Role of Minor Constituents in the Photooxidation of Virgin Olive Oil

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ABSTRACT: Virgin olive oil was photooxidized at 2 and 40°C and at fluorescent light intensities of 0, 620, 2710, and 5340 lux. As expected, higher fluorescent light intensities induced higher peroxide formation in the oil. The thiobarbituric acid reactive substances (TBARS) were found to be good indicators of photooxidation during the early stage of the reaction. Pheophytin A and β-carotene were light- and temperature-sensitive, whereas α-tocopherol and total polyphenols were mostly affected by light. Pheophytin A functioned as a photosensitizer, resulting in rapid oxidation of the oil. β-Carotene was a strong natural inhibitor of photooxidation for all light intensities at 2°C, suggesting quenching properties for singlet oxygen. However, β-carotene antioxidant activity was reduced at 40°C because of its rapid destruction.

JAOCS 75, 837–843 (1998).

KEY WORDS: β-carotene, pheophytin, photooxidation, polyphenols, α-tocopherol, virgin olive oil.

Virgin olive oil (VOO), obtained from the fruits of the tree *Olea europaea* by mechanical means and without any chemical treatment, is one of the few vegetable oils consumed in its natural state, without any refining. As a consequence, VOO has a distinctive odor. However, its high concentration of chlorophyll pigments makes it unstable in storage under lighted conditions; according to Werman and Neeman (1), VOO is less resistant to daylight than refined soybean and linseed oils. This phenomenon could be explained by the photosensitizing effect of chlorophylls in VOO. However, refined olive oil was reported to be more stable to photooxidation than soybean and linseed oils (1).

Photooxidation of VOO in the presence of naturally occurring chlorophyll pigments produces singlet oxygen, which reacts with unsaturated fatty acids and produces fatty acid hydroperoxides (2,3). Decomposition of these hydroperoxides initiates a free-radical type of autoxidation. This photooxidation results in a change in color and, because of the formation of hydroperoxide decomposition products, develops undesirable odor and flavor constituents (2–5). Thus, prevention of photooxidation in VOO is of great importance to ensure palatability, economy, and nutritional value.

Previous lipid photooxidation studies reported in the literature (6–10) were related almost exclusively to linoleic acid in relation to chlorophyll-sensitized photooxidation, possibly because this fatty acid occurs in almost all vegetable oils in relatively high concentrations. Few studies have been carried out on purified olive oil and other purified vegetable oils from which pigments, peroxides, phenols, and other minor constituents had been eliminated. Such model systems do not take into consideration the possible interactions between components that may exist in a real system. Because VOO contains, among other compounds, photosensitizers, singlet oxygen quenchers, and phenolic antioxidants, it was of interest to investigate the behavior of these components in the photooxidation process at different temperatures and various light intensities and time periods. In the present study, pheophytin A (PHE A), β-carotene (β-C), α-tocopherol (α-T), total phenols, and polyphenols were investigated to elucidate their roles in the photooxidation process of VOO.

EXPERIMENTAL PROCEDURES

Materials. VOO from the fruits of the Moroccan olive tree cultivar "Picholine" was obtained by centrifugal separation of oil after milling of the fruits and malaxation of the paste.

Eight to 12 g of oil was placed in uncovered disposable Petri dishes (90 × 15 mm) and exposed to fluorescent light intensities of 0, 620, 2710, and 5340 lux using 20-watt cool-white fluorescent tubes, for 0, 6, 12, 24, 48, 72, and 120 h and at 2 and 40°C. Individual Petri dishes were used for each storage time. Fluorescent light intensity (FLI) on the surface of the samples was measured by a LI-COR 185 B quantum flux meter with a LI-190 SB quantum sensor (LI-COR Inc., Lincoln, NE).

