Studies in Photooxidation of Olive Oil

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ABSTRACT

Photooxidation of olive oil, bleached to remove most non-triglyceride components, was studied to elucidate the role of added chlorophyll a, pheophytin a and b, α- and β-carotene, d-α-tocopherol and nickel dibutyldithiocarbamate.

Chlorophyll functioned as a photosensitizer resulting in rapid oxidation of the oil and the added components and loss of color. α- and β-carotene acted essentially equally as singlet oxygen quenchers. α-tocopherol had little apparent effect on the oxidation rate. Carotenes and tocopherols apparently were destroyed more rapidly when chlorophyll was present. The ratio of peroxide value to conjugated dienoic acids formed developed greater values when chlorophyll was present, thus suggesting a singlet oxygen effect in the system. Pheophytin also proved to be an oxidation promoter.

INTRODUCTION

Olive oil in its natural state contains many constituents other than triglycerides. The color of the oil depends on the kind and amounts of pigments and other substances which vary with the stage of maturation of the olive fruit when crushed for oil. Olive oil of good quality is not physically or chemically altered, and the pigments transferred from the fruit to the oil may have a profound effect on storage stability of the oil.

Renewed interest has been shown in the effect of naturally occurring pigments on oxidative stability of the oil (1-5). Chlorophyll may act as a sensitizer to form singlet oxygen in the photooxidation mechanism, while carotenes may retard photooxidation by quenching singlet oxygen (1,5,6,7). Tocopherols and other phenolic compounds may act as antioxidants under suitable circumstances, and tocopherols also may quench singlet oxygen. Photooxidation with singlet oxygen is not controlled by the antioxidants commonly used to inhibit autoxidation (5). Singlet oxygen quenchers may function by reacting chemically with singlet oxygen or perhaps by acting as a screening agent to reduce the ability of incident radiation to transfer energy necessary to form singlet oxygen. Carlsson et al. (5) demonstrated that nickel chelates could retard photooxidation of unsaturated food oils exposed to UV radiation and visible light. The inherent absorption capability of these colored compounds possibly could screen out the active wavelengths during irradiation and thus would protect the oils by light absorption rather than by quenching singlet oxygen.

Since olive oil contains naturally occurring photosensitizers, singlet oxygen quenchers and phenolic antioxidants, it was of interest to examine the role of some of these in olive oil and learn how they relate to quality of the oil as affected by light. Substances studied for this purpose were chlorophyll, pheophytin, β-carotene and α-tocopherol. The behavior of nickel chelate during photooxidation of olive oil also was studied.

EXPERIMENTAL PROCEDURES

Virgin olive oil extracted by the Pieralisi centrifugal system from olive fruits of the cultivar "Koroneiki" was obtained in sealed tin cans from the island of Crete (Greece). The oil had an initial peroxide value of 15. Absorbance ($E^{1\,cm}_{1\,\%}$) at 232 nm and 270 nm were 2.5 and 0.2, respectively. These values indicated that oxidation already had begun in the oil. Bleached oil was prepared from the virgin olive oil. Bleaching was accomplished by placing a plug of glass wool in the bottom of a 2 cm glass column which was then filled with a mixture of 35 g activated charcoal (Darco-G60$_7$ MCB Mfg., Cincinnati, Ohio), 50 g Tonsil Optimum Extra (L.A. Salomon Co., Port Washington, New York), 15 g 60-100 mesh florisil (Fisher Scientific Co.) and 25 g Hyflo Supercel (Fisher Scientific Co.) and capped by a 1 cm layer of infusorial earth. About 150 ml hexane was percolated through the column prior to addition of 100 g of the oil in 150 ml hexane. The column was washed with 200 ml hexane to elute the remaining oil. The hexane was removed from the eluate in a vacuum evaporator at 40 C. A stream of nitrogen was applied to the top of the column throughout the treatment. The efficiency of bleaching was monitored by measuring the color of the oil by the AOCS spectrophotometric method (8).

