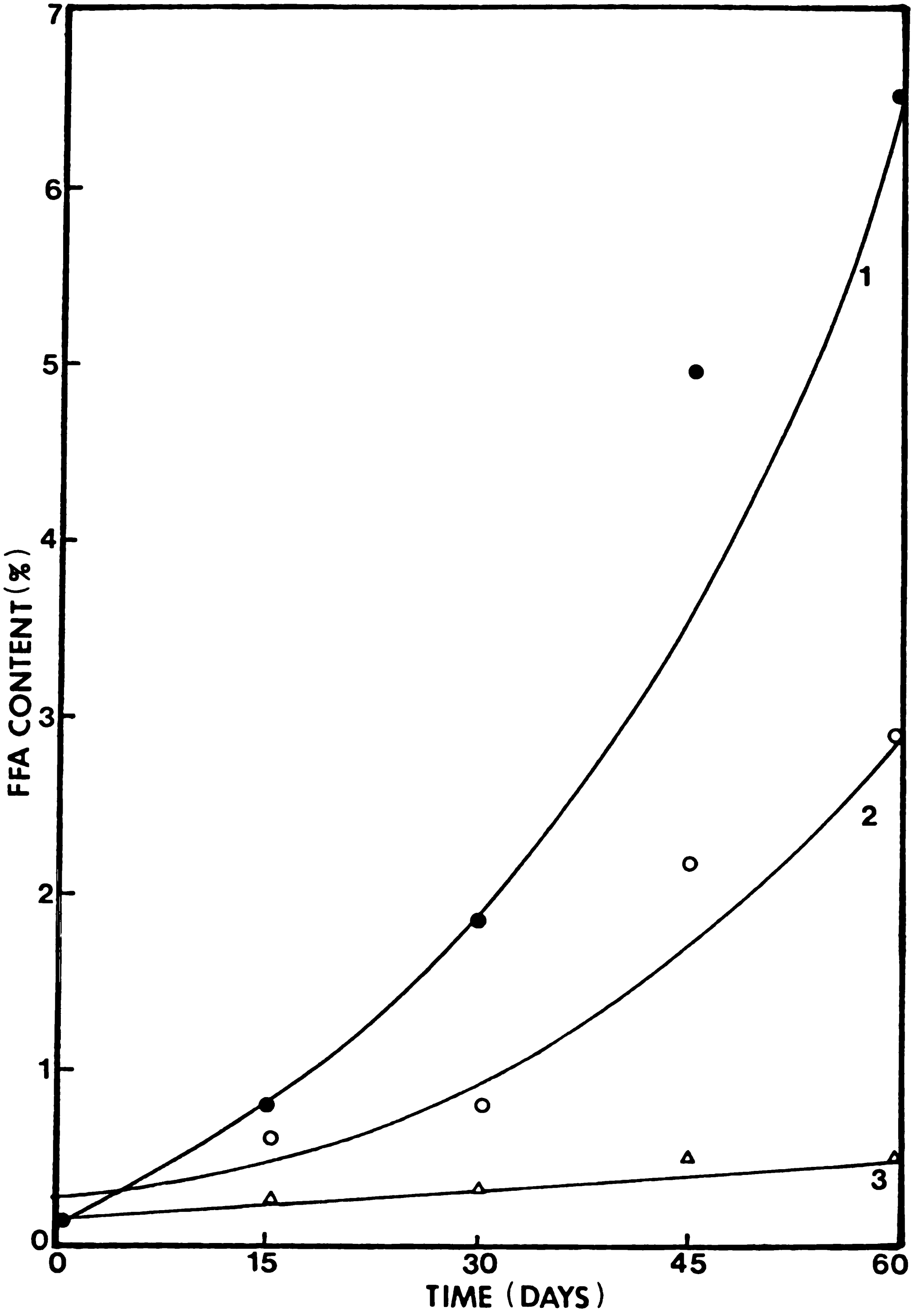

Figure 2 Free fatty acid content of olive oil as affected by time and method of fruit collection (●: lower net layer, o: upper net layer, △: tree).

upper layer and even less for the samples collected directly from the trees.

The increase in the free fatty acid content of the samples collected from the tree is probably due to the presence of the enzyme lipase which is activated with the process of maturation (Martinez, 1975). According to Newnschuander and Michelakis (1978), even the dacus fly infestation can cause an increase in free fatty acid content. The holes formed by the exit of larvae provide the starting points for fungus development during improper storage of the fruit. Fungi in turn liberate the enzyme lipase which causes hydrolytic deterioration of the oil.

Martinez (1975) reported that olives collected from the tree are of better quality than the ones gathered from the ground and should be processed separately.

The high degree of hydrolytic deterioration of oil extracted from samples from the lower net layer was related to "bad" fruit conditions. The starting of fungus development in these fruits was apparent by visual inspection.

Fruits collected from spots where rain water remained on the surface of the ground were in worse condition. Thus olive fruits on the lower net layer, forced to fall by shaking the trees, were the ones that showed higher free fatty acid content since they remained on the nets longer, therfore underwent greater hydrolytic deterioration.

Data in Figure 2 dealing with fruits obtained from the upper layer of nets show that the free fatty acid content of the oil was less than one when they remained on the nets up to a month. From that time on, free fatty acids increased and reached a value of 3.03% when this study was terminated (two months after initiation).

While this oil was still considered as "virgin" (ordinary), according to quality critieria of COI (1966), it was not the type of oil that consumers prefer. They prefer oils with free fatty acid content <1. Such oils are known as "extra virgin."

Data in Table 4 do not show any significant statistical difference in the change in the free fatty acid content of oil, as affected by collection time, for fruits collected from the trees. Despite the fact that we did not have a significant difference in the free fatty acid content of the oil extracted from fruits collected from the tree (Table 4), we may have had some differences in the aroma constituents of the oil. According to Martinez (1975), the best aromatic characteristics of the oil exist at the optimum stage of fruit maturation which can be established visually when the fruits begin to darken. Many of the fruits collected from the trees had passed this stage of maturity.

Results dealing mainly with the hydrolytic deterioration of olive oil, obtained from another experiment in two consecutive years appear in Table 5. The same trees were used for both years.

The results shown in Table 5 indicate that the rate of the increase in the free fatty acid content of the oil was not the same for the two years. The free fatty acid content of the oil, during the time that fruits remained on the tree, increased from 0.8% to 1.7% for the first year, while for the second it was increased from 0.2% to 1.2% during one month.

Oxidative Deterioration of Olive Oil

Table 6 presents data on the peroxide value of oil from the various samples. These data demonstrate that the oxidative deterioration of olive oil like the hydrolytic (Figure 1) was also higher in the samples from the lower net layer than from the upper one. The tree samples had the lower peroxide value.

Table 7 shows that in one month the peroxide value of oil samples from another experiment changed from 0 to 10. This value was lower than the upper limit (20) proposed by the COI (1966) for virgin olive oil.

Data of Table 6 for the oil from the upper and lower net layer had passed this limit after a month. On the contrary, in the samples collected from the tree, the peroxide value was relatively low when this study was terminated (Table 6).

Table 4 Free fatty acid content of olive oil as affected by time and method of fruit collection.

Origin of Olive fruit	Free Fatty Acid (%)					Differences
	Collection time in days					
	0	15	30	45	60	
Tree	0.14	0.27	0.33	0.44	0.53	NS
Upper net layer	-	0.62	0.78	2.24	3.03	*
Lower net layer	0.19	0.47	1.87	5.02	6.96	**

NS: No significant difference with collection time at P=0.05.

*: Significant difference with collection time at P=0.05.

**: Highly significant difference with collection time at P=0.05.

Table 5 Free fatty acid content of olive oil as affected by collection time of fruit from the upper net layer.

Collection Time (Days)	Free Fatty Acid (%)	
	Production year	
	1979-80	1980-81
0	0.0	0.0
3	0.8	0.2
7	0.9	0.5
15	1.1	1.0
22	1.4	1.1
29	1.7	1.2

Table 6 Peroxide value of olive oil as affected by time and method of fruit collection.

Origin of olive fruit	Peroxide Value ($meq\ O_2/Kg$ oil)					Differences[a]
	Collection time in days					
	0	15	30	45	60	
Tree	5.7	5.7	3.0	10.0	7.0	*
Upper net layer	-	10.8	4.7	32.7	22.5	*
Lower net layer	6.7	11.6	6.7	47.7	43.8	**

*: Significant difference with collection time at P=0.05.
**: Highly significant difference with collection time at P=0.05.

Table 7 Peroxide value of olive oil, as affected by collection time of fruit from the upper net layer.

Collection time (Days)	Peroxide Value ($meq\ O_2/Kg$ oil)
0	0.0
3	5.0
7	8.0
15	8.5
22	9.0
29	10.0

These data indicate that the oxidative deterioration of olive oil during the time that fruits remain on the tree does not increase much. Probably the epidermis of olive fruits protects the oil from coming in contact with the air. During commercial harvesting, however, the skin is damaged and the oxygen of the air may interact with the oil.

Oil and Moisture Changes in the Olive Fruits

As is shown in Table 8, samples collected from the nets (lower-upper) contained more oil expressed (in raw weight) than the ones obtained from the tree. This is probably due to loss of water from the fruits. In fact, as Table 9 shows, the moisture content of fruits regardless of the origin was almost the same initially (0 months). Two months later, however, samples collected from the nets had about one-half the moisture content of the initial. The moisture content of fruits collected from the tree did not change appreciably.

Figure 3 shows that the oil content of fruits collected from the tree, 15 days later from the time that this experiment was initiated, started decreasing but not significantly. Martinez (1975) reported that as maturity advances the fruit increases in weight until it is fully developed. This state is maintained for a certain time and then the fruit begins to lose weight and its oil yield is affected.

Table 8 Oil content of fruit as affected by time and method of fruit collection.

Origin of olive fruit	Oil Content (%)					Differences[a]
	Collection time in days					
	0	15	30	45	60	
Tree	30.6	33.3	31.9	31.0	29.5	NS
Upper net layer	-	37.4	35.8	36.5	43.8	*
Lower net layer	30.8	35.2	33.7	35.5	45.0	*

NS: No significant difference with collection time at P=0.05.
*: Significant difference with collection time at P=0.05.
**: Highly significant difference with collection time at P=0.05.

Table 9 Moisture content of olive fruit as affected by time and method of fruit collection.

Origin of Olive fruit	Moisture Content (%)	
	First Sampling	Last Sampling
	0 Months	2 Months
Tree	41.73	43.54
Upper net layer	41.50	21.40
Lower net layer	41.65	17.33

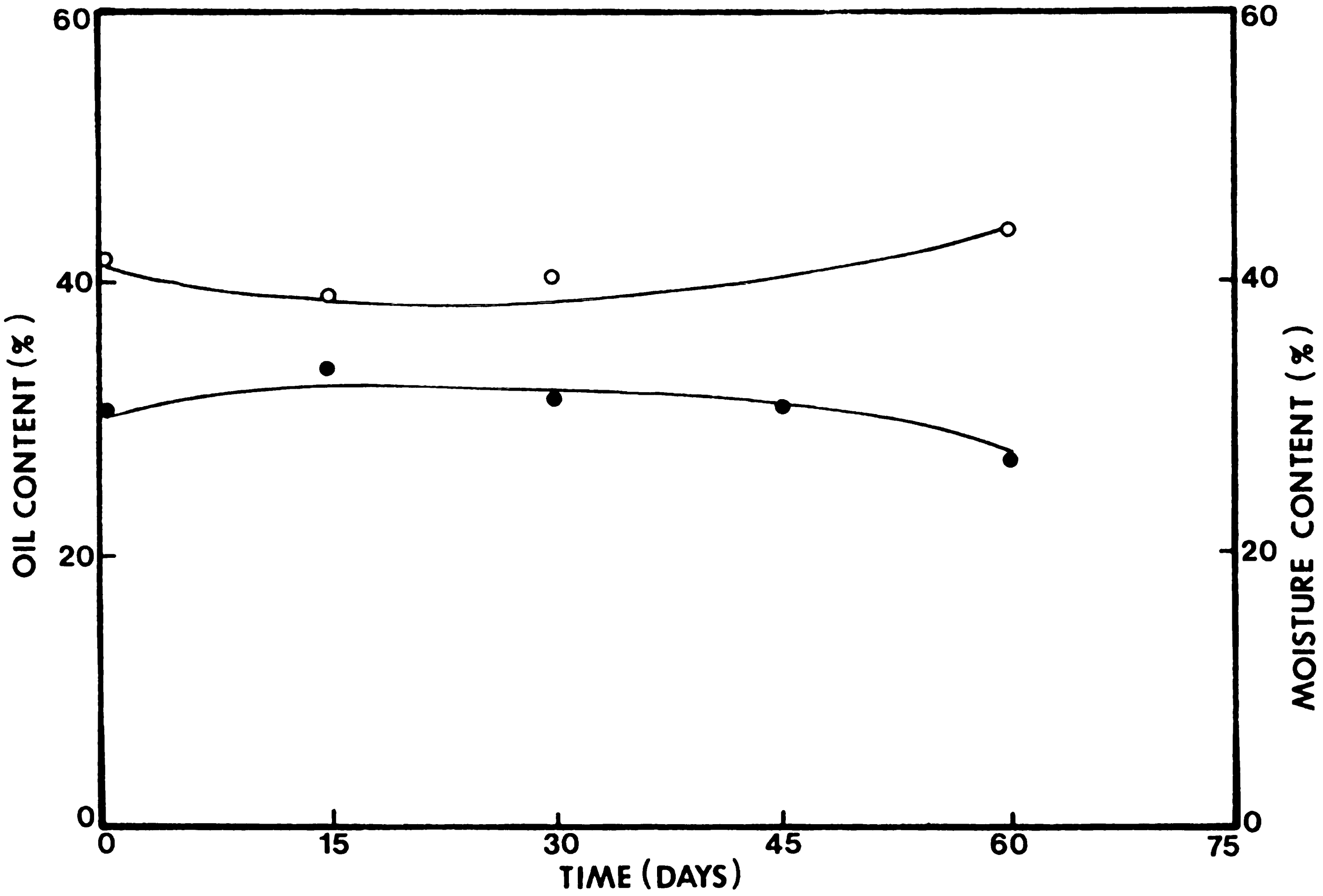

Figure 3 Oil and moisture content of olive fruits from tree as affected by collection time. (●: oil, ○: water)

II. Comparison of Extraction Systems

Initial Quality of Olive Oil as Affected by the Extraction System

Table 10 presents results from the initial analysis of olive oil samples obtained during olive fruit processing by three different systems. The olives used were from the cultivar "Koroneiki." As soon as they were brought to the Institute at Chanea they were mixed well and were divided into three parts. One part of the fruits was processed by the Pieralisi system, another by the Hiller, and the third one by the Rapanelli (Sinolea-Decanter) system. As is shown in Table 10, the initial peroxide value in all of the oil samples obtained was less than 10. These data agree with those from previous experiments (Table 6) and show that olive oil undergoes some oxidative deterioration, but not high, before the removal of the fruits from the tree. With such low peroxide values (Table 10) we can not attribute any effect on oxidation from the extraction systems.

Free fatty acid content of all samples did not exceed one. With such values the oil was rated as "extra virgin" (COI, 1966). Moisture content on the other hand was lower than 0.5%. This is in agreement with the value reported in the literature by Cucurachi (1975).

Since Table 10 shows no significant differences in the free fatty acid, peroxide value, foreign materials and moisture content for the oil obtained by the three different systems, from six trials, we did not attribute any effect of the extraction systems on the initial quality of the oil.

Table 10 FFA, PV, foreign materials, and moisture of olive oil obtained by 3 different extraction systems.

Trials	Extraction System	Data of Analysis			
		FFA (%)	PV $meqO_2/Kg$ oil	Foreign materials (%)	Moisture (%)
A	Rapanelli-Sinolea	0.30	6.0	traces	0.3
	Rapanelli-Decanter	0.31	6.25	"	0.3
	Pieralisi	0.31	7.0	"	0.2
B	Rapanelli-Sinolea	0.19	8.0	0.2	0.4
	Rapanelli-Decanter	0.24	8.4	0.3	0.3
	Pieralisi	0.15	7.8	0.2	0.3
C	Rapanelli-Sinolea	1.0	8.5	0.3	0.4
	Pieralisi	1.0	10.0	0.3	0.3
D	Pieralisi	0.29	7.0	0.2	0.3
	Hiller	0.36	7.5	0.2	0.4
E	Rapanelli-Sinolea	0.80	6.5	0.2	0.3
	Rapanelli-Decanter	1.00	7.5	0.2	0.3
	Pieralisi	0.82	6.5	traces	0.2
	Hiller	0.72	6.7	0.2	0.3
F	Rapanelli-Sinolea	0.60	7.0	traces	0.2
	Rapanelli-Decanter	0.70	8.0	"	0.3
	Pieralisi	0.60	7.0	"	0.2

*Olive fruits from each trial were of the same quality and were collected directly from the trees.

A difference was observed only in the color of the Rapanelli oils (Sinolea-Decanter). Decanter oil appeared darker than Sinolea oil due to its higher chlorophyll content. Chlorophyll determination was made at the end of storage, in darkness studies. At that time the Sinolea and the Decanter oils contained 5.0 and 9.95 ppm chlorophyll respectively. Carocci (1963) noted that the high chlorophyll content of the Decanter oil is probably the result of extended malaxation.

Oxidation of Oil Obtained by the 3 Extraction Systems

Some of the data obtained from this study were statistically analyzed and the results are presented in Tables 11, 12, and 13. The values of the coefficients in these tables showing the increase of hydroperoxides, were higher in samples stored in light (direct or diffused) than in darkness. The values of coefficients corresponding to direct light were greater than that corresponding to diffused light (Tables 12 and 13). This indicates that oxidation proceeded to a higher degree in the case of direct light.

Figures 4 and 5 show the increase in peroxide value per unit time, for Pieralisi and Rapanelli (Sinolea-Decanter) oils stored in darkness and in diffused light. The slopes of the three lines for the diffused light are obviously larger than the ones corresponding to darkness.

Table 11 Correlation coefficient and regression equation between peroxide value's and storage time in olive oil stored in darkness.

Extraction System	Correlation Coefficient (r)	Regression equation	
		a	b*
Pieralisi	0.92	8.17	0.61^a
Rapanelli-Sinolea	0.76	7.13	0.91^a
Rapanelli-Decanter	0.97	7.20	1.63^b
Pieralisi	0.87	9.74	0.73^a
Rapanelli-Decanter	0.99	6.26	2.17^b

*Values b with different superscripts are statistically different at P=0.05.

Table 12 Correlation coefficient and regression equation between peroxide value's and storage time in olive oil stored in diffused light.

Extraction System	Correlation Coefficient (r)	Regression equation	
		a	b*
Pieralisi	0.87	11.10	3.39^a
Rapanelli-Sinolea	0.78	16.16	5.18^b
Rapanelli-Decanter	0.77	14.48	3.56^a
Pieralisi	0.93	18.02	2.57^a
Rapanelli-Decanter	0.95	7.40	2.90^a

*Values b with different superscripts are statistically different at P=0.05.