Standards. Chlorophyll A (CHL A) chlorophyll B (CHL B), α-T, β-tocopherol (β-T), and β-C standards were purchased from Sigma Chemical Company (St. Louis, MO). PHE A and pheophytin B (PHE B) were prepared from the corresponding chlorophylls by the method of Schwartz and Von Elbe (11). In this method, ether solutions of CHL A and CHL B were acidified with 13% HCl. The acid was removed by washing the ether layer twice with an equal volume of 5% Na_2SO_4 and evaporated under a stream of nitrogen gas. The

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pheophytin/ether mixture was dried over anhydrous Na_2SO_4 and evaporated under nitrogen. The prepared pheophytins were analyzed by high-performance liquid chromatography (HPLC) according to the method of Rahmani and Saari Csallany (12). Their purity was further confirmed by comparing their visible spectra and molar extinction coefficients to those of pure pheophytins reported in the literature (13,14).

Quantitation of pigments. Chlorophylls A and B and pheophytins A and B, and β-C were determined in the oil according to the HPLC method developed by Rahmani and Saari Csallany (12).

Oxidation index. Peroxide values were determined by a modified Wheeler method (15). Specific extinction values ($E^{1\%}_{1\,cm}$ were measured at 232 nm on 1% solution (wt/vol) of VOO in isooctane (16). The thiobarbituric acid (TBA) test was carried out according to the method of Jacobson *et al.* (17) at a moderate temperature (60°C), with no added acid so as to minimize breakdown of hydroperoxides. Percentage of acidity, expressed in terms of oleic acid, was determined in the oil samples according to the American Oil Chemists' Society method Ca 5a-40 (18). Composition of fatty acids was determined according to the method described by Vigneron *et al.* (19). Total phenols and polyphenols were determined by the method of Vazquez *et al.* (20) and were expressed as ppm tannic acid. The tocopherols were determined by the HPLC method according to Carpenter (21).

Statistical analysis. Two-factor analysis of variance was carried out to estimate the effects of FLI and time on the levels of peroxide and TBA-reactive substances (TBARS) formed in VOO during the photooxidation experiments.

A linear squares regression analysis was used to find correlation coefficients between peroxide values and relative concentrations of the remaining PHE A and β-C in VOO photooxidized at 2 and 40°C.

RESULTS AND DISCUSSION

The initial peroxide value, conjugated dienes as measured by extinction value at 232 nm, and acidity of the VOO indicated that oxidation had already started in the oil (Table 1). However, these values are within the limits of the International Olive Oil Council established for extra-type olive oil quality (22). Fatty acid composition of the VOO was within the range of values reported by Fedeli (23). Levels of total polyphenols, tocopherols, PHE A, and β-C (Table 1) were within reported ranges of these VOO constituents (23,24). PHE A was the only chlorophyll pigment detected in the VOO sample.

The effects of photooxidation from FLI on peroxide formation at 2 and 40°C in VOO are shown in Figure 1. At 2 and 40°C, photooxidation was similar at FLI 0, 620, and 2710 lux, and there appears to be no induction period at both temperatures. At both temperatures the analysis of variance revealed that both FLI and the time of irradiation had a significant effect ($\alpha < 0.001$) on VOO oxidation. At FLI 5340 lux irradiation after 6-h exposure, photooxidation of VOO proceeded with a significantly greater ($\alpha < 0.001$) rate at 40 than at 2°C. The extent of photooxidation of VOO depended on three factors: FLI, time, and temperature.

Increased peroxide formation due to higher FLI is consistent with the findings of Endo *et al.* (6) in methyl linoleate model system. The quantity of hydroperoxides formed during the photooxidation of unsaturated fatty acid methyl esters, in model systems, is directly proportional to the total amount of light absorbed (25).

The evolution of TBARS, the secondary oxidation products of lipid oxidation, in VOO photooxidized at 2 and 40°C in the presence of various FLI values is presented in Figure 2. Light-exposed VOO showed significantly higher TBARS concentrations ($\alpha < 0.001$) than the unexposed samples, both at 2 and 40°C. However, at 40°C, no statistical difference was found in the formation of TBARS at 0 and 620 lux ($\alpha = 0.01$), indicating a temperature rather than a FLI effect. Higher FLI and/or temperature caused greater TBARS formations during the early stages of photooxidation. Under these circumstances, the measurement of TBARS could be used as an indicator for the peroxidation of VOO. After 24 h at higher FLI exposure at 40°C, the TBARS concentration started to level off. This may be attributed to the decomposition of the secondary oxidation products that react with the TBA reagent to form TBARS. Thermal lability of TBARS at 80°C has previously been reported in the literature (26).