Additives used included chlorophyll a, α and β carotene and D-α-tocopherol (Sigma Chemical Co.), pheophytin a + b (ICN Pharmaceuticals Inc., Plainview, New York) and nickel-dibutyldithiocarbamate (Pealtzs' Bauer Inc., Stamford, Connecticut). These were prepared in stock solutions of 1 ml acetone with sufficient hexane to make 10 ml.

Reactions were carried out in 250 ml beakers, containing 25 or 50 g oil plus additives, in a stainless steel box (50×22×5 cm) lined with aluminum foil. Two 20 watt cool-white fluorescent tubes were suspended approximately 6 cm above the samples. The remaining open space was covered with aluminum foil. The fluorescent radiation at the level of the samples was 4500 lux measured with a light meter (Luna Pro Gossen, W. Germany). Temperature inside the container was maintained at 30 ± 2 C. The radiation source was turned off for sampling. The position of the samples was rearranged at each sampling in order to equalize illumination as much as possible.

Peroxide values were determined by the official AOCS method (9). The official AOCS method (10) for conjugated dienoic acids (CDA) was used, and percentages of conjugated dienoic acids were calculated from

$$CDA\% = 0.84\left(\frac{As}{bc} - K_o\right)$$

where K_o = 0.07 absorptivity for esters, As = observed absorbance at 233 nm, b = cell width in cm and c = concentration in g/liter.

The method of Sidwell et al. (11) was used for the thiobarbituric acid (TBA) test.

RESULTS AND DISCUSSION

The bleached oil had zero absorbance at the four wave lengths 460, 550, 620 and 670 nm and was completely colorless by visual inspection. The unbleached oil was found to have 4.2 ppm β-carotene determined by the AOAC method (12), which is in the range reported by others (13). The α-tocopherol content of unbleached oil was 20.69 ppm as determined by HPLC (14). The absorbance at 670 nm due to chlorophyll was consistent with a finding of 5.5 ppm by the AOCS method (15). The bleaching process removed all peroxides and reduced free fatty acids from a value of 0.70% to 0.035%. Tocopherol content of the bleached oil was not determined. Vianni (22) showed that a bleaching process similar to that used here decreased α-tocopherol content of soybean oil from 4.08 to 1.55 mg/100 g and γ-tocopherol from 116.5 to 11.3 mg/100 g.

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TABLE I

Peroxide Formation in Bleached Olive Oil Containing Different Additives During Illumination With Fluorescent Light at 32 C ± 2 C

Illumination time (hrs)	Peroxide values (meq O_2/Kg oil) Control	Covered with aluminum foil	4 ppm chlorophyll	6 ppm chlorophyll	4 ppm β-carotene	6 ppm β-carotene	6 ppm α-carotene
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
24	38.0	13.0	59.0	61.0	32.0	28.5	31.0
48	73.5	30.0	82.0	90.5	61.0	56.0	59.0
60	105.0	45.0	98.0	111.0	87.0	82.0	86.0
84	205.0	62.0	189.0	200.0	180.0	155.0	175.0

TABLE II

Peroxide Formation in Bleached Olive Oil Containing Different Levels of Added D-α-tocopherol During Illumination with Fluorescent Light at 32 C ± 2 C

Illumination time (hrs)	Peroxide value (meq O_2/Kg oil) Control	50 ppm D-α-tocopherol	100 ppm D-α-tocopherol	150 ppm D-α-tocopherol
0	0.0	0.0	0.0	0.0
12	23.0	22.5	21.0	18.5
24	38.0	33.0	32.5	33.5
48	73.5	73.0	68.5	65.0
60	105.0	92.0	84.0	81.0
84	205.0	160.0	145.0	135.0