Table 13 Correlation coefficient and regression equation between peroxide values and storage time in olive oil obtained by different systems and stored in direct light.

Extraction System	Correlation Coefficient (r)	Regression equation	
		a	b*
Pieralisi	0.90	1.21	4.93^a
Rapanelli (Sinolea)	0.99	7.21	5.18^a
Rapanelli (Decanter)	0.84	30.44	3.91^a
Pieralisi	0.98	12.49	6.64^a
Rapanelli (Decanter)	0.98	3.60	28.67^b

*Values b with different superscripts are statistically different at $P=0.05$.

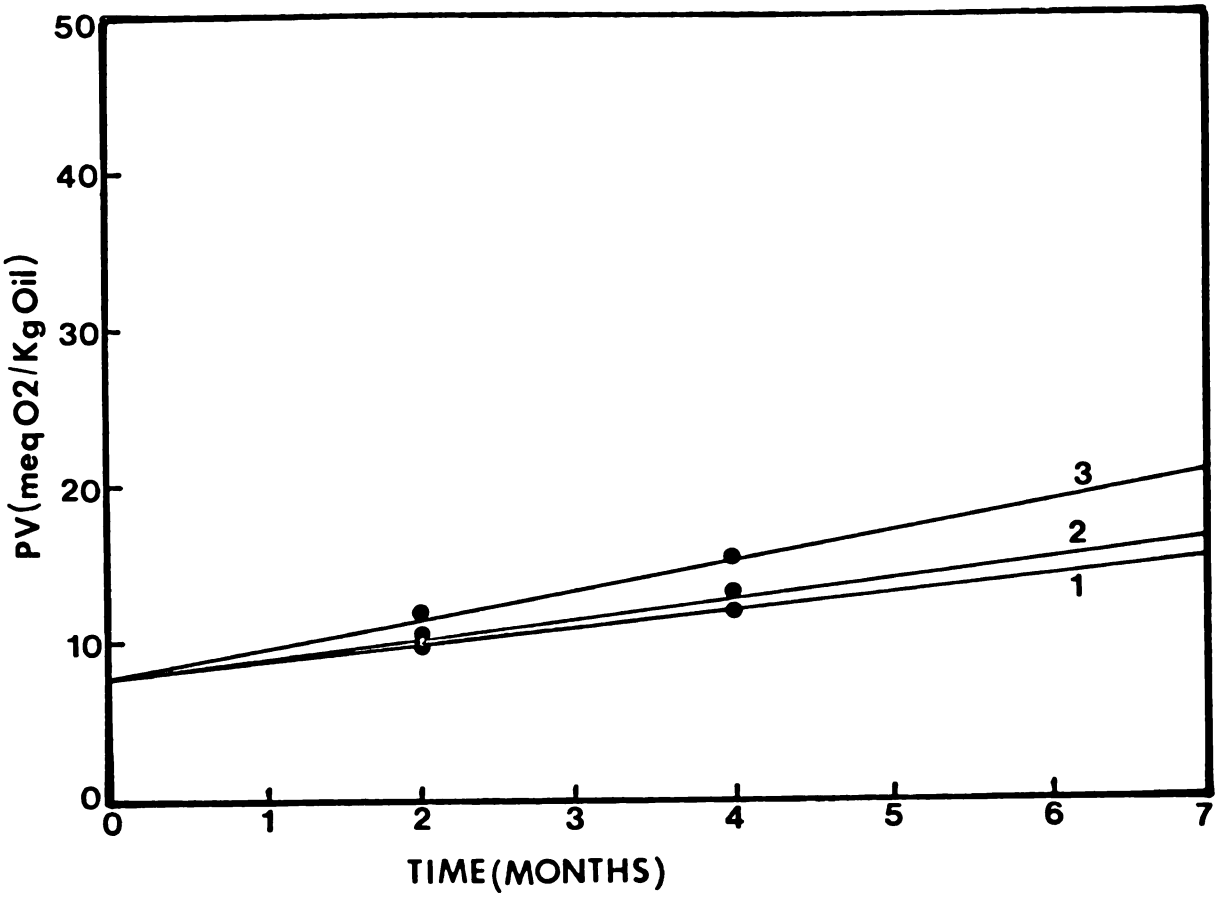

Figure 4 Peroxide value of olive oil obtained by the Pieralisi and Rapanelli (Sinolea-Decanter) systems during storage in darkness. (1:Pieralisi, 2:Rapanelli-Sinolea, 3:Rapanelli-Decanter)

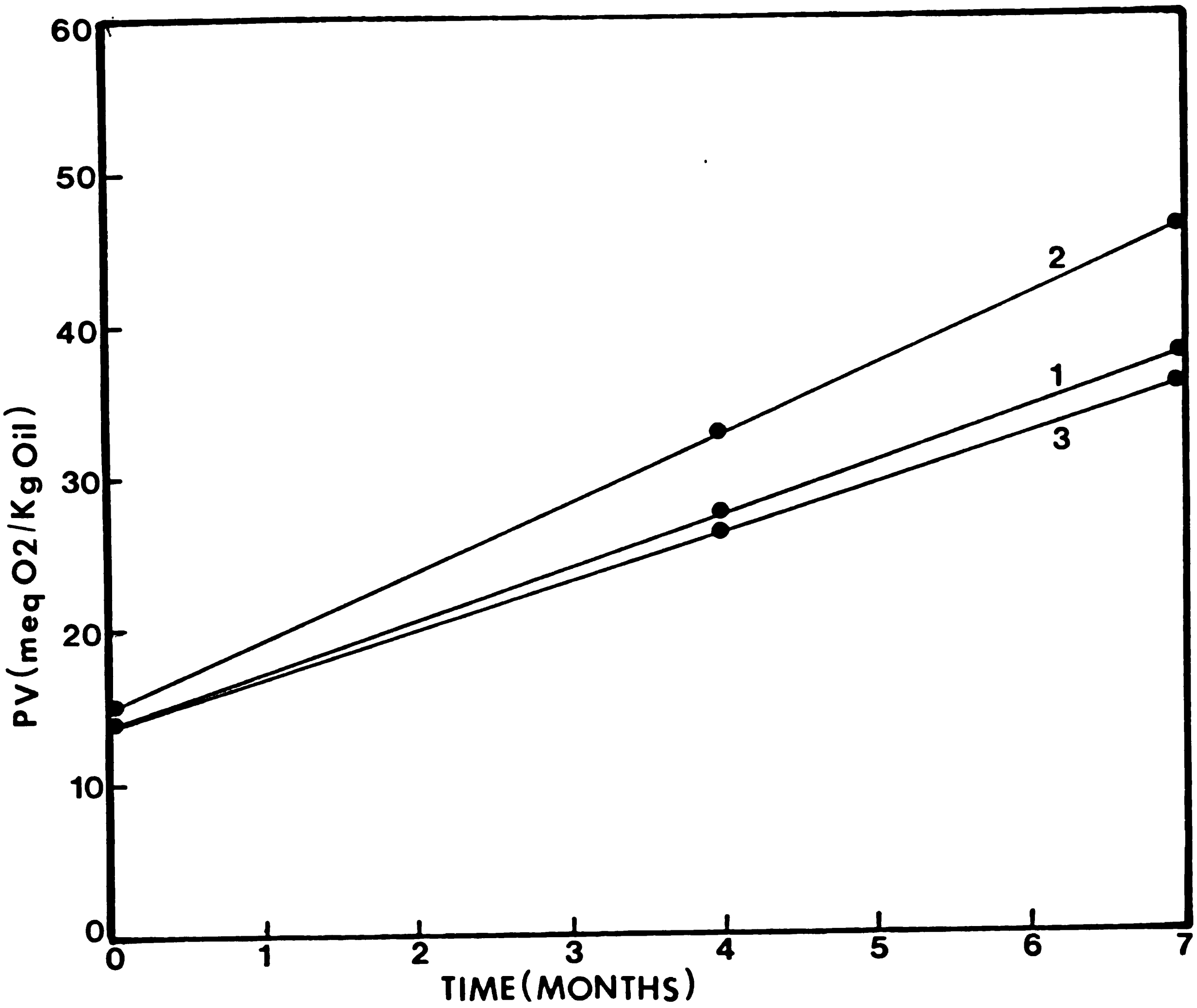

Figure 5 Peroxide Value of olive oil obtained by the Pieralisi and Rapanelli (Sinolea-Decanter) systems during storage in diffused light. (1:Pieralisi, 2:Rapanelli-Sinolea, 3:Rapanelli-Decanter)

It is interesting that, although the Rapanelli-Sinolea oil contained a lesser amount of chlorophyll (5.0 ppm) than the Rapanelli-Decanter oil (9.9 ppm), the latter was oxidized to a greater degree in darkness. These results do not agree with the findings of Interesse et al. (1971), who reported that chlorophyll acted as an antioxidant in darkness.

As shown in Figure 6, oil obtained by the Hiller system showed different degrees of oxidative deterioration during storage, under different light conditions. The oxidation proceeded at the lowest rate in darkness with a higher rate in diffused light and the highest rate in direct light (Figure 6). The same pattern was observed with the oils obtained by the Pieralisi system (Figure 7). However, in the case of Pieralisi oil, no great difference in the degree of oxidation between the levels direct and diffused light, was observed.

Statistical analysis of the oils showed significant differences in peroxide value between samples stored in darkness and in diffused light for all three systems.

Resistance to Oxidation of Olive Oil Obtained by the Rapanelli Sinolea and Rapanelli Decanter System

For evaluation of the two types of oil (Sinolea-Decanter) obtained by the Rapanelli system, the resistance of the oil to oxidation during storage was studied. Only conditions of darkness and diffused light were used.

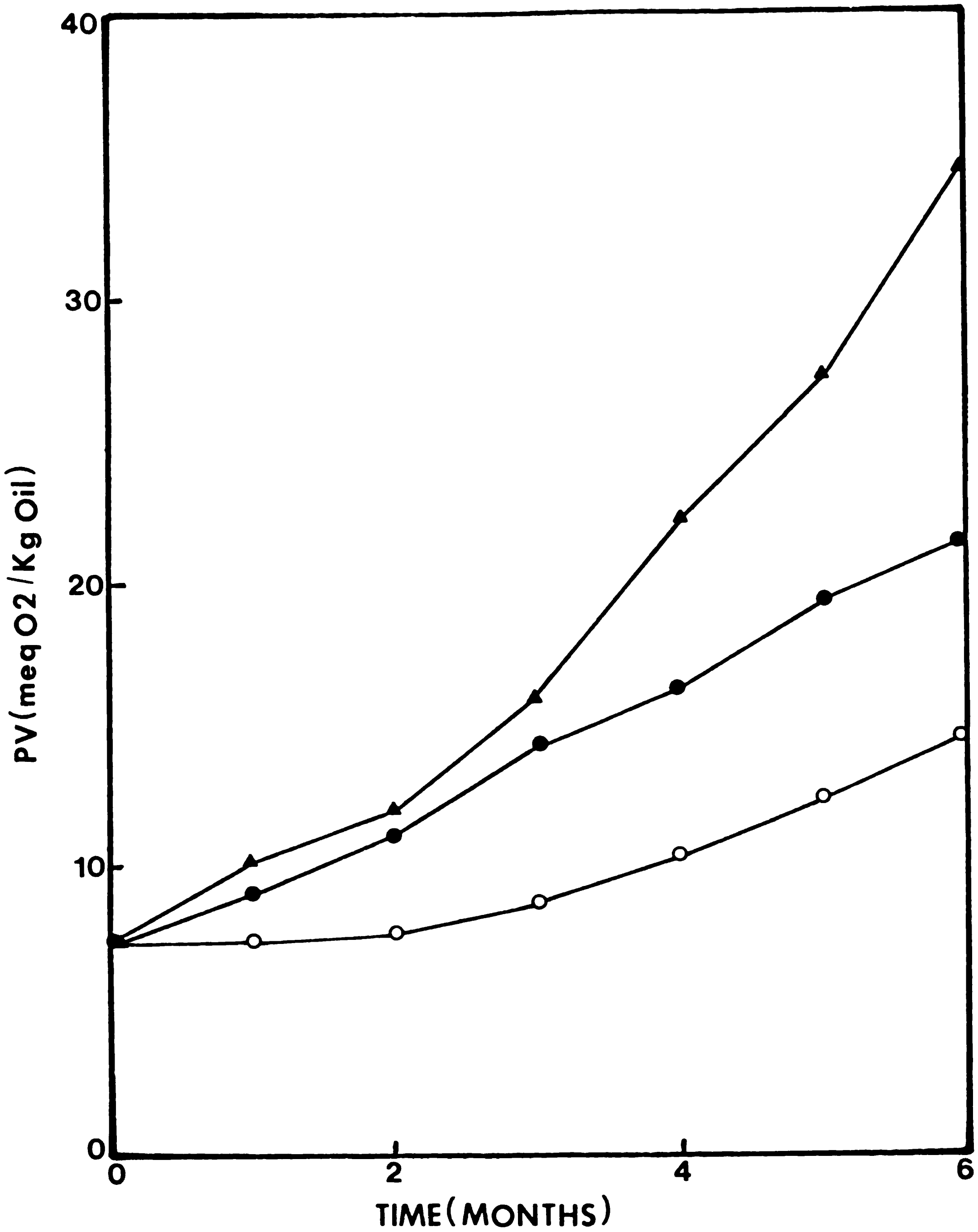

Figure 6 Peroxide value of olive oil obtained by the Hiller system during storage under different light conditions. (o:Dark, ●:diffused light, ▲:direct sunlight)

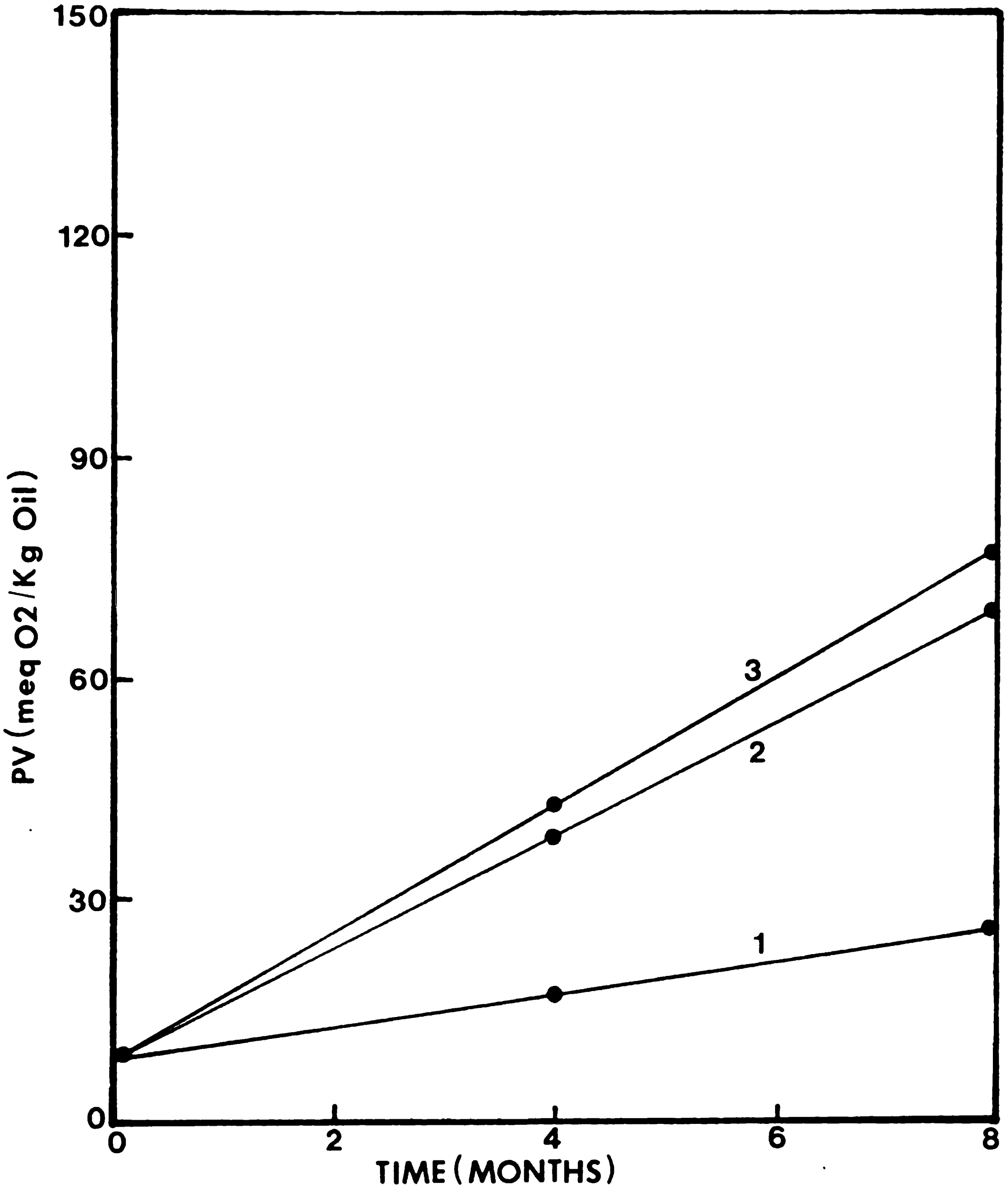

Figure 7 Peroxide value of olive oil obtained by the Pieralisi system, during storage under different light conditions. (1: Dark, 2: Diffused light, 3: Direct sunlight)

As shown in Table 14, significant differences were observed in the peroxide value between the oils. It is interesting that Rapanelli-Sinolea oil was oxidized to a lower degree than Rapanelli-Decanter oil during storage in darkness. Under diffused light conditions, however, the reverse was observed. The different behavior of these two oils was probably due to the different composition. Rapanelli-Decanter oil contained 9.9 ppm chlorophyll, while the Rapanelli-Sinolea contained 5.0 ppm. With these chlorophyll values however, a higher degree of oxidation would be expected under light conditions, for Rapanelli-Decanter than Rapanelli-Sinolea oil. It seems that other components of the oil may play an important role.

Table 14 Correlation coefficient and regression equation between peroxide value's and storage time in Rapanelli oils stored in darkness and diffused light.

Extraction System	Light Conditions	Correlation Coefficient (r)	Regression equation	
			a	b*
Rapanelli Sinolea	Dark	0.94	7.11	1.12^a
	Diffused Light	0.94	10.78	4.41^b
Rapanelli Decanter	Dark	0.98	6.79	1.19
	Diffused Light	0.83	11.86	3.07

*Values b with different superscripts are statistically different at P=0.05.

Based on the observation that the two types of oil (Sinolea and Decanter) obtained by the Rapanelli system showed, each one, different resistance to oxidation under dark and light conditions, a new study was initiated. In this study the photooxidation of olive oil containing added chlorophyll and carotene or certain other components was studied. Results of this study will be discussed in part IV.