Effect of minor constituents. As expected, PHE A and β-C in VOO were light- and temperature-sensitive (Tables 1 and 2, Figs. 3, 4). In this study, linear correlations of –0.942 and –0.934 were obtained between peroxide values and the relative concentrations of the remaining β-C in VOO photooxidized at 2°C at 2710 and 5340 lux, respectively. Coefficient *A* represents the disappearance of β-carotene (Table 3). This indicates that β-C serves as a strong natural inhibitor of pho-

TABLE 1
Characteristics of Virgin Olive Oil

Quality indices		Fatty acids	%	Pigments	μg/g	Antioxidants	μg/g
Peroxide value	14.8	16:0	9.3	Pheophytin A	8.54	Total polyphenols[a]	49.7
$E^{1\%}_{1\,cm}$, 232 nm	1.0	16:1	1.3	Pheophytin B	0.00	α-Tocopherol	129.4
Acidity	1.0	18:0	2.3	β-Carotene	0.54	β-Tocopherol	Trace
(%, oleic acid)		18:1	76.5				
TBARS/mg oil[b]	0.1	18:3	1.4				

[a]Expressed as tannic acid.
[b]Expressed as malondialdehyde equivalents. TBARS, thiobarbituric acid-reactive substances.

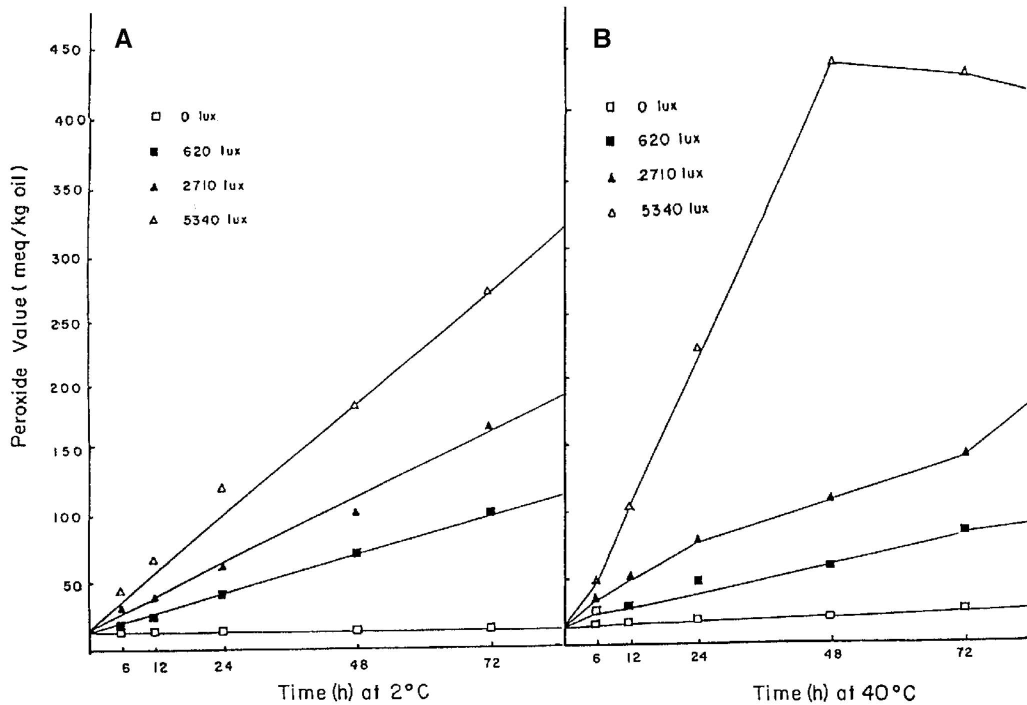

FIG. 1. Peroxide values of virgin olive oil exposed to various fluorescent light intensities at 2 (A) and 40°C (B). Variations between duplicate determinations were less than 3.0 meq/kg.

tooxidation at 2°C, but not as strong at 40°C because of its rapid destruction at that temperature (Table 2).