Table I shows that bleached olive oil oxidized rapidly in the presence of light and oxidized substantially even in the dark during 84 hr at 30 ± 2 C. The presence of 4 or 6 ppm chlorophyll increased the rate of oxidation as shown by peroxide values in the first 24 hr, but the values at 48, 60 and 84 hr were little different from those developed in the control sample. The presence of 4 or 6 ppm β-carotene or 6 ppm α-carotene appeared to have only a slight inhibiting effect for the first 12 hr, but the peroxide values at 84 hr were only minimally less than those of the control at that time. Little difference in effect was observed between α- and β-carotene. The increased peroxide formation in the presence of chlorophyll is consistent with the role of chlorophyll as a sensitizer in the photooxidation mechanism (1, 3,5). The diminishing effect of chlorophyll at later stages suggests that chlorophyll was degraded and no longer participated in the sensitizing role required for photooxidation. The decreased peroxide formation in the presence of carotenes in the early stages of the study suggests that carotenes acted as singlet oxygen quenchers in phootoxidation or perhaps were oxidized selectively, thus sparing the olive oil until the carotenes are destroyed in the oxidation. Another role proposed for carotenes is to filter out active wavelengths of incident radiation, thus protecting against activation of oxygen by light (16). Terao et al. observed that β-carotene disappeared rapidly from soybean oil during irradiation, but the presence of β-carotene can be prolonged in irradiated oil by the presence of α-tocopherol (17).

Table II shows that varying the added α-tocopherol content had little effect on development of peroxides in olive oil illuminated with fluorescent light. The data are so similar that it would be inappropriate to ascribe either an antioxidant, prooxidant or singlet oxygen quenching effect to the tocopherol role in these samples.

Effect of Sensitizers and Singlet O_2 Quenchers on Photooxidation of Bleached Olive Oil

Bleached olive oil oxidized readily when exposed to fluorescent light as shown in Table III. The presence of only small quantities, e.g. 6 ppm, of chlorophyll enhanced oxidation somewhat. β-carotene and nickel chelates each inhibited oxidation substantially in the first hours of illumination, thus supporting the concept that chlorophyll brings about formation of singlet oxygen which is quenched or its formation inhibited by β-carotene and nickel chelates.

TABLE III

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/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 with aluminum foil	0	4.0	7.0	8.0	8.5
B + 6 ppm Chl + 100 mg/kg carotene	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 chelates	0	10.0	16.0	25.0	47.0

B = bleached oil, Chl = chlorophyll.

The rapid oxidation that occurred in samples containing both chlorophyll and α-tocopherol indicates that tocopherol, a free radical scavenger, is rapidly destroyed during photooxidation, as has been reported in Terao et al. (17).

The role of light in the oxidation process is clearly exemplified by the low rate of oxidation in samples protected from light by aluminum foil (Table III). The average peroxide value of samples protected from light was 15% of the peroxide value formed in comparable samples exposed to light.