Oxidation of Olive Oil Obtained by Different Systems and Stored in Tin Containers

This study reveals results concerning oxidation of oil samples originating from the same mass of fruits extracted with different systems. The oil was transferred into tin containers (5 Kg each) and stored at room temperature. A 2 cm head space of air was left in each sample. The tins were tightly closed.

Figure 8 presents data obtained from this study. After seven months of storage, only the oil of Rapanelli-Sinolea had a peroxide value less than 20 and was therefore considered virgin (COI, 1966). Rapanelli-Decanter oil had exceeded this value after the sixth month of storage. Peroxide values of the samples when this study was terminated were found to be in the following order: Rapanelli-Decanter>Pieralisi>Hiller>Rapanelli-Sinolea. At the end of the test period only the Rapanelli-Decanter oil smelled rancid. Figure 9 shows that the oxidation rate in oils obtained by the above systems differ somehow but not much.

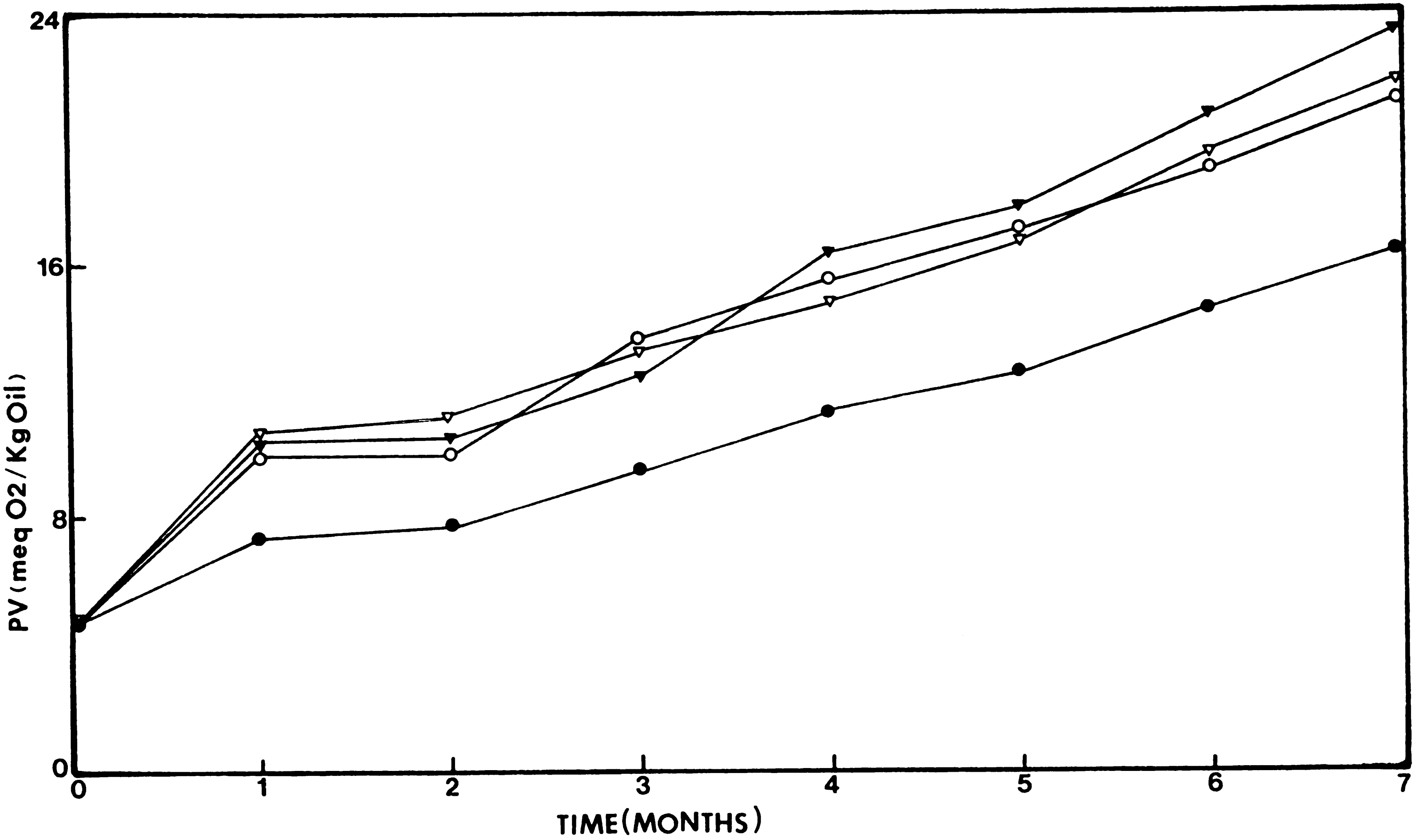

Figure 8 Peroxide value of olive oil obtained by different extraction systems and stored in tin containers at room temperature. (●: Rapanelli-Sinolea, ▲: Rapanelli-Decanter, △: Pieralisi, ○: Hiller)

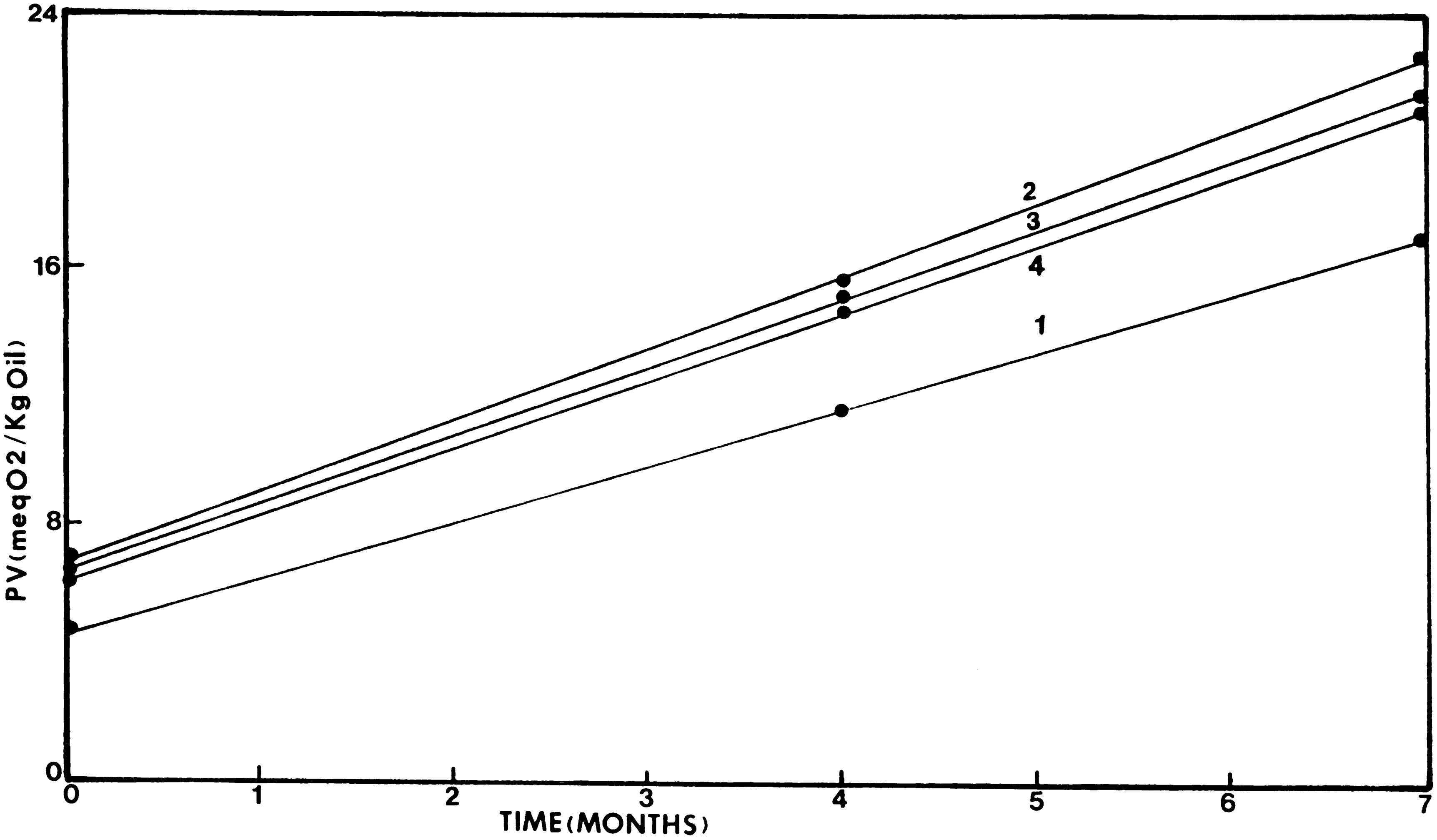

Figure 9 Slopes of increases of peroxide value in olive oil obtained by different extraction systems and stored in containers at room temperature. (1: Rapanelli-Sinolea, 2: Rapanelli-Decanter, 3: Pieralisi, 4: Hiller)

Since all the oils were obtained from the same quality of fruits but with different systems, it seems logical that the small differences in the resistance of oils to oxidation was the effect of the extraction system. Probably they affected the oil composition. In fact the procedures and the requirements for operation differ among the systems. Other systems require more, others a lesser amount of water for the malaxation of olive paste step. Among the two procedures in Rapanelli system the Rapanelli-Sinolea does not require addition of water for malaxation of paste. The Rapanelli-Decanter process, however, requires a lot of water.

Montedoro et al. (1978) reported that the decrease of phenolic constituents during the extraction process may be explained by the solubilization effect of the vegetable water. The same worker noted that among the factors which can affect the phenolic constituents of the oil, the extraction process plays a very important role. Felici et al. (1979) determined lower polyphenol content in oils from centrifugal systems than from the traditional (press). Press systems require less water than centrifugal for operation.

According to Fedeli (1977) most of the phenolic constituents present in olive fruits go into the aqueous phase as the oil is processed in the mill. A fraction however remains in the oil and favors its oxidative stability (Cantarelli, 1961; Notte and Romito, 1971; and Fedeli, 1977).

The fact that the Rapanelli-Sinolea oil showed higher resistance to oxidation than the Rapanelli-Decanter (Figure 8) could be attributed to the different phenolic composition of the two oils since water was added at the malaxation step only for the Decanter type.

III. Effect of Different Packaging and Storage Conditions on the Quality of Olive Oil

Results of the effect of different packing materials along with storage conditions (dark-diffused and direct sunlight) on quality of olive oil are discussed in this part of the present study.

Six different samples of olive oil obtained during a collection season were used for storage studies. They were numbered as olive oils No. 1, No. 2, No. 3, No. 4, No. 5, and No. 6. In all the cases fruits from the cultivar "Koroneiki" were used. The oils No. 1 - No. 4 were extracted by the Pieralisi system while the No. 5 and No. 6 by the system Rapanelli-Sinolea and Rapanelli-Decanter, respectively.

Figures 10 and 11 present data obtained from samples of olive oil No. 1 put in plastic bottles and stored in diffused and direct light. Five cm head space was left in each bottle. One-half of the samples in each case, were covered with aluminum foil.

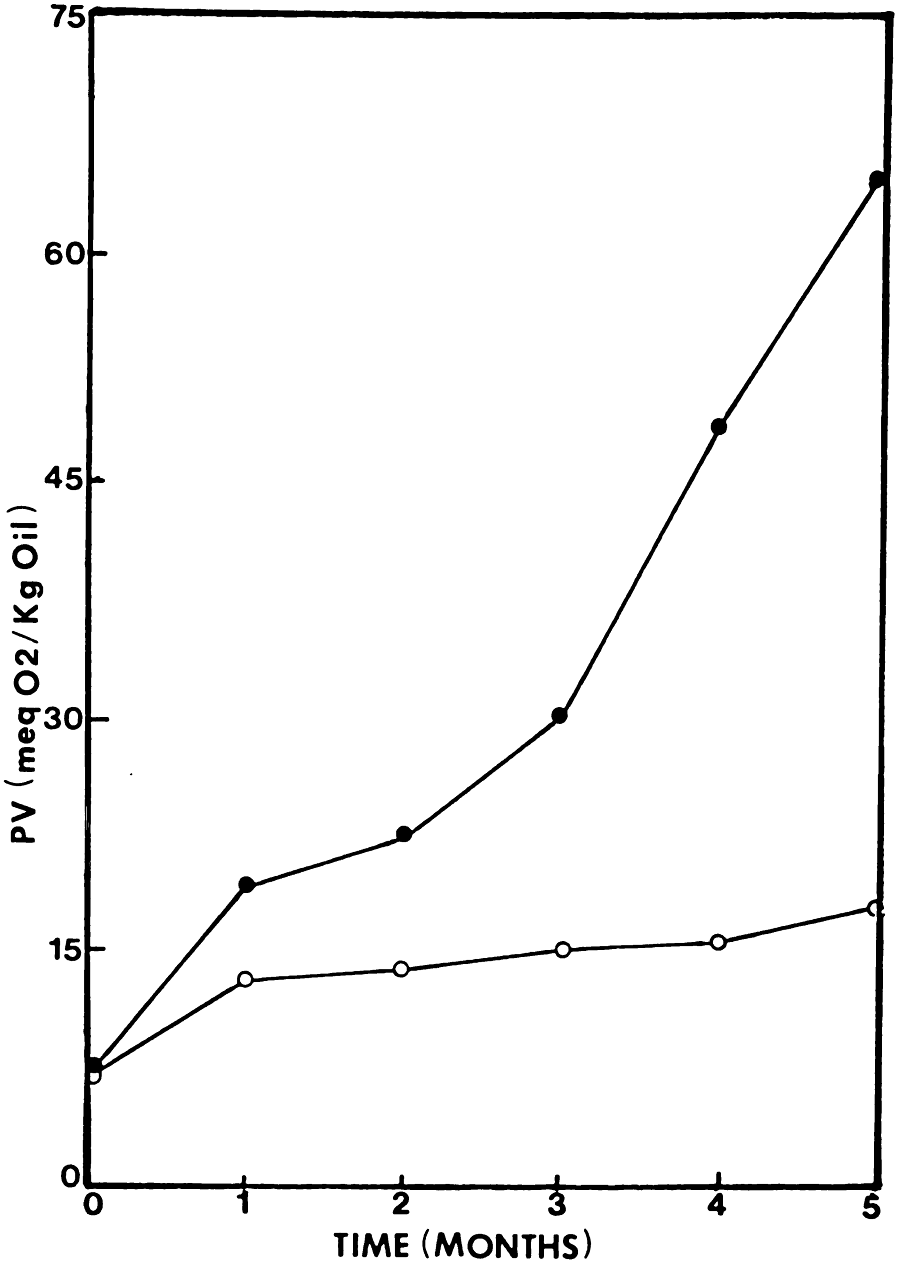

Figure 10 Effect of diffused light on peroxide formation in olive oil stored in plastic bottles. (●: Plastic bottles, o: Plastic bottles covered with alum. foil)

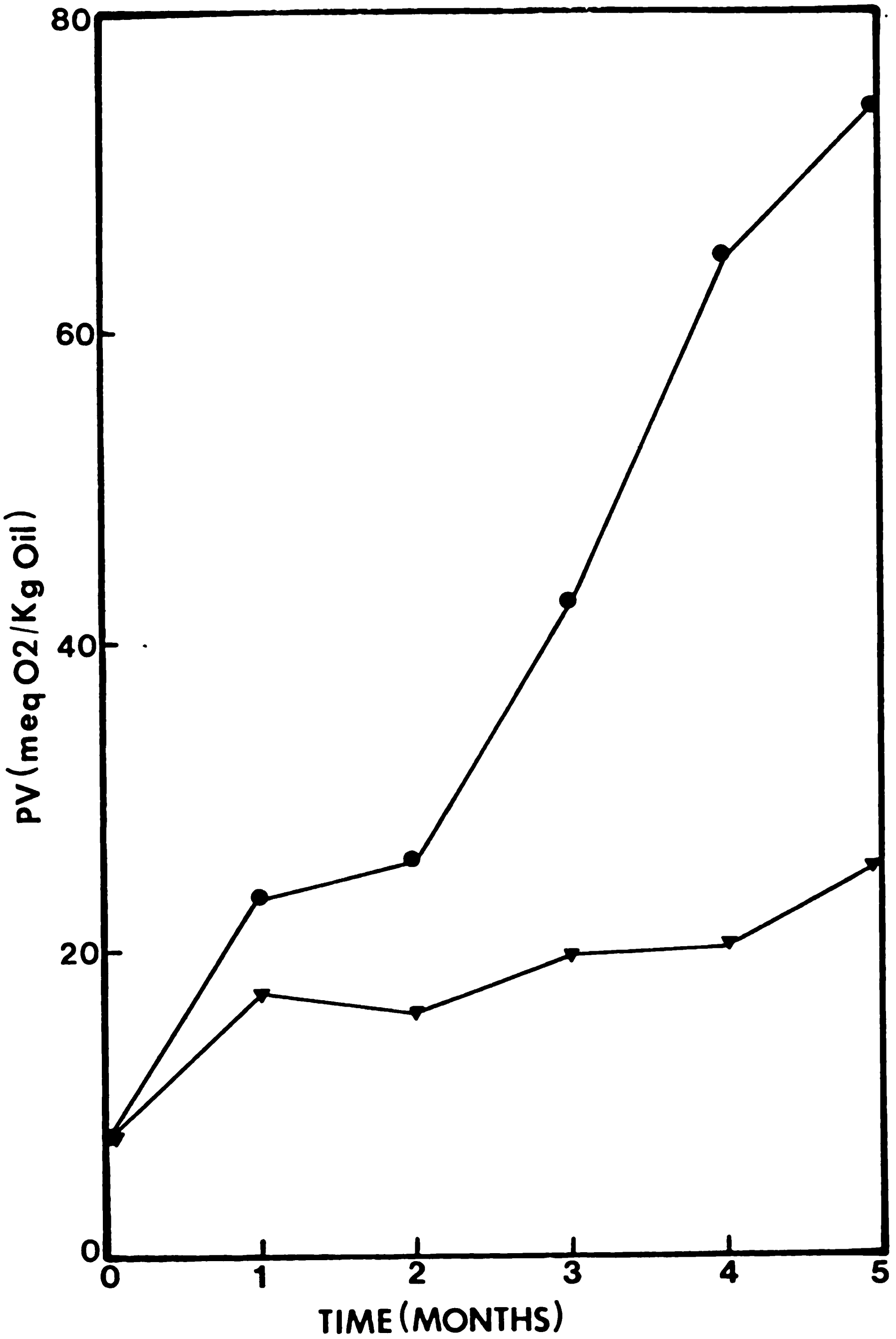

Figure 11 Effect of direct sunlight on peroxide formation in olive oil stored in plastic bottles. (●: Plastic bottles, ▲: Plastic bottles covered with alum. foil)

Under both light conditions (diffused-direct light) the samples in plastic bottles covered with aluminum foil developed lower peroxide values than those not covered. When this study was terminated, the peroxide value of samples covered with aluminum foil and stored in diffused or direct sunlight was 18 and 26, respectively. In the uncovered samples it was 65 and 75, respectively.