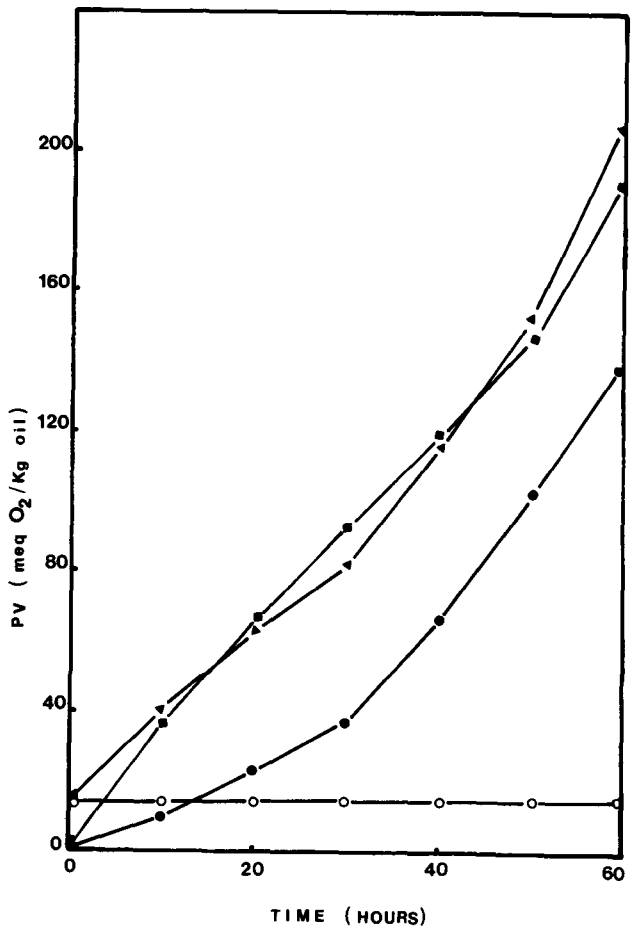

FIG. 1. Peroxide development in bleached and unbleached olive oil in the presence of fluorescent light. -●-●-, bleached oil; -■-■-, bleached oil plus 10 ppm chlorophyll; -▲-▲-, unbleached oil; -○-○-, unbleached oil protected by aluminum foil.

The oxidation of bleached and unbleached olive oil exposed to fluorescent light is shown in Figure 1. The added chlorophyll increased the peroxide value of unbleached oil, while protection from light effectively retarded the oxidation of unbleached oil. Peroxide values in oxidizing unbleached oil ultimately reached higher values than in bleached oil containing added chlorophyll. The peroxides formed through oxidation by singlet oxygen undergo scission (5) to radical species which accelerate the rate and ultimate level of peroxide formation which is greater than would occur in systems having no additives to affect $^1O_2$ formation. As shown in Table III, nickel chelates functioned in this system essentially equivalent to β-carotene, thus demonstrating a probable role as singlet oxygen quencher by each. Carlsson (5) suggested, however, that nickel chelates may function by absorption of light at wavelengths which activate oxidation.

Conjugated diene formation from the linoleic acid component showed more dramatic differences with time of illumination than were shown by the peroxide values. In Table IV, it is shown that conjugated dienes already were present in the unbleached oil and doubled in 72 hr. The bleached oil which had no initial conjugated dienes reached a conjugated diene content equal to that of unbleached oil in 20 hr and greatly exceeded that in unbleached oil at 72 hr. Addition of chlorophyll to bleached oil caused conjugated diene formation to accelerate rapidly, while complete protection from light resulted in only a very slow development of conjugated dienes, as expected.

Photooxidation of dienoic fatty acids results in formation of hydroperoxides which are conjugated as in products formed by autoxidation and in formation of hydroperoxides which are both conjugated and non-conjugated as a result of oxidation by singlet oxygen (18,19). Although the dienoic acids are in lesser quantity than monoenoic acids in olive oil, the early oxidation takes place primarily in the