While the difference in peroxide value between samples exposed to light versus protected from light is large, the difference between samples exposed to diffused versus direct (only 4 hours/day) light is not great.

The results obtained when samples were covered with aluminum foil agree with findings of others (Gutierrez and Jimenez, 1970). In similar studies with polyethylene bottles, they observed that samples stored in light were oxidized to a higher degree than those stored in darkness.

The role of light in oxidation of olive oil has been studied by Pretzch (1970) and Interesse *et al*. (1971). They demonstrated that exposure of olive oil to light causes an increase in the oxidation rate. It seems that the natural pigments present in olive oil under the action of light develop an oxidizing activity (Interesse *et al*., (1977).

It is interesting to note that, according to Valentinis and Romani (1960), direct sunlight in the absence of air, caused a decrease in the peroxide value of olive oil during storage.

Figure 12 presents data from another experiment concerning the relative effect of plastic and glass bottles on the oxidation of olive oil No. 2 exposed to diffused light.

As shown, samples in polyethylene bottles developed higher peroxide values than those in glass bottles. When this study was terminated the peroxide value of samples in plastic and glass bottles was 48 and 32, respectively. When samples were covered with aluminum foil lower peroxide value were recorded than in uncovered ones. Samples in covered glass bottles had lower peroxide values than those in covered plastic bottles after five months of storage.

Our results agree with Gutierrez's (1975) findings. He reported higher peroxide values in polyethylene bottles than in glass bottles, but not much higher.

It is interesting to note however that, according to Unal (1978), samples of olive oil in glass bottles showed a decrease in peroxide value during storage. On the other hand he observed an increase in peroxide value in samples stored in PVC bottles. He attributed that to the oxygen permeability of PVC.

Table 15 contains data for olive oil stored in transparent polyethylene bottles and exposed to diffused light for a period of three months. Although the initial peroxide value of the oil was relatively low (8.7), in one month this value exceeded 20. At this point the oil

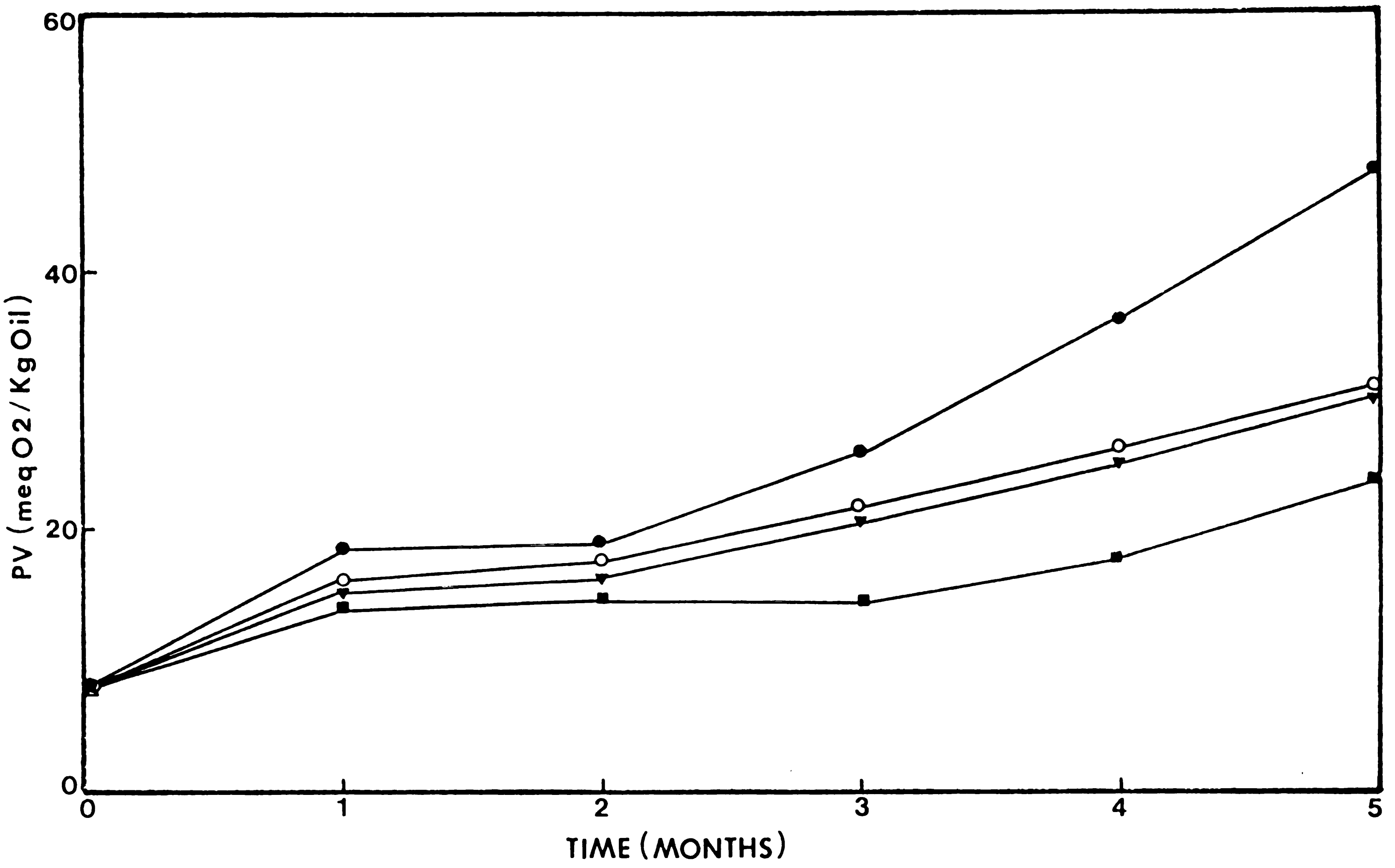

Figure 12 Effect of diffused light on peroxide formation in olive oil stored in plastic or in glass bottles. (●: Plastic bottles, ▲: Plastic bottles covered with alum. foil, o: Glass bottles, ■: Glass bottles covered with alum. foil)

had an off taste. The oil would no longer be considered as virgin according to COI (1966). Peroxide values continued to increase and reached 52.5 after three months storage. Besides becoming rancid, the oil had almost lost its original color.

Table 15 Peroxide value of olive oil stored for 3 months in diffused light in polyethylene plastic bottles.

Storage time (months)	Initial FFA content (%)	Peroxide value (meq O_2/Kg oil)
0	0.9	8.7
1	0.9	24.6
2	0.9	39.5
3	0.9	52.5

Ramunni (1964) demonstrated that olive oil stored in colorless glass bottles in the presence of light quickly lost the chlorophyll and about the 70% of the carotene present.

This study indicates that commercial bottling of olive oil in transparent plastic containers is undesirable because it can be oxidized easily during the long time it is displayed in the stores. During storage, air may enter due to the permeability of plastic containers and will be involved in the oxidation mechanism. In addition, the presence of diffused light in stores may promote oxidation.

Table 16 presents data from oils (No. 4, 5 and 6), obtained by different extraction systems, stored in darkness in hermetically closed glass bottles with little head space. The peroxide value had not changed after two years of storage at room temperature.

These results support Gutierrez's (1975) suggestions concerning storage of olive oil in containers impermeable to atmospheric oxygen and light conditions. According to Gutierrez (1975) however, the elimination of these two factors during olive oil storage, is not always sufficient to maintain quality during storage. Thus he implies that other factors also have a long term effect.

These results support the observations of other workers that the material used for olive oil packaging have a great effect on its quality. Olive oil is very sensitive to light and this factor can cause a significant oxidative deterioration of the oil, during storage, if oxygen is present. Therefore packaging materials which exclude the light and atmospheric oxygen should be used for olive oil. These will favor maintenance of olive oil quality during storage.

Table 16 Peroxide value of olive oil obtained by different extraction systems and stored for 2 years in darkness in glass bottles with little head space.

No. of oil	Extraction System	Date of Storage	Date of final analysis	Peroxide value (meq O_2/Kg oil)	
				Initial value	Final value
4	Pieralisi	1/2/79	1/8/81	7.8	8.4
5	Rapanelli Sinolea	1/20/79	"	5.9	6.8
6	Rapanelli Decanter	1/23/79	"	4.2	5.2

IV. Photooxidation Studies

It has been noted in the literature that certain natural pigments found in oils can act as sensitizers, in the presence of light, initiating the photooxidation mechanism. This mechanism involves the formation of singlet oxygen which then leads to peroxide development without the participation of free radicals (Rawls and Van Santen, 1970; Clements *et al*., 1973).

According to Carlsson *et al*. (1976), chromophoric impurities such as chlorophyll are assumed to act as photosensitizers generating $^1O_2$ by the transfer of excitation energy.

One of the purposes of this work was to study the roles of chlorophyll, pheophytin, carotene and tocopherol, which occur naturally in olive oil in the photooxidation process of this oil.

Efficiency of Bleaching Technique

Because the natural pigments present in olive oil may affect the photooxidation mechanism, bleached olive oil was prepared from virgin oil and subsequently used as the basic material for the photooxidation studies.

Several bleaching materials were used in various combinations. The following mixture was found to give satisfactory results: charcoal (35 g), extra tonsil (50 g), florisil (15 g), hyflo supercel (25 g), infusorial earth (ca 1 cm in the column).

The efficiency of the bleaching process was monitored by measuring the color of the oil by the AOCS (1973) spectrophotometric method. The results of bleaching experiments are shown in Table 17.

As was expected, unbleached* olive oil had high absorbance at 460 nm, which is attributed to the presence of β-carotene and other carotenoids.

Separate colorimetric determination of β-carotene, showed that unbleached olive oil contained 4.2 ppm. This value is within the range of values reported by others (Gracian, 1968).

The absorbance of the oil at 670 nm was high due to the presence of chlorophyll which absorbs the maximum at that wavelength. The absorbance at 620 nm was also attributed to the chlorophyll.

Generally the color of unbleached olive oil depends on the stage of maturity of the fruits and therefore of the kind of pigments carried with the oil during the extraction process.

The zero absorbance values obtained for bleached olive oil at the four wavelengths (Table 17) indicated that the bleaching technique was very efficient in removing chromophoric pigments. The bleached oil appeared to be completely colorless by visual inspection.

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*For the rest of this text the term unbleached will be used for virgin olive oil.

Table 17 Absorbance of unbleached and bleached olive oil at different wavelengths.

(nm)	Absorbance	
	Unbleached Olive Oil	Bleached Olive Oil
460	0.918	0.0
550	0.062	0.0
620	0.083	0.0
670	0.582	0.0

(Bleaching agent: charcoal + tonsil + florisil + hyflosupercel + infusorial earth).

Chlorophyll Content

The chlorophyll content of unbleached olive oil was found to be 5.45 ppm. This value is within the range of 0.0-9.7 ppm reported by others (Vitagliano, 1960).

Felice et al. (1970) in their studies with traditional (press) and centrifugal systems found somewhat higher chlorophyll values in the oil obtained from centrifugal systems.

The chlorophyll content of olive oil depends generally on the degree of maturation. Unripe fruits contain more chlorophyll than ripe ones. The olive oil used for this study was extracted from semiripe fruits.

α-Tocopherol Content

The α-tocopherol content of unbleached olive oil was determined by HPLC (Carpenter, 1978) and was found to be 20.69 ppm.

Gracian and Arevalo (1965) identified only α-tocopherol in olive oil. They proposed that γ-tocopherol, sometimes present, must be considered as a product of α-tocopherol oxidation. According to Vitagliano (1960), α-tocopherol in olive oil ranges from 12-162 ppm. Boatella (1945) however reported higher concentrations of α-tocopherol (70-150 ppm) in olive oil.

Total Phenols

Olive oil is considered stable to autoxidation because of the presence of phenolic constituents which vary depending on cultivation procedures and environmental factors (Vazquez *et al*., 1975, 1976).

Cantarelli and Montedoro (1969, 1972) observed that polyphenols extracted from olive oil inhibited rancidity in other oils.

Gutfinger (1981) reported that olive oils produced commercially by mechanical processes exhibited lower stability to oxidation than olive oils extracted with solvent (chloroform/methanol mixture). The higher resistance to oxidation of the solvent-extracted oils is probably due to their high polyphenol content in particular to that of ortho-diphenols which are considered as better antioxidants (Gutfinger, 1981).

In this study, an effort was made to determine the total poylphenol content of the unbleached oil with the Folin-Ciocalteau reagent. Figure 13 shows the standard curve used for this determination.

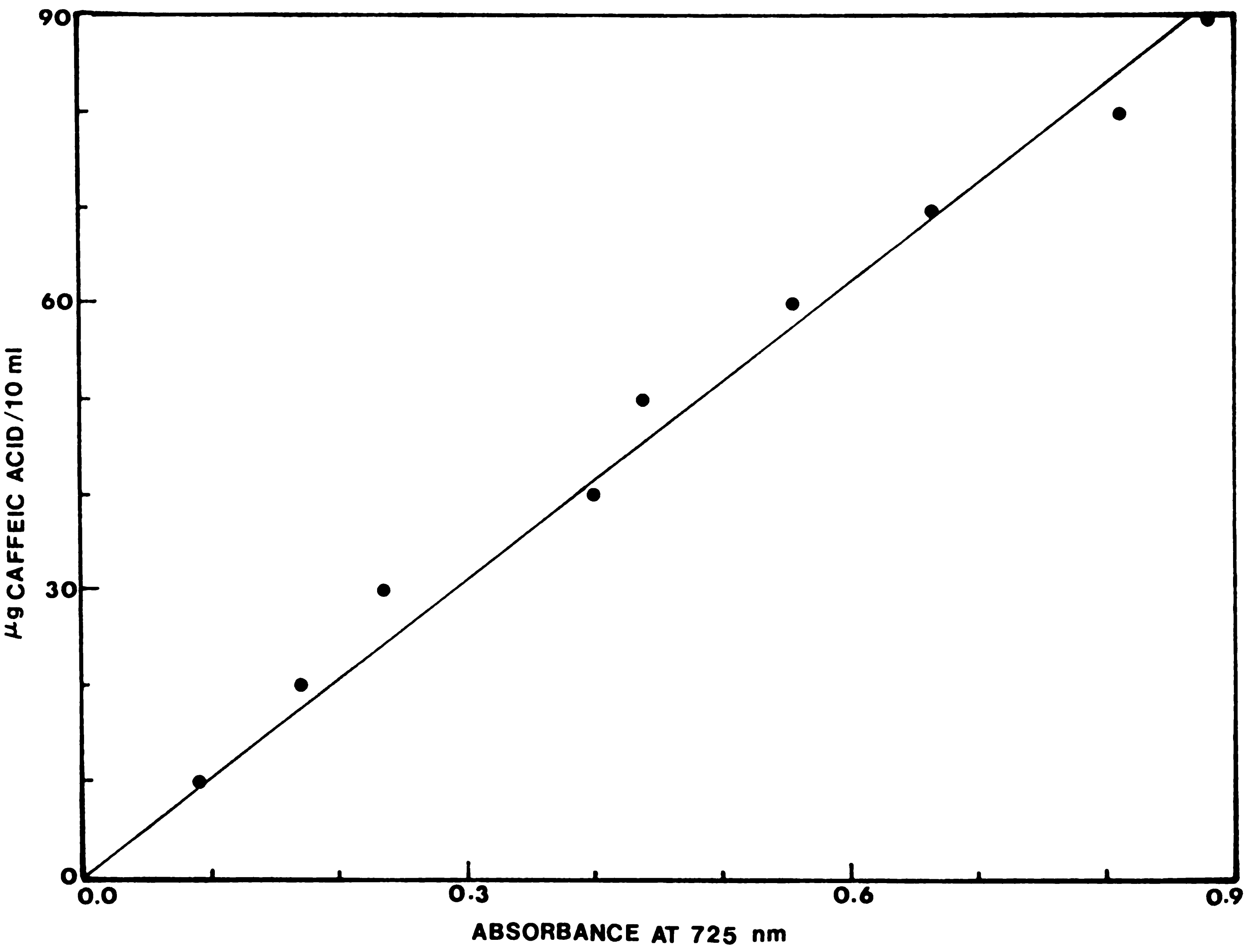


Figure 13 Standard curve for determination of phenols.

The average of several determinations gave a value of 120 ppm expressed as caffeic acid. This value is within the range of values reported by others (Felice *et al.*, 1979; Gutfinger, 1981). Gutfinger (1981) reported a range in total polyphenols in virgin olive oil from 50-157 ppm. The values in solvent extracted oils ranged from 321-574 ppm.

Fatty Acid Composition of Unbleached Olive Oil

The fatty acid composition of olive oil as determined by GLC is shown in Table 18. The results are close to some values reported in the literature (Gutfinger, 1981). He determined the same fatty acids in olive oil but reported values higher in linoleic and lower in oleic acids than the oil used here. As expected, the oil contained a small amount of linolenic acid ($C_{18:3}$) and a high percentage of oleic acid ($C_{18:1}$).

Total Free Fatty Acids in Unbleached and Bleached Olive Oil

Titration of the unbleached olive oil revealed a total free fatty acid content of 0.70%, as oleic acid. The bleached oil had a lower free fatty acid content, than unbleached oil apparently due to partial retention of free fatty acids in the bleaching column.

Table 18 Fatty acid composition of unbleached olive oil.

Fatty Acid	Percentage
C16:0	11.7
C16:1	0.9
C18:0	2.4
C18:1	77.7
C18:2	6.5
C18:3	0.8

Number following the : denotes the number of double bonds in each fatty acid.

Peroxide Value of Samples

Initial peroxide value of the unbleached olive oil used for the first experiment in photooxidation studies was found to be 15 meq O_2/Kg oil. This value increased slightly (up to 4 units) with the time it was held in the laboratory.

The peroxide value of bleached olive oil was zero due to the removal of hydroperoxides during the bleaching operation.

Ultraviolet Absorption Values of Unbleached Olive Oil

The $E_{1cm}^{1\%}$ at 232 nm and 270 nm and the ΔK value were determined.

$$\Delta K = K_{268} - \frac{K_{262} + K_{274}}{2}$$

From the absorption measurements of the oil at 232, 262, 268, 270 and 274 nm the following values were obtained.

$$E^{1\%}_{232} = 2.5,\ E^{1\%}_{270} = 0.2 \text{ and } \Delta K = 0.005$$

These values indicate that the unbleached oil was slightly oxidized, although the values are still lower than the upper limits set by the International Olive Oil Council (1966).

Metal Analysis

Prooxidant metals such as iron and copper present in olive oil, mainly as a result of processing, affect the stability of the oil by catalyzing the oxidation mechanism. Fedeli (1973) observed that the oxygen absorption rate in autoxidized olive oil was related to the amount of catalytic metals (Fe, Cu, Ca, Ni and Mg) present in the oil.

In the present study, the Fe and Mg content of the unbleached olive oil was determined.

The results obtained are presented in Table 19. These values are the average of duplicate determinations

Table 19 Iron and magnesium content of olive oil.

Metals	Concentration (ppm)
Iron	0.026
Magnesium	0.135

Unbleached olive oil had a low Fe content due to the fact that the oil was extracted in a centrifugal Pieralisi system, where most of the system was constructed of stainless steel.

The values obtained concerning the iron content of the oil are even lower than these reported by Italian researchers (Felice *et al*., 1979). In a comparative study, they found higher iron content in olive oil extracted by hydraulic systems than in that obtained from centrifugal systems. They found Fe values from 0.68-1.73 ppm for oil obtained by press systems and from 0.46-1.13 ppm for oil obtained by centrifugal systems.

Cu values obtained by Felice *et al*. (1979) for press and centrifugal systems ranged from 15-20 ppb to 10-20 ppb, respectively.

Effect of Fluorescent Light on Peroxide Formation in Unbleached and Bleached Olive Oil Containing No Additives

Fluorescent light is widely used in storage and supermarkets to illuminate displays of many kinds of foods, including fats and oils. The storage of olive oil in transparent plastic containers has become common practice. This material allows the passage of considerable amounts of light energy, and thus may be sufficient to initiate photooxidation of unbleached olive oil containing pigments such as chlorophyll and pheophytin.

To gain knowledge of the effect of light on the photooxidation of olive oil, samples of unbleached and bleached oil, with or without additives were illuminated with fluorescent light. The total intensity of the light above the samples was 7500 Lux.

Figure 14 shows the results of peroxide value determinations on samples of unbleached and bleached olive oil with no additive. As is shown in this figure, higher peroxide values were developed in unbleached olive oil than in bleached oil.

Vianni (1980), in his studies with soybean oil, observed higher resistance to oxidation in unbleached (refined) than bleached soybean oil when both were exposed to fluorescent light. He attributed the greater amount of oxidation in the bleached oil to the removal of natural antioxidants during the bleaching process. Our results showed higher resistance to oxidation in case of bleached than unbleached olive oil. Probably in the case of olive oil, and in the presence of light, the prooxidative effect of the natural sensitizers (chlorophyll and pheophytin) was higher than the effect of the antioxidants (tocopherols and phenols) present in the oil. This agrees with the findings of Carlsson _et_ _al_. (1976). According to Carlsson _et_ _al_., the rates of hydroperoxide formation during exposure to light of samples containing natural sensitizers are unaffected by efficient peroxy radical scavengers such as hindered phenols.

When containers of unbleached and bleached olive oil were covered with aluminum foil, the oxidation proceeded less actively than in uncovered samples (Figure 14).

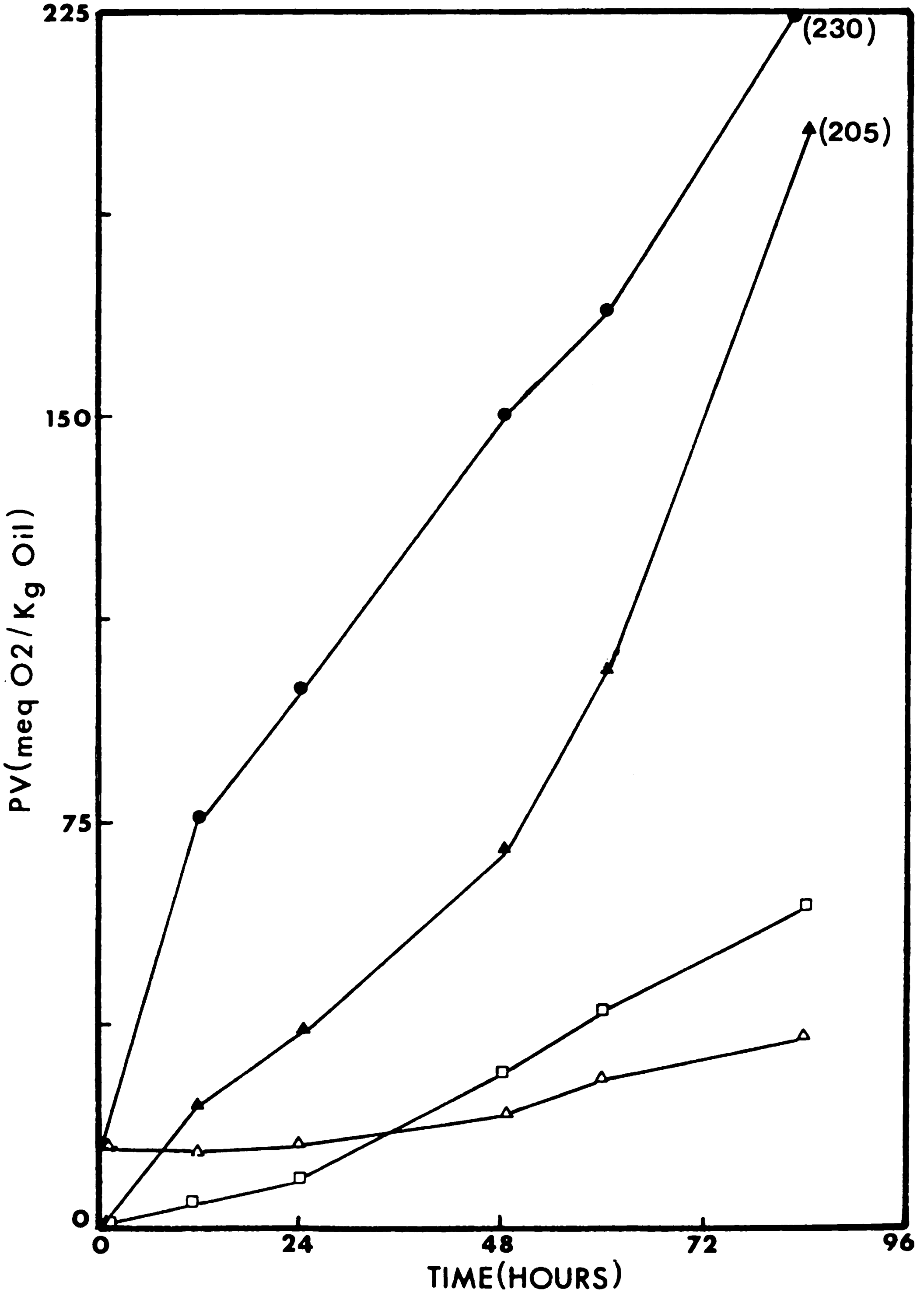


Figure 14 Effect of fluorescent light on peroxide value in unbleached and bleached olive oil. (●: Unbleached olive oil, ▲: Bleached olive oil, □: Bleached cover with alum. foil, △: Unbleached cover with alum. foil)

It is interesting to note that, in the absence of light (samples shielded with aluminum foil), bleached olive oil was oxidized to a higher degree (PV=62) than unbleached (PV=35) although the initial peroxide value of the unbleached sample was higher than that of bleached sample (Figure 14). These results suggest that the natural antioxidants (phenols and tocopherols) present in olive oil have an effect in the absence of light but have little or no effect in the presence of light.

In order to determine if chlorophyll had any antioxidant effect in the absence of light, an experiment was run using bleached oil with added chlorophyll. Results of this study will be discussed later.

Effect of Fluorescent Light on Peroxide Formation in Bleached Olive Oil Containing Chlorophyll-a, and α and β-Carotene

Samples of bleached olive oil with added chlorophyll -a or α and β-carotene in doses similar to the values determined in unbleached olive oil were illuminated with fluorescent light for 84 hrs.

Results obtained from this experiment are presented in Table 20. This shows that all samples were oxidized to a high but different degree. Up to 48 hrs illumination the oxidative deterioration of samples was in the following order: Samples with 6 ppm chlorophyll > samples with 4 ppm chlorophyll > samples containing no additive (control) > samples containing 4 ppm β-carotene > samples

Table 20 Peroxide formation in bleached olive oil containing different additives, during illumination with fluorescent light, at 32° ± 2°C.

Illumin. time (hrs)	Peroxide value (meq/Kg oil)									
	Control	Dark Control[a]	4 ppm chl[b]	6 ppm chl	4 ppm β-carot.	6 ppm β-carot.	6 ppm α -carot.	50 ppm d-α-tocop.	100 ppm d-α-tocop.	150 ppm d-α-tocop.
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	23.0	5.5	46.5	50.0	15.0	12.0	16.0	22.5	21.0	18.5
24	38.0	13.0	59.0	61.0	32.0	28.5	31.0	33.0	32.5	33.5
48	73.5	30.0	82.0	90.5	61.0	56.0	59.0	73.0	68.5	65.0
60	105.0	45.0	98.0	111.0	87.0	82.0	86.0	92.0	84.0	81.0
84	205.0	62.0	189.0	200.0	180.0	155.0	175.0	160.0	145.0	135.0

a: Sample containers covered with aluminum foil.

Chl[b]: Chlorophyll

containing 6 ppm α-carotene > samples containing 6 ppm β-carotene. The same order was observed when this study was terminated at 84 hrs.

As is shown in Table 20, higher peroxide values were found in bleached olive oil with addition of chlorophyll-a at the level of 6 than at 4 ppm. These results indicate that the degree of photooxidation is related to the amount of chlorophyll present. However in the case of autoxidation, according to Fedeli and Brillo (1975), there is no numerical relation between the chlorophyll concentration and the rate of oxidation in virgin olive oil.

As in the case of chlorophyll, β-carotene showed different effects in bleached olive oil at the levels of 4 and 6 ppm. Lower peroxide values were determined when carotene was used at the higher level.

Terao _et_ _al_. (1979) reported that the addition of β-carotene retarded the photooxidation of methyl linoleate efficiently. β-Carotene also efficiently inhibited the deterioration of soybean oil which was exposed to photoirradiation in the presence of tocopherols.

According to Satter and Deman (1978) the inhibitory effect of β-carotene on the photooxidation of oils may be as a filter screening out radiation of active wavelengths.

β-Carotene showed a more pronounced effect in preventing oxidation than α-carotene at the 6 ppm level. The oxidation inhibition weakened toward the end of the incubation period, probably due to carotene destruction.

Terao et al. (1979) demonstrated that β-carotene disappeared rapidly during irradiation and so its inhibitory effect was not maintained. The effectiveness of β-carotene was prolonged in the presence of δ-tocopherol. The latter seemed to protect β-carotene from oxidation. According to Terao et al. (1979), tocopherols and especially δ-tocopherol should be added together with β-carotene, when this pigment is used as an additive for protecting the oils from photooxidation.

Effect of Fluorescent Light on Peroxide Formation in Bleached Olive Oil Containing Different Levels of D-α-Tocopherol

The effect of fluorescent light on the peroxide formation of bleached olive oil with added tocopherol is shown in Table 20. The data indicate that all samples containing D-α-tocopherol developed essentially the same perixide values as those of the control up to 48 hrs illumination. From then on the increase in peroxide value was lower for the samples with added tocopherol.

As the addition of α-tocopherol increased (from 50 to 100 to 150 ppm) the rise in peroxide value was slightly reduced. This is at least partially consistent with the observation of Oliver et al. (1944) that the upper limit of tocopherol was not reached since a prooxidant effect occurs when an excess amount is present.

Vianni (1980) found that, in the absence of chlorophyll and under fluorescent light, α and γ-tocopherols both gave good protection against photooxidation of bleached soybean oil.

Carlsson *et al*. (1976) reported that tocopherol undergoes oxidation when exposed to $^1O_2$ in oil solutions. According to them although α-tocopherol can quench $^1O_2$ and prevent hydroperoxide formation it undergoes rapid peroxidation itself. Terao *et al*. (1979) found that α-tocopherol scarcely inhibited the production of monohydroperoxides (MHP) in photooxidation studies because it disappeared rapidly during photooxidation. Matsushita and Terao (1980) found that α-tocopherol disappeared completely after 12 hrs of irradiation. Our results showed a higher effect by α-tocopherol at the longer period of illumination.

Effect of Fluorescent Light on Peroxide Formation of Olive Oil Containing Chlorophyll, α , β-Carotene, D-α-Tocopherol and Ni-Chelate

The effect of β-carotene, D, α-tocopherol and Ni-chelate, which are generally considered as singlet oxygen quenchers, are discussed here. Bleached olive oil containing 6 ppm added chlorophyll was used. All the quenching agents were used at the level of 100 ppm.

Results of this study are presented in Table 21. Samples containing 6 ppm chlorophyll and those containing 6 ppm chlorophyll + 100 ppm D-α-tocopherol showed almost similar changes in peroxide value throughout the illumination period. Our results agree with those of Vianni (1980). He found that both α and γ tocopherols were unable to protect

Table 21 Peroxide formation in bleached olive oil containing added chlorophyll and other additives during illumination with fluorescent light at 32°C ± 2°C.

Treatments	Peroxide Value (meq O_2/Kg oil)				
	Illumination time (hours)				
	0	8	16	24	32
B (Control)	0	23.0	37.0	48.0	66.0
B + 6 ppm Chl	0	27.0	45.0	51.0	72.5
B + 6 ppm Chl covered with aluminum foil	0	4.0	7.0	8.0	8.5
B + 6 ppm Chl + 100 mg/Kg carotene β	0	5.0	16.0	26.0	57.0
B + 6 ppm Chl + 100 mg/Kg D-α-tocopherol	0	31.0	54.0	63.0	75.0
B + 6 ppm Chl + 100 mg/Kg Ni Chelate	0	10.0	16.0	25.0	47.0

B: Bleached oil
Chl: Chlorophyll

bleached soybean oil, to which chlorophyll was added, from oxidation in the presence of light. These tocopherols, however, were effective in preventing oxidation of bleached oil only in the absence of light.

Results of this experiment do not agree with those of previous experiments where some effect of D-α-tocopherol was noted during the last hrs of illumination. Probably in the presence of chlorophyll, D-α-tocopherol was easily destroyed as it has been reported by others (Terao _et_ _al_., 1979).

β-Carotene kept the peroxide value of the samples at a very low level for the first 8 hrs. Subsequently the peroxide value of these samples increased more rapidly in a way parallel to that of the samples containing Ni-chelate.

The fact that β-carotene prevented peroxide formation to a high degree only for the first hours of illumination is probably due to the destruction of this pigment. Terao _et_ _al_. (1979) reported reduction of β-carotene after 5 hrs of irradiation. The inhibitory effect of this pigment, however, was maintained for 8 hrs in the presence of δ-tocopherol (Terao _et_ _al_., 1979)

When Ni chelate was added to the system, the peroxide value of the samples remained low. These samples had lower peroxide values than those containing β-carotene or D-α-tocopherol at the end of the study period. According to Carlsson _et_ _al_. (1976) nickel chelates are able to retard photooxidative deterioration of unsaturated food oils by near UV and visible light while the peroxy radical scavengers (phenols) aren't efficient. The latter interfere with the free radical chain oxidation process (Dugan, 1976).

Our results demonstrate lesser effects from nickel chelate than these observed by Carlsson *et al*. (1976), probably due to differences in systems and in the concentrations used. With a Ni-chelate concentration of 0.24% they were able to provide some protection to olive oil from photooxidation. According to Carlsson *et al*. (1976), the inherent absorption of these colored compounds could simply screen out the active wavelengths during irradiation and so would protect the oils solely by light absorption rather than by quenching singlet oxygen.

It is interesting to note that samples containing 6 ppm chlorophyll in the absence of light (shielded with aluminum foil) developed very low peroxide values at 32 hrs illumination. The peroxide value of the samples was 8.5 when this study was terminated (Table 21).

Effect of Fluorescent Light on Peroxide Formation and Conjugated Diene Formation in Olive Oil Containing Chlorophyll

This study shows the effect of light on peroxide formation and formation of conjugated dienes in unbleached and bleached (with and without chlorophyll) olive oil. Fifty g samples instead of 25 g were used in this experiment.

The peroxide values obtained from this particular study are presented in Figures 15, 16 and 17. Higher peroxide values were determined in samples of bleached olive oil to which chlorophyll was added than in the controls (Figure 15).

It should be noted however that, in unbleached olive oil containing no additives, higher peroxide value were produced finally than in bleached olive oil to which 10 ppm chlorophyll was added (Figure 15 and 16). This observation led to the belief that other pigments present (like pheophytin) may play an important role in the photocatalytic oxidation of olive oil since more chlorophyll (10 ppm) than that naturally present (5.2 ppm) was added to the bleached oil. Thus a new study was initiated in which a mixture of pheophytin a and b was added to bleached olive oil. Results of this study are discussed later.