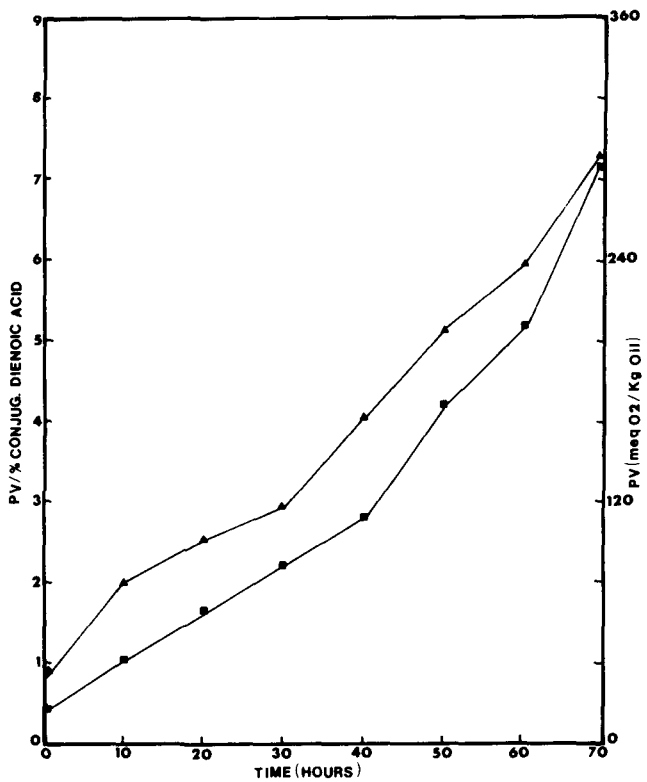

FIG. 2. Peroxide value and peroxide value/% conjugated dienoic acid ratios for unbleached olive oil illuminated with fluorescent light. -■-■-, peroxide value; -▲-▲-, PV/% CDA ratio.

TABLE IV

Increase in % Conjugated Dienoic Acid of Unbleached and Bleached Olive Oil Containing Chlorophyll During Illumination with Fluorescent Light

Samples	% Conjugated dienoic acid Illumination time (hr)							
	0	10	20	30	40	50	60	72
Unbleached oil	19.50	19.80	24.60	28.60	28.70	31.70	34.50	40.00
Bleached oil	0.00	9.80	24.00	28.00	38.00	60.00	65.00	95.00
Bleached oil w/ aluminum foil	0.00	7.00	8.00	8.50	8.70	10.00	11.00	12.90
Bleached oil + 10 ppm chlorophyll	0.00	16.00	31.00	35.00	47.00	60.00	80.00	97.70

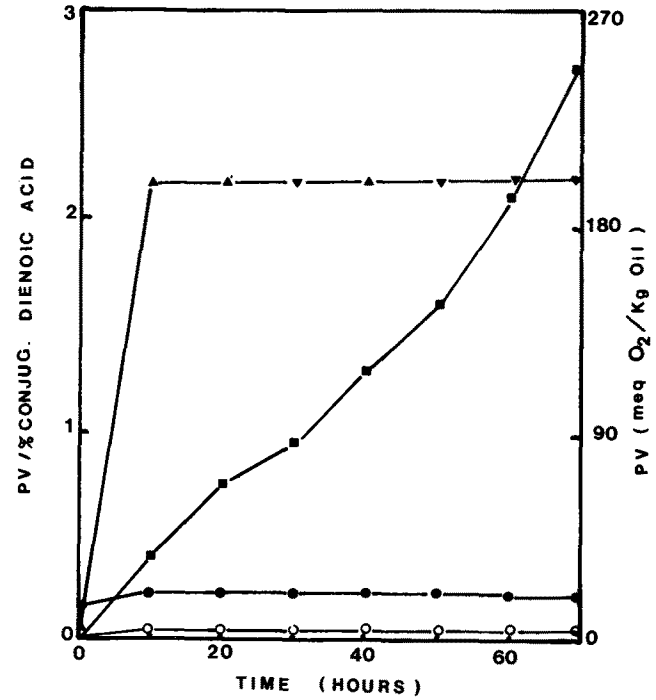

FIG. 3. Peroxide value and peroxide value/% conjugated dienoic acid ratios for bleached olive oil non-illuminated and illuminated with fluorescent light. -■-■-, PV for illuminated oil containing 10 ppm chlorophyll; -▲-▲-, PV/% CDA for illuminated oil containing 10 ppm chlorophyll; -○-○-, PV for non-illuminated oil containing no additives; -●-●-, PV/% CDA for non-illuminated oil containing no additives.

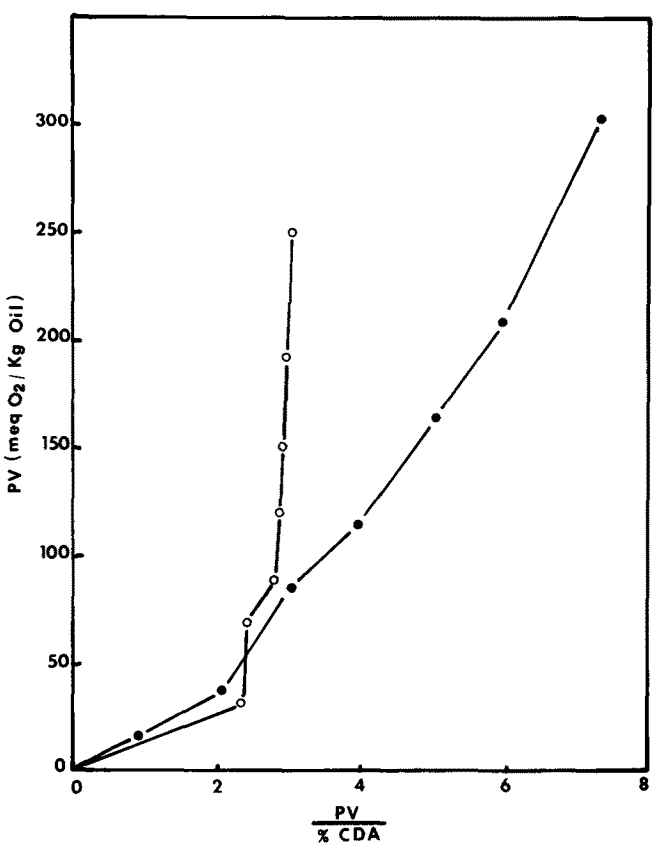

FIG. 4. Peroxide value versus peroxide value/% conjugated dienoic acid ratio for unbleached oil and for bleached oil containing added chlorophyll. -●-●, unbleached oil; -○-○-, bleached oil with 10 ppm chlorophyll.

dienoic acids. Consequently, it was of interest to examine the ratio of peroxide value to % conjugated dienes to aid in determining the role of singlet oxygen in olive oil oxidation. In an autoxidizing dienoic system, the ratio of PV to % CDA would remain fairly constant over a broad range of early oxidation, whereas it would increase in a system in which oxidation occurred essentially as a result of singlet oxygen oxidation forming both conjugated and non-conjugated hydroperoxides.

The peroxide value increased and the ratio PV/%CDA also increased when unbleached olive oil was illuminated for 70 hr as shown in Figure 2. The peroxide value in bleached oil containing added chlorophyll increased steadily as shown in Figure 3. The ratio of PV/%CDA, however, accelerated rapidly in the early stages of photooxidation and then remained constant. The leveling of the curve coincided with the disappearance of chlorophyll from the system as evaluated by visual inspection. As shown in Figure 3, both the PV and the PV/%CDA ratio for bleached oil containing no additives remained essentially constant when no light was permitted to reach the system. These phenomena thus indicate a probable role for singlet oxygen in photooxidation of olive oil.

Figure 4 presents the data of PV versus PV/%CDA for unbleached olive oil and bleached olive oil containing added chlorophyll. The PV vs PV/%CDA in unbleached oil showed a continuous increase. In bleached oil, the ratio approached a constant value after more than two hours of illumination, probably due to destruction of chlorophyll. These results