Figure 17 shows the effect of light and darkness on the oxidation of unbleached olive oil. It is apparent that unbleached olive oil is very sensitive to photooxidation but stable to autoxidation. These results agree with previous work (Kiritsakis _et_ _al_., 1977)

The resistance of olive oil to autoxidation is accounted for probably by the low percentage of polyunsaturated fatty acids (Table 18) and the presence of natural antioxidants such as phenols, tocopherols and

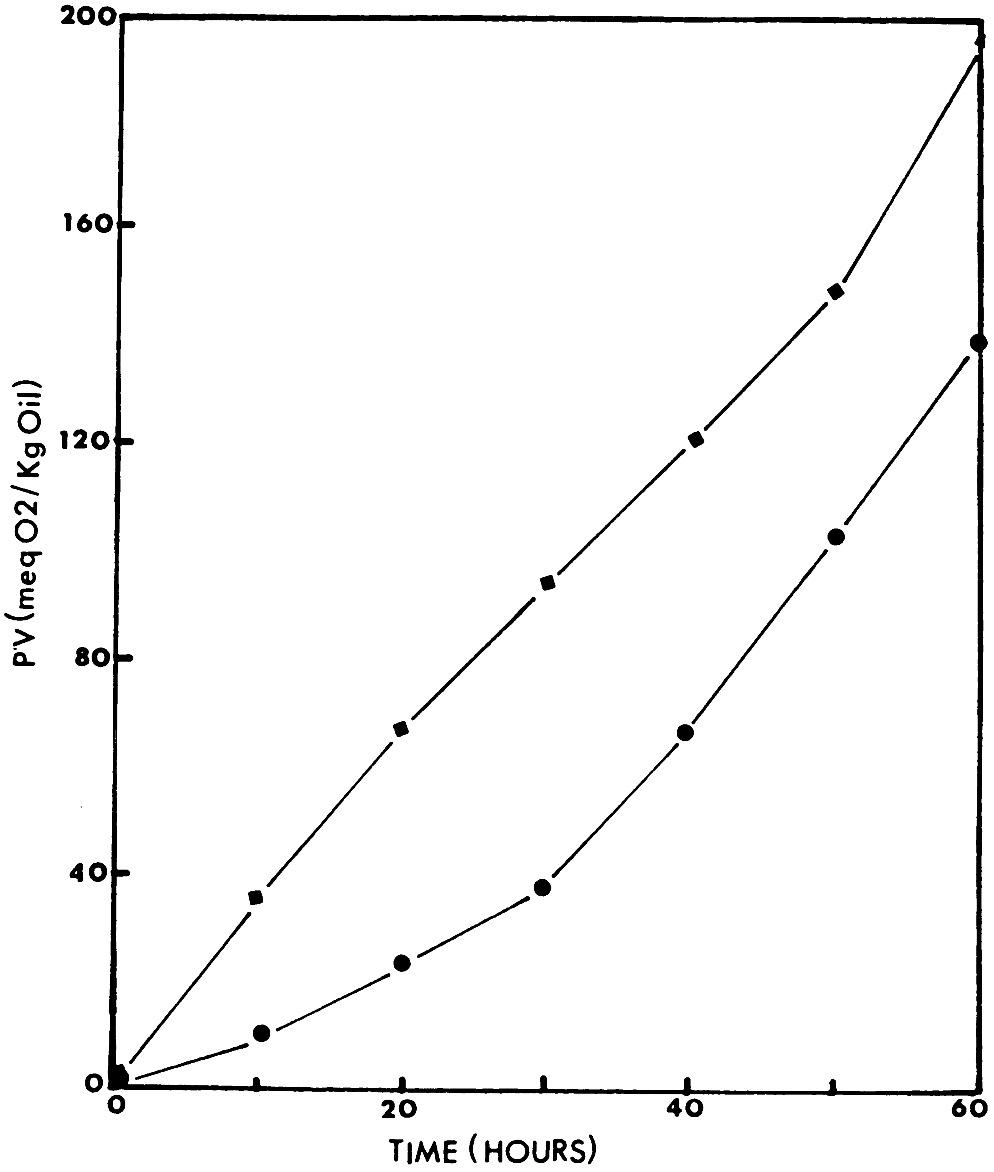


Figure 15 Effect of fluorescent light on peroxide value of bleached olive oil containing added chlorophyll. (■: Bleached olive oil + 10 ppm Chl ●: Bleached olive oil)

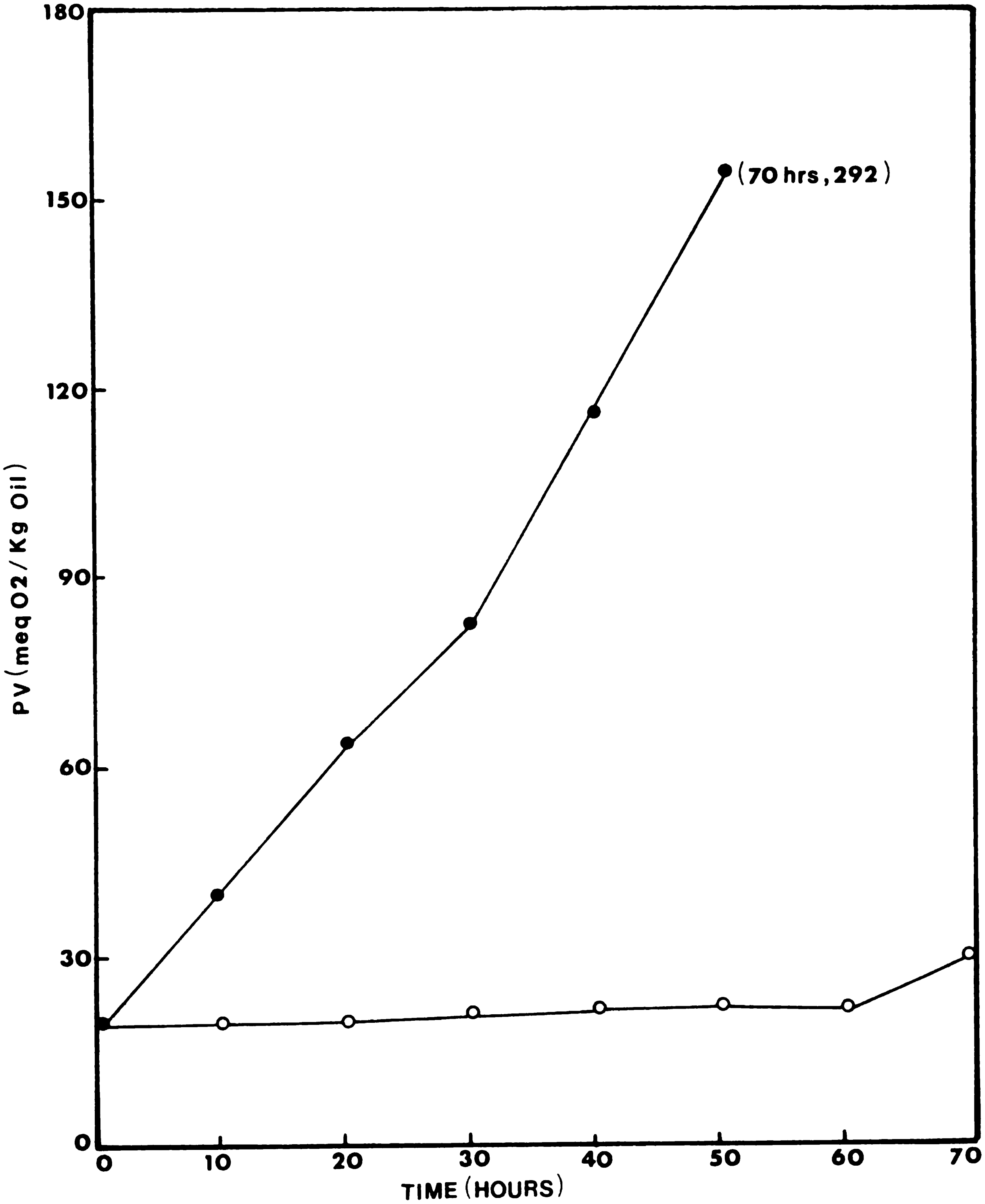


Figure 16 Effect of fluorescent light on the peroxide value of unbleached olive oil. (●: Unbleached olive oil, o: Unbleached olive oil with alum. foil)

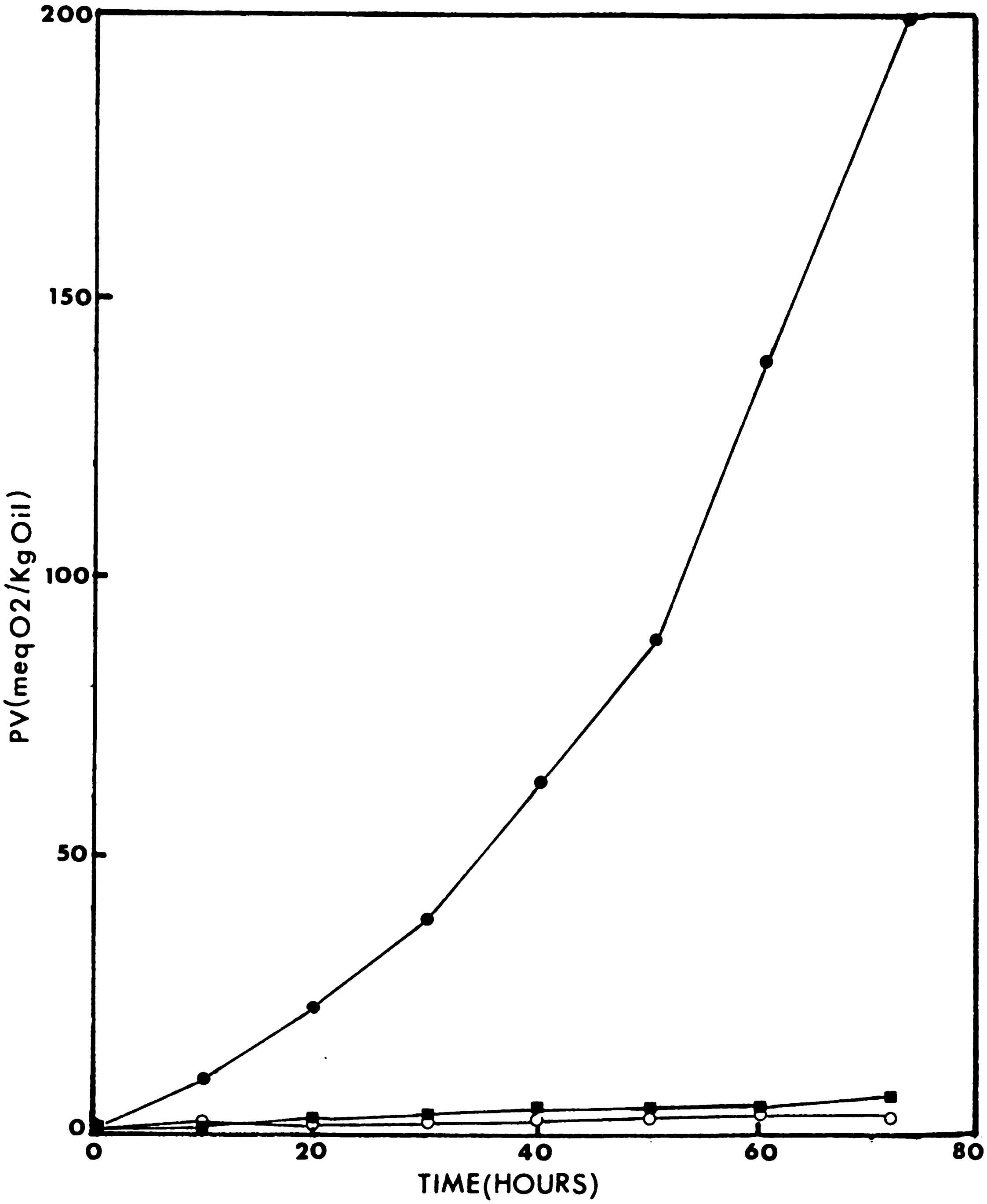


Figure 17 Effect of fluorescent light on peroxide value in bleached olive oil. (●: Bleached olive oil, ■: Bleached olive oil + 10 ppm Chl with alum. foil, o: Bleached olive oil with alum. foil)

sterols. The α-tocopherol content of the olive oil used was 20.69 ppm, while the phenol content was 120 ppm.

Boskou and Morton (1976) observed some antioxidant effect by Δ^{-5}-anemasterol present in olive oil and Cantarelli and Montedoro (1979) demonstrated an antioxidant effect of the phenols present in olive oil. They observed that removal of phenols from olive oil caused its rapid oxidation.

Gutfinger (1981) also reported an antioxidant effect by the phenols present in olive oil and in particular by the 0-diphenols.

To study if chlorophyll exhibits antioxidant activity in the absence of light, samples of bleached olive oil with and without chlorophyll added were placed in beakers covered with aluminum foil and a control sample illuminated with fluorescent light.

In the absence of light, no oxidation occurred up to 70 hours in bleached olive oil, no matter whether chlorophyll was present or not (Figure 17).

Table 22 shows the increase in the percentages of conjugated dienoic acid in unbleached olive oil, bleached olive oil and bleached oil to which 10 ppm chlorophyll was added, during illumination with fluorescent light. The same Table shows the increase in the percentage of conjugated dienoic acid in bleached olive oil containing no chlorophyll in the absence of fluorescent light. In all

Table 22 Increase in % conjugated dienoic acid of unbleached and bleached olive oil containing chlorophyll during illumination with fluorescent light.

Samples	% Conjugated dienoic acid							
	Illumination time (hrs)							
	0	10	20	30	40	50	60	70
Unbleached olive oil	19.50	19.80	24.60	28.60	28.70	31.70	34.50	40.00
Bleached olive oil	0.00	9.80	24.00	28.00	38.00	60.00	65.00	95.00
Bleached /w alum. foil cover	0.00	7.00	8.00	8.50	8.70	10.00	11.00	12.90
Bleached + 10 ppm Chlorophyll	0.00	16.00	31.00	35.00	47.00	60.00	80.00	97.70

samples an increase in the values was observed throughout the illumination period.

Khan et al. (1954) observed that conjugated hydroperoxides were formed during autoxidation of unsaturated fatty acids. They also observed that when methyl linoleate was illuminated in the presence of chlorophyll two types of hydroperoxides (conjugated and nonconjugated) were developed. Among them only the conjugated hydroperoxides absorb at 233 nm.

The observed increase in the percentage of the conjugated diene acid in our samples was probably due to the formation of conjugated diene hydroperoxides mostly from the linoleic acid present in olive oil. The oil used as GC analysis showed contained only a small amount of linolenic acid thus we did not expect much involvement of this acid in the diene conjugated values. Since nonconjugated linoleate hydroperoxides do not absorb at 233 nm, it seems logical that there was an accumulation of more hydroperoxides in unbleached olive oil which remained undetected.

Based on findings of others (Khan et al., 1954; Rawls and Van Santen, 1970; Frankel et al., 1982) that photooxidation with $^1O_2$ involves the formation of two types of hydroperoxides, the ratio was determined:

$$\frac{\text{Peroxide Value (PV)}}{\text{\% Conjugated Dienoic Acid (CDA)}}$$

in order to get an indication of possible involvement of singlet oxygen in our studies. It was assumed that the value of % CDA in our samples would be related to the presence of diene conjugated hydroperoxides. Thus in a system containing linoleate and chlorophyll, like olive oil, during autoxidation the ratio PV/% CDA would remain constant since all the hydroperoxides formed would be of conjugated type (Khan _et_ _al_., 1954) and therefore all would be detected at 233 nm. However if this system is exposed to light, the ratio would increase, along with the presence of chlorophyll, since the nonconjugated hydroperoxides formed by singlet oxygen would not be detected at 233 nm.

Our results showed that in the unbleached olive oil containing natural chlorophyll, the ratio PV/% CDA continued increasing along with the peroxide value (Figure 18) as long as the chlorophyll color remained persistent. In contrast, in bleached olive oil containing added chlorophyll (10 ppm), while the peroxide value increased continually with incubation time, the ratio of PV/% CDA increased up to 10 hrs and then leveled off (Figure 19). The subsequent leveling of the ratio coincides with the disappearance of chlorophyll as observed by visual inspection.

When the light was excluded during illumination of bleached olive oil, both the peroxide value and the PV/% CDA ratio remained rather consistent throughout the incubation

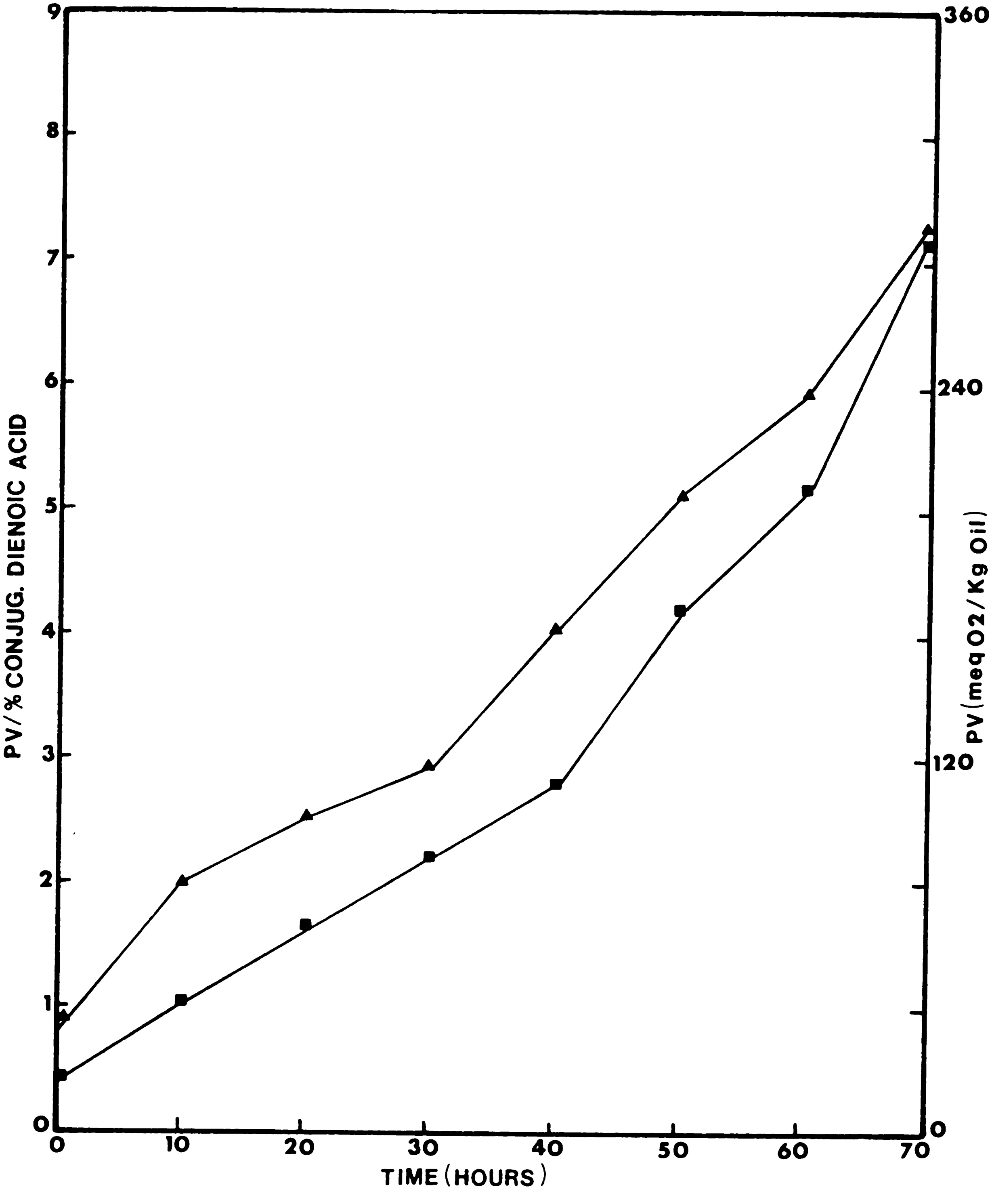


Figure 18 Peroxide value and PV/% CDA ratio in unbleached olive oil illuminated with fluorescent light. (■: PV, ▲: PV/% CDA)