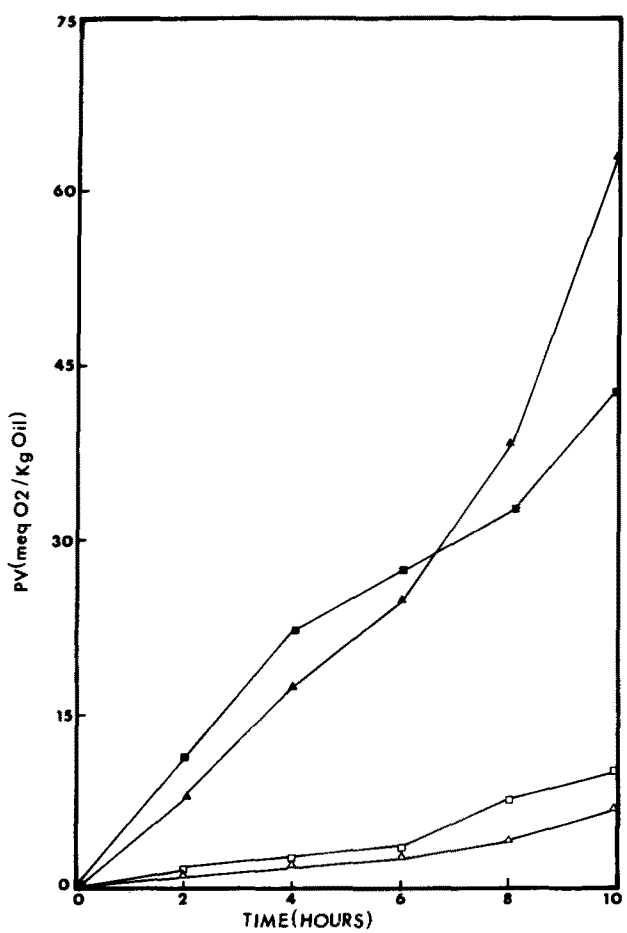

FIG. 5. Peroxide development in the presence of fluorescent light of bleached olive oil containing added chlorophyll or pheophytin. -△-△-, bleached oil; -▲-▲-, bleached oil with 10 ppm pheophytin a + b; -□-□-, bleached oil containing 10 ppm chlorophyll and protected with aluminum foil; -■-■-, bleached oil with 10 ppm chlorophyll a.

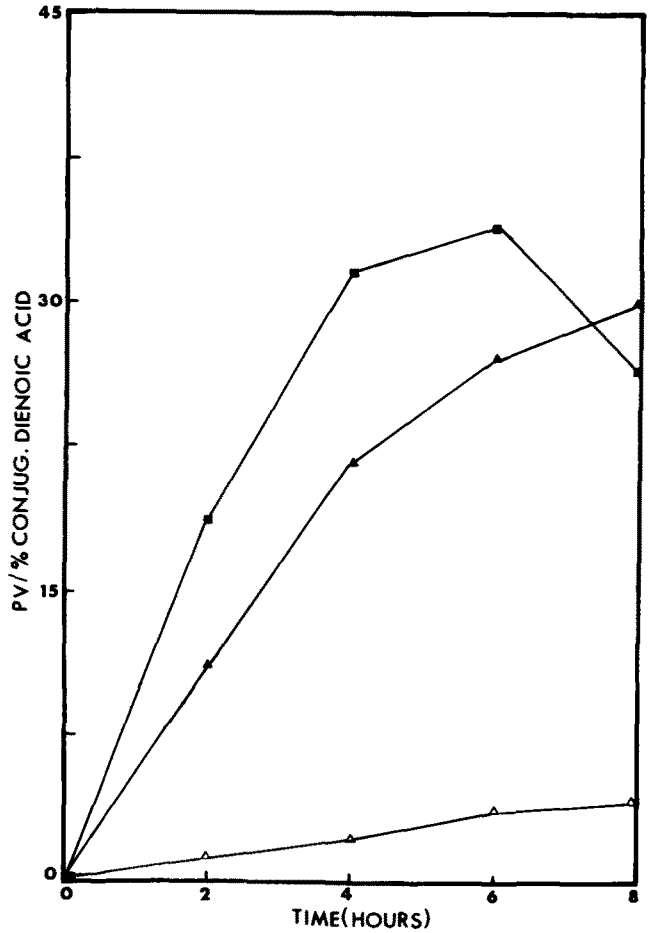

FIG. 6. Peroxide value/% conjugated dienoic acid ratio versus time of bleached olive oil containing added chlorophyll or pheophytin and illuminated with fluorescent light. -△-△-, bleached oil; -▲-▲-, bleached oil with 10 ppm pheophytin a + b; -■-■-, bleached oil with 10 ppm chlorophyll a.

TABLE V

PV and TBA Absorption Values of Bleached Olive Oil Containing Chlorophyll, in the Presence or Absence of Fluorescent Light

	Bl + 10 ppm chl		Bl + 10 ppm chl with alum. foil	
Hours	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 oil, Chl = chlorophyll.

suggest that singlet oxygen was involved during photooxidation of olive oil containing natural or added chlorophyll. However, the oxidation in unbleached olive oil also was regulated by carotenes, tocopherols and other naturally occurring constituents of olive oil.

Role of Pheophytin in Photooxidation of Olive Oil

The presence of pheophytin may be as important in the photooxidation of olive oil as in chlorophyll. Figure 5 shows that chlorophyll a and pheophytin a and b are essentially equivalent in the first 6-8 hr of illumination but, after that, peroxide formation developed more rapidly in the samples containing pheophytin. Visual observation revealed that the color due to chlorophyll was essentially absent after 6-8 hr whereas the color of the samples containing pheophytin was not changed appreciably in that time. The PV/%CDA ratio continued to increase after 6 hr in the samples containing pheophytin, while the same ratio for samples containing added chlorophyll reached a maximum at 6 hr (Fig. 6).

TBA Reactive Compounds Formed During Photooxidation

Photooxidation of dienoic acids in olive oil can lead to β,γ unsaturated hydroperoxides as well as α,β unsaturated hydroperoxides. The β,γ systems presumably can undergo cyclization and fission to form malonaldehyde, which can give the TBA (thiobarbituric acid) reaction (20,21). As shown in Table V, the TBA absorption values developed in bleached olive oil containing 10 ppm chlorophyll were approximately double the values for similar samples which were permitted no light. These observations corroborate results from studies on formation of peroxide values and conjugated diene content that indicated a probable role for singlet oxygen in the photooxidation of olive oil.

REFERENCES

1. Rawls, H.R., and P.J. VanSanten, JAOCS 47:121 (1970).
2. Koch, E., Anal. Chem. 45:2120 (1973).
3. Clements, A.H., R.H. VanDenEngh, D.J. Frost, K. Hoogenhout and J.R. Nooi, JAOCS 50:325 (1973).
4. Chan, H.W.S., JAOCS 54:100 (1977).
5. Carlsson, D.J., T. Suprunchuk and H.M. Wiles, JAOCS 53:656 (1976).
6. Foote, C.S., Science 162:963 (1971).
7. Kaplan, M.L., Chem. Technol. 1:621 (1971).
8. Official and Tentative Methods of the American Oil Chemists' Society, Color Photometric Method cc 136 (1973).
9. Ibid., Peroxide Value, Vol. 8 (1973C).
10. Ibid., Spectrophotometric Determination of Conjugated Dienoic Acid. Ti. Ta-64 (1973).
11. Sidwell, C.G., H. Salwin, M. Benca and J.H. Mitchell Jr., JAOCS 31:603 (1954).
12. AOAC Official Methods of Analysis. 12th ed., Association of Official Analytical Chemists. Washington, DC (1975).
13. Gracian, J., In: Analysis and Characterization of Oils and Fat Products. Boekenoogen, H.E., ed., Intern. Publ. Inc., London, NY (1968).
14. Carpenter, A.P. Jr., JAOCS 55:668 (1978).
15. Official and Tentative Methods of the American Oil Chemists' Society, Chlorophyll Method cc 130-55 (1973).
16. Sattar, A., and J.M. DeMan, JAOCS 53:473 (1976).
17. Terao, J., R. Yamaguchi, H. Murakami and S. Matsushita, J. Food Proces. and Preser. 4:79 (1980).
18. Khan, N.A., W.E. Tolberg, D.H. Wheeler and W.O. Lundberg, JAOCS 31:460 (1954).
19. Frankel, E.N., W.E. Neff, E. Selke and D. Weisleder, Lipids 17:11 (1982).
20. Dahle, L.K., E.G. Hill and R.T. Holman, Arch. Biochem. Biophys. 98:253 (1962).
21. Pryor, W.A., J.P. Stanley and E. Blair, Lipids 11:370 (1978).
22. Vianni, R., Photooxidation studies on soybean oil. Dissertation. 1980. Michigan State University.

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