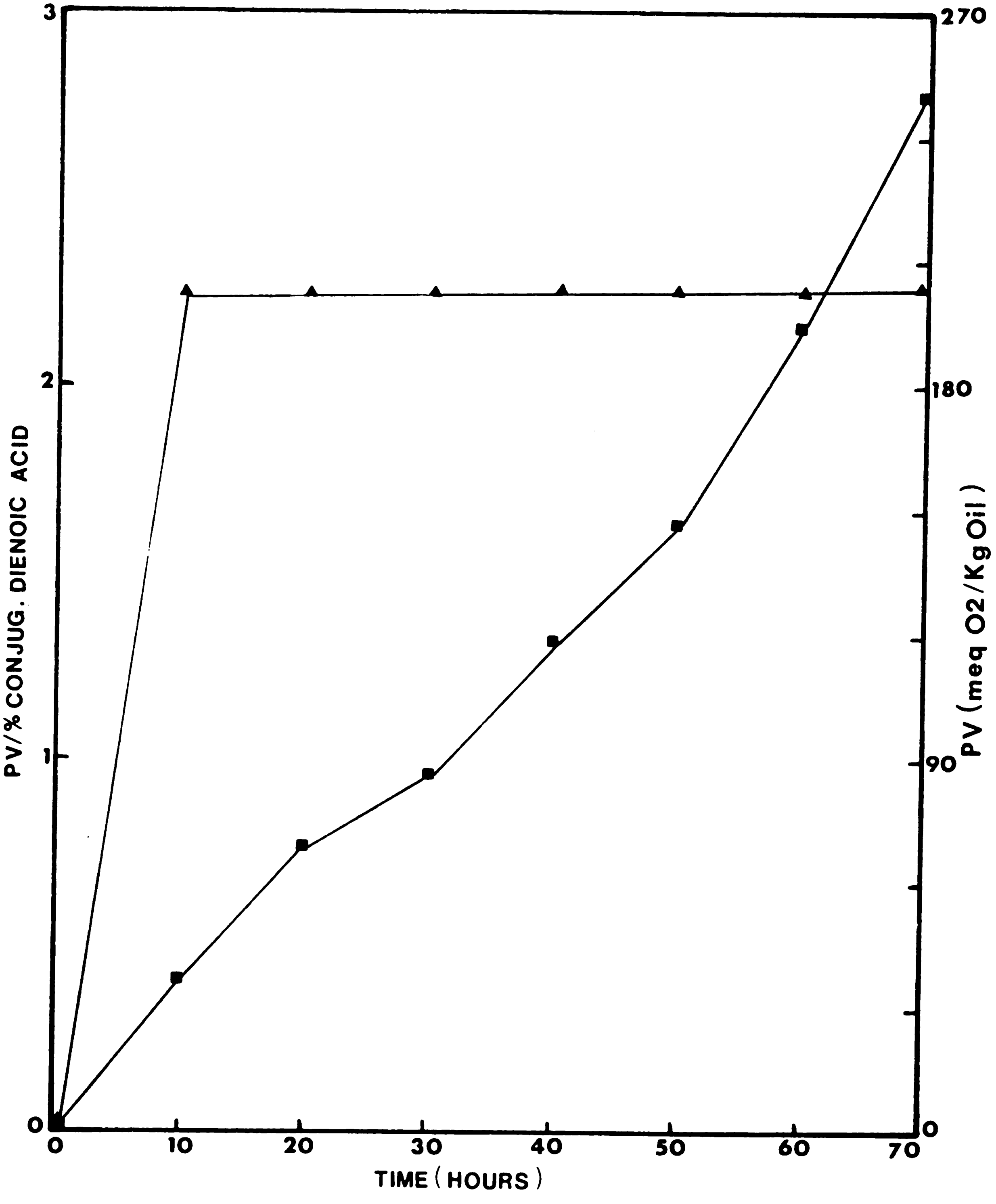


Figure 19 Peroxide value and PV/% CDA in bleached olive oil containing added chlorophyll and illuminated with fluorescent light. (▲: PV/% CDA, ■: PV)

(Figure 20). This indicates that the few hydroperoxides formed during autoxidation were conjugated.

Data of Figures 18 and 19 suggest that $^1O_2$ was involved in the photooxidation of bleached (with added chlorophyll) and unbleached olive oil which has naturally occurring sensitizers.

Effect of Fluorescent Light on Peroxide Formation in Olive Oil Containing Chlorophyll-a and Pheophytin a and b

Based on the observation that the increase in the ratio PV/% CDA for samples with added chlorophyll happened at the early illumination period, an experiment was conducted using bleached olive oil to which chlorophyll or pheophytin was added. Peroxide value, diene conjugation and TBA tests (for some samples) were conducted every 2 hrs throughout the illumination period.

Figure 21 demonstrates the effect of fluorescent light on peroxide formation in samples containing either chlorophyll or pheophytin and exposed to light. The same figure demonstrates the role of chlorophyll in the absence of light.

Pheophytin as well as chlorophyll promoted oxidation of bleached olive oil in the presence of light. It is obvious that both molecules catalyzed olive oil photooxidation.

Rawls and Van Santen (1970) using chlorophyll a and pheophytin (a + b) and methyl linoleate as substrate observed

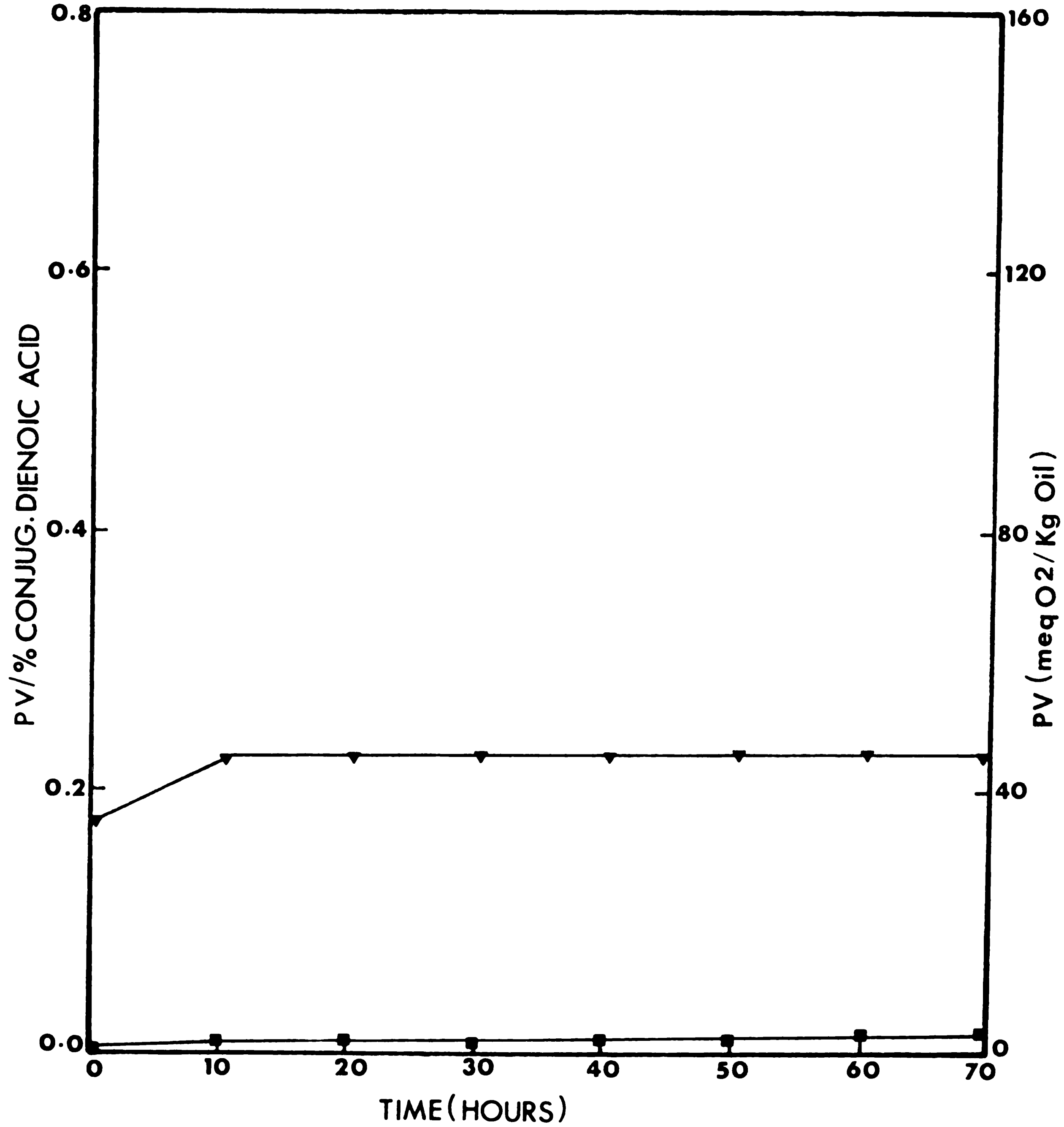


Figure 20 Peroxide value and PV/% CDA ratio in bleached olive oil containing no additives in the absence of fluorescent light. (■: PV, ▲: PV/% CDA)

Figure 21 Effect of fluorescent light on the peroxide value of bleached olive oil containing added chlorophyll and pheophytin.
(▲: Bleached olive oil + 10 ppm pheop a + b,
■: Bleached olive oil + 10 ppm Chl a,
□: Bleached olive oil + 10 ppm Chl a with alum. foil, △: Bleached olive oil)

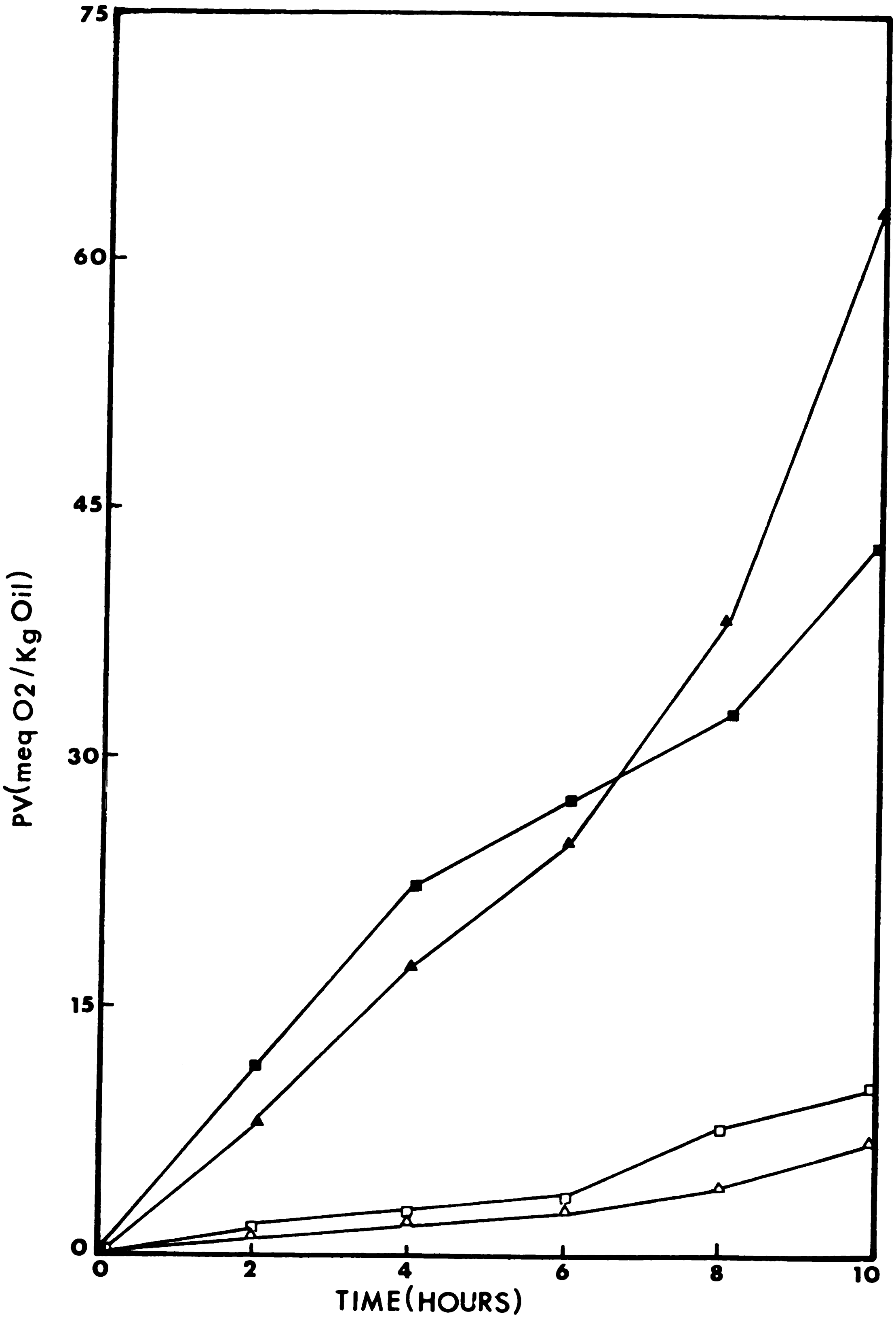


Figure 21

that the photooxidation products in both cases showed a very similar pattern and the results were very close.

In the studies with the two pigments, pheophytin and chlorophyll, the latter exhibited a greater prooxidant effect during the first six hrs of illumination (Figure 21). From then on the samples containing pheophytin showed a greater increase in peroxide formation.

The more pronounced effect of chlorophyll in the first hrs of illumination could be attributed to the Mg contribution to the energy distribution of the pigment. Both pigments have a porphyrin structure and the only important structural difference between them is that chlorophyll contains magnesium while in the case of pheophytin that element has been replaced by hydrogen as is shown below:

$$\text{Chlorophyll} + 2H^{+} \longrightarrow \text{Pheophytin} + Mg^{++}$$

Actually chlorophyll is considered a very labile molecule. The fact that a higher oxidation effect resulted from pheophytin than from chlorophyll in the last hrs of illumination was probably due to chlorophyll destruction. It was obvious by visual inspection that chlorophyll was completely destroyed (disappearance of the green color) after six hrs of illumination, whereas the color of the pheophytin containing samples did not change appreciably. Rawls and Van Santen (1970) observed destruction of chlorophyll during their photooxidation studies but they reported a low rate of destruction. Ramunni (1964) reported that even the natural chlorophyll present in olive oil undergoes complete decomposition during storage of olive oil in the presence of light.

Sastry et al. demonstrated that bleaching of chlorophyll occurs during photooxidation. He proposed the following mechanism for the bleaching of chlorophyll in the presence of visible light:

$$
\begin{array}{lll}
\mathrm{Chl} \xrightarrow{h\nu} & \longrightarrow & |\mathrm{Chl}|^{*} \longrightarrow {}^{3}\mathrm{Chl} \\
{}^{3}\mathrm{Chl} + {}^{3}\mathrm{O}_2 & \longrightarrow & {}^{1}\mathrm{O}_2 + \mathrm{Chl} \\
{}^{1}\mathrm{O}_2 + \mathrm{RH} & \longrightarrow & \mathrm{ROOH} \\
\mathrm{ROOH} \xrightarrow{h\nu} & \longrightarrow & \mathrm{ROO}\cdot + \mathrm{H}\cdot \\
\mathrm{ROO}\cdot + \mathrm{Chl} & \longrightarrow & \mathrm{ROOH} + \mathrm{Chl}\cdot \\
\mathrm{Chl}\cdot + {}^{1}\mathrm{O}_2 & \longrightarrow & \mathrm{Chl}\cdot - \mathrm{O}_2 \\
\mathrm{Chl}\cdot - \mathrm{O}_2 + \mathrm{H}\cdot & \longrightarrow & \mathrm{Chl} - \mathrm{O}_2 \text{ (bleached)}
\end{array}
$$

Hydroperoxides are formed due to the action of singlet oxygen and these may give rise to peroxy radicals on exposure to light. The peroxy radicals abstract hydrogen atoms from chlorophyll, thus disturbing its conjugated electron system. The resulting peroxy radical of chlorophyll combines with a proton and is stabilized itself to a stable peroxide.

Results concerning the role of chlorophyll in darkness disagree with the findings of Interesse et al. (1971), who reported that chlorophylls a and b act as antioxidants.

Figure 22 shows a drop in the ratio PV/% CDA for samples containing chlorophyll after six hrs illumination time. This was probably due, as mentioned previously, to chlorophyll destruction. Samples containing pheophytin however showed a continous increase in that ratio (PV/ % CDA). These samples had maintained some of their initial color when this study was terminated.

The increase in the ratio PV/% CDA was more pronounced at the first hrs of illumination (Figure 22) probably due to the higher activity of the pigments.

Photooxidation by $^1O_2$ form isomeric hydroperoxides at each end of the double bond systems in unsaturated fatty acids. Consequently when linoleic acid, which is present in olive oil, is oxidized by $^1O_2$, hydroperoxides can be formed at C_9, C_{10}, C_{12} or C_{13} with double bonds at 10-11 and 12-13 for C_9, at 8-9 and 12-13 for C_{10}, 9-10 and 13-14 for C_{12} and at 9-10 and 11-12 for C_{13}. It is obvious that the 10-OOH is β-γ to the 12-13 double bond whereas the 12-OOH is β-γ to the 9-10 double bond. Thus, during the photooxidation by $^1O_2$ there is formation of hydroperoxides, which can lead to the development of β-γ systems.

According to Dahle <u>et</u> <u>al</u>. (1969) and Pryor <u>et</u> <u>al</u>. (1976) the hydroperoxide possessing double bonds β, γ to the peroxide group is capable of undergoing cyclization and fission with ultimate formation of malonaldehyde.

When TBA values were assessed along with peroxide value, the samples containing bleached olive oil and 10

Table 23 Peroxide value and TBA absorption values of bleached olive oil containing chlorophyll, in the presence or in the absence of fluorescent light.

Hours	Bl + 10 ppm Chl		Bl + 10 ppm Chl with alum. foil	
	PV	TBA	PV	TBA
0	0.0	0.040	0.0	0.040
2	11.7	0.078	1.0	0.045
4	22.2	0.148	2.3	0.073
6	27.5	0.158	3.8	0.086

Bl: Bleached Olive Oil
Chl: Chlorophyll

Table 24 Ratios of PV/% CDA and PV/TBA of bleached olive oil containing chlorophyll in the presence or in the absence of fluorescent light.

Hours	Ratio: $\frac{PV}{\%CDA}$		Ratio: $\frac{PV}{TBA}$	
	Bl + 10 ppm Chl.	Bl + 10 ppm Chl with alum. foil	Bl + 10 ppm Chl	Bl + 10 ppm Chl with alum. foil
0	0.00	0.00	0.00	0.00
2	1.87	0.14	150.00	22.22
4	3.19	0.31	150.00	31.50
6	3.38	0.50	174.00	44.20

Bl: Bleached olive oil
Chl: Chlorophyll

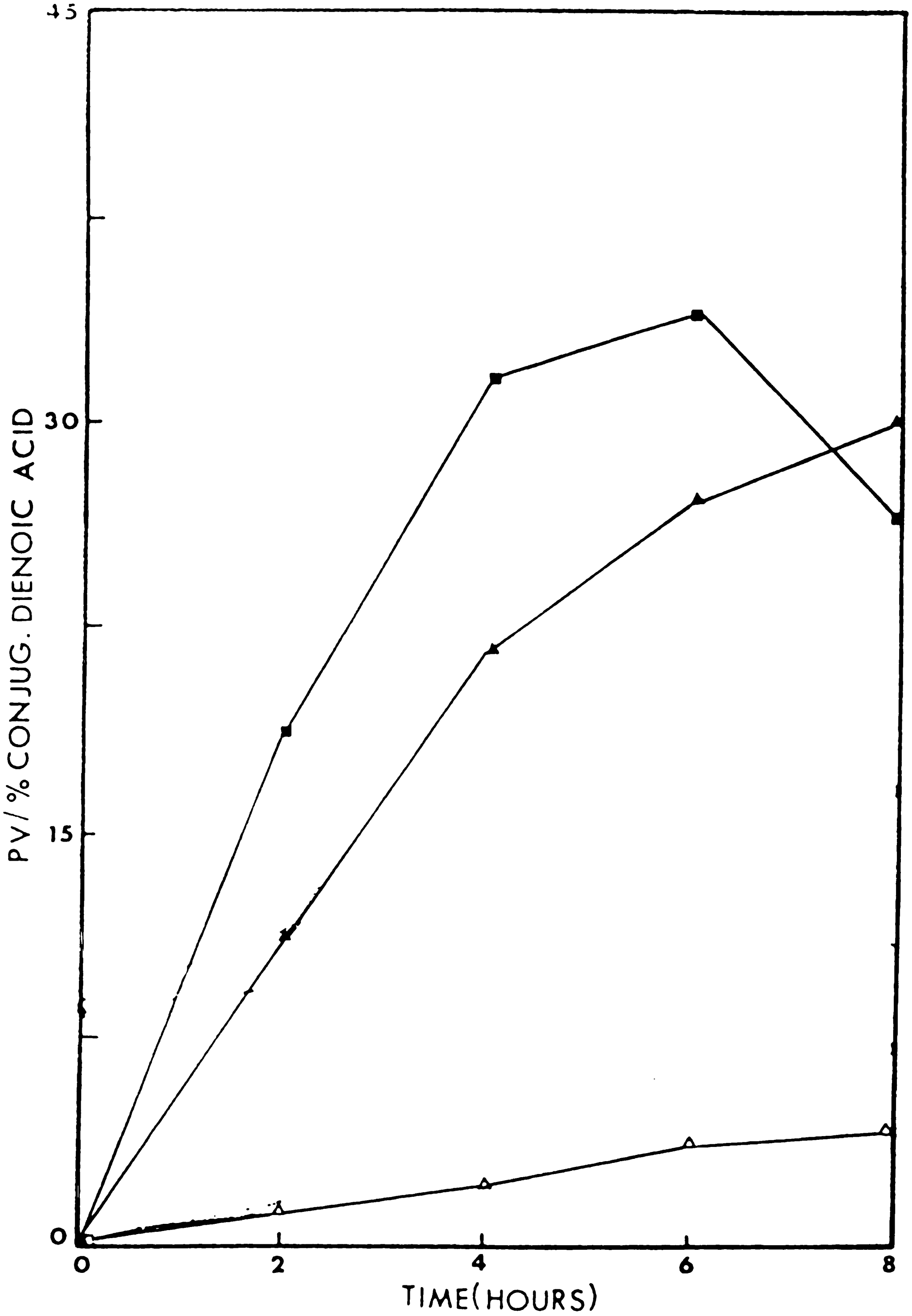


Figure 22 PV/% CDA ratio vs time of bleached olive oil containing added chlorophyll and pheophytin. (△: Bleached, ▲; Bleached + 10 ppm pheop (a + b), ■: Bleached + 10 ppm Chl a)

ppm chlorophyll showed higher peroxide values and TBA values than the samples containing the same amount of chlorophyll and covered with aluminum foil (Table 23).

The ratio of PV/% CDA and PV/TBA for the above samples are shown in Table 24. These ratios in both groups of samples were increased with the illumination time. The rate of the increase in the PV/TBA ratio as in the case of PV/% CDA (Figure 22) was more pronounced at the first hrs of illumination (Table 24). The values of these two ratios were higher in the case of samples containing 10 ppm chlorophyll and exposed to light than the ones with the same amount of chlorophyll but protected from the light.

Although the TBA values of the bleached olive oil containing 10 ppm chlorophyll and exposed to fluorescent light were higher than those of bleached olive oil containing the same amount of chlorophyll and protected from the light (shielded with aluminum foil), we can not say that these small differences in TBA values (Table 23) were the result of $^1O_2$ participation. However, these results corroborate somewhat the finding from peroxide value and diene measurement which gave an indication that singlet oxygen was involved in the photooxidation of olive oil.

SUMMARY AND CONCLUSIONS

The relative quality of olive oil extracted from fruits collected directly from the tree and from plastic nets was studied. The effects of the extraction systems: Pieralisi, Hiller and Rapanelli (Sinolea-Decanter) and of packaging (glass-plastic) and storage conditions (dark, diffused, and direct light) were studied as well. In addition, the effect of fluorescent light on the photooxidation of olive oil containing either natural or added (after bleaching) substances was investigated.

During the time the fruits remained on the tree, neither hydrolytic nor oxidative deterioration of the oil were noticeable. These deteriorations, however, became significant in oil extracted from fruits which remained on the collection nets longer than a month. Beyond a month, the free fatty acid content of the oil exceeded one and the peroxide value exceeded 20, thus it was no longer considered as "virgin" oil.

There was no significant effect of the various processing systems on the initial quality of the oil, as evaluated by the peroxide values, free fatty acids, moisture and foreign materials.

Some difference in the color of Rapanelli Decanter oil was observed. It was darker than the others as it contained more chlorophyll.

The peroxide values of the oil extracted by the systems Pieralisi, Hiller and Rapanelli-Sinolea after seven months of storage in darkness did not differ significantly among the systems. The peroxide values of Rapanelli-Decanter oil however were higher than the others and differed significantly (P=0.05).

The oil obtained from the above systems was oxidized to a different degree in darkness, in diffused light and in direct sunlight. The samples stored in darkness were significantly less oxidized when compared with those stored in diffused and direct sunlight. The samples stored in diffused and in direct sunlight were not oxidized differently from each other. Under diffused light conditions, the oil extracted by the Rapanelli-Decanter system was oxidized to a lesser degree than that of Rapanelli-Sinolea. The reverse, however, was observed when the two oils were stored in darkness.

The packaging materials influenced the olive oil stability during storage in diffused light. Glass bottles provided better protection from oxidation than plastic bottles of polyethylene. Exclusion of light with aluminum foil resulted in lower peroxide values in both types of oil containers. The peroxide value of the oil in commercial polyethylene bottles, stored in diffused light, was greater than 20 in one month. Therefore this oil was no longer considered as "virgin" oil. Even the color of the oil was destroyed after 3 months storage probably due to chlorophyll destruction. Indeed, relatively rapid destruction of the chlorophyll added to the bleached olive oil was observed in photooxidation studies.

The olive oil used for photooxidation studies with fluorescent light was satisfactorily bleached with a mixture of different bleaching agents. Zero absorbance values were determined at 460 nm, 550 nm, 620 nm and 670 nm. The bleaching procedure reduced the peroxide value of the oil to zero. The free fatty acid content of the oil was also reduced.

Unbleached olive oil was oxidized to a greater degree than the bleached olive oil in the presence of fluorescent light. In the absence of fluorescent light, however, the reverse was observed. This indicated that the natural antioxidants (tocopherols and phenols) present in olive oil had a pronounced effect in the absence of light, while they had little or no effect in the presence of light. Apparently photooxidation destroys these compounds quite rapidly.

At the level of 4 ppm, chlorophyll added to the bleached olive oil showed a lower prooxidative effect than at the level of 6 ppm. Therefore there is an apparent relationship between the chlorophyll concentration and the degree of photooxidaiton. Since unbleached olive oil contained 5.2 ppm chlorophyll and the added chlorophyll at the level of 4 ppm promoted oxidation, it seems logical that the chlorophyll content of olive oil was sufficient to catalyze photooxidation.

Both, chlorophyll a and pheophytin a + b, increased the oxidation rate significantly in the bleached olive oil when present at the level of 10 ppm. Higher oxidation rates were achieved with chlorophyll than with pheophytin during the first 6 hrs of incubation. The effect of pheophytin was more pronounced from that point on. This reversal in rates was probably due to chlorophyll destruction. It was obvious that after six hrs illumination time the green color of the chlorophyll containing samples faded considerably. This pigment did not seem to function as an antioxidant in darkness.

β-Carotene at the concentration of 4 ppm prevented the photooxidation of bleached olive oil to a lesser degree than at the level of 6 ppm. At the same level of concentration (6 ppm), α-carotene was less effective than β-carotene in preventing photooxidation of bleached olive oil. The protective effect of both carotenes was more pronounced in the first hrs of incubation. The weakening of the effect toward the end of the incubation period was probably due to destruction of the carotene.

D-α-tocopherol showed some inhibition of oxidation of bleached olive oil containing no chlorophyll after 48 hrs of incubation. As the addition of α-tocopherol increased (50 to 100 to 150 ppm) the rise in peroxide value was slightly reduced. D-α-tocopherol in the presence of chlorophyll did not prevent peroxide formation in bleached olive oil illuminated with fluorescent light. This was probably due to destruction of the tocopherol by photooxidation.

Ni-chelate provided some protection against the photooxidation of bleached olive oil containing 6 ppm added chlorophyll. At the concentration of 100 ppm, Ni-chelate afforded a greater protecting effect than β-carotene. This indicates that Ni-chelate may exhibit a greater singlet oxygen quenching effect.

The values of the PV/% CDA ratio indicated that both conjugated and nonconjugated hydroperoxides were formed when bleached olive oil containing chlorophyll or pheophytin was exposed to fluorescent light. Also conjugated and nonconjugated hydroperoxides probably were formed when unbleached olive oil containing natural chlorophyll was illuminated with fluorescent light. These results provide additional evidence for the probable implication of singlet O_2 in oxidation of olive oil when sensitizers are present.

The conclusions drawn from this study are summarized as follows:

1) The oxidative and hydrolytic deteriorations of olive oil are not appreciable during the time the fruit remains on the trees.

2) Oil from olives which remain on collection nets longer than a month suffers oxidative and hydrolytic deterioration.

3) The compared extraction systems (Pieralisi, Hiller, Rapanelli-Sinolea and Rapanelli-Decanter) did not affect the initial quality of the oil appreciably.

4) The small differences in the degree of olive oil oxidation during storage in darkness indicated that all the systems, except the Rapanelli-Decanter, had similar effects on the tendency of the oil to oxidation.

5) The oxidation of olive oil proceeds slowly in darkness, more readily in diffused light and even greater in direct sunlight.

6) Glass packaging materials give better protection against oxidation than polyethylene plastic bottles during storage of olive oil in diffused light. Olive oil should not be bottled in transparent plastic bottles in order to minimize oxidative deterioration during storage.

7) The natural substances (phenols and tocopherols) present in olive oil exhibit an appreciable antioxidant effect only in the absence of light.

8) Chlorophyll did not exhibit antioxidant activity in darkness under conditions of tests carried out here. In contrast, it acted as a photosensitizer under light conditions promoting oxidation to a high degree.

9) Pheophytin also appears to participate in the photooxidative mechanism and to undergo less degradation than chlorophyll upon exposure to fluorescent light. This indicates that there may be a higher photooxidative effect in olive oil from pheophytin, if present, than from chlorophyll.

10) Ni-chelate exhibits a greater effect in preventing photooxidation of olive oil than the β-carotene does and thus may be a better quencher of singlet oxygen.

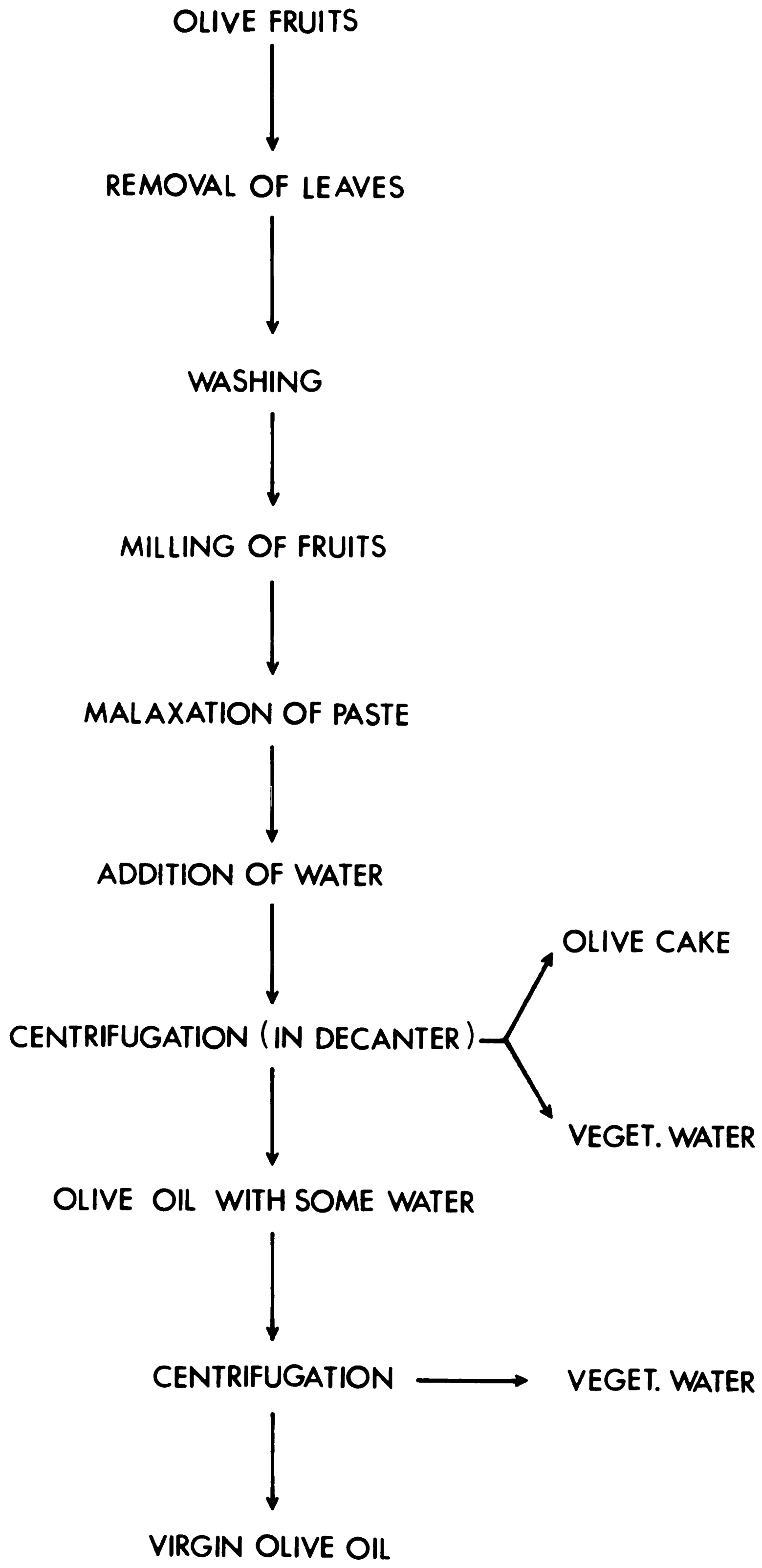
11) The presence of conjugated and nonconjugated hydroperoxides indicated that singlet ($^1O_2$) oxygen could have been involved in the photooxidation of olive oil, when chlorophyll or pheophytin was present.

12) It appears that the best way to avoid oxidative degradation in olive oil is protecting it from light and oxygen.

APPENDICES

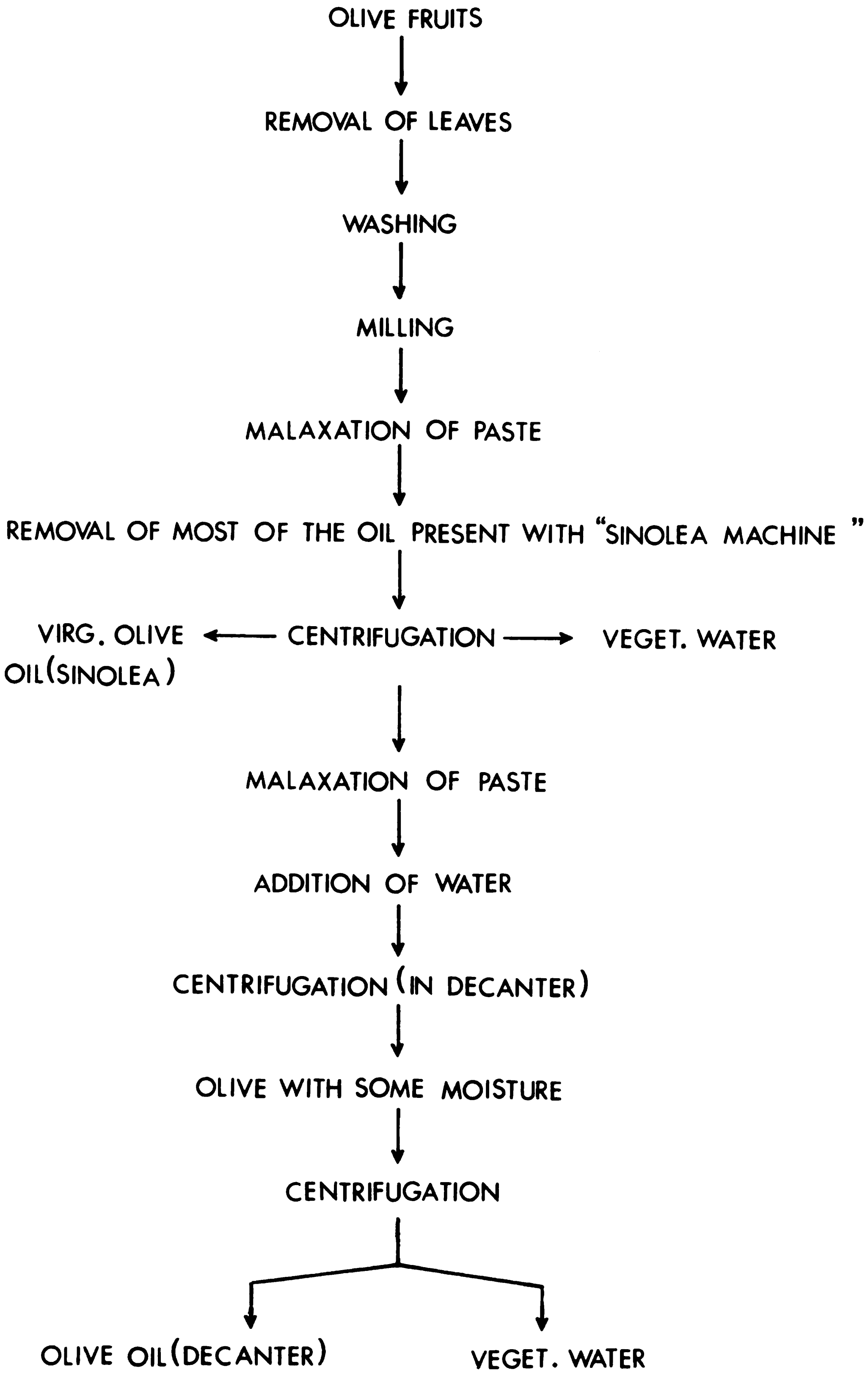
APPENDIX 1

STEPS IN EXTRACTION OF OLIVE OIL BY THE PIERALISI PROCESS



APPENDIX 2

STEPS IN EXTRACTION OF OLIVE OIL BY THE RAPANELLI PROCESS



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