Changes in α-T and polyphenol concentrations were also observed during the various light exposure periods. At 5340 lux FLI, about 50% of the initial α-T concentration disappeared after the first 6 h of irradiation at both 2 and 40°C (Fig. 5). Because the decrease of the concentration of α-T was about the same at 2 and 40°C at the same FLI, its destruction was due to light and not to temperature.

The poor inhibitory effect of α-T on photosensitized oxi-

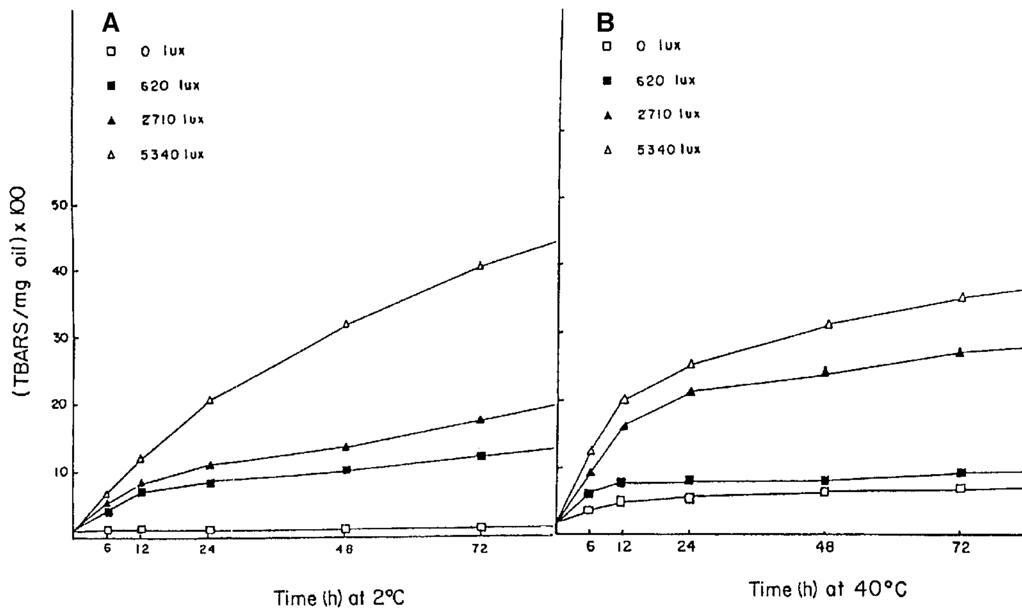

FIG. 2. Total thiobarbituric acid-reactive substances (TBARS) in virgin olive oil exposed to various fluorescent light intensities at 2 (A) and 40°C (B). Variations between duplicate determinations were less than 0.002 total TBARS/mg oil.

TABLE 2
Parameters and Correlation Coefficients (*r*) of the Exponential Equations[a] for Pheophytin A and β-Carotene Degradation Curves at 2 and 40°C

Fluorescent light intensity at surface (lux)	2°C *a*	2°C *b*	2°C *r*	Time (h)	40°C *a*	40°C *b*	40°C *r*	Time (h)
Pheophytin A								
0	0.004	4.561	0.979	(0–120)	−0.002	4.592	0.990	(0–120)
2710	−0.023	4.565	0.998	(0–120)	−0.005	4.475	0.934	(0–120)
5340	−0.031	4.394	0.968	(0–48)	−0.062	4.525	0.998	(0–48)
β-Carotene								
0	0.005	4.611	0.913	(0–120)	−0.004	4.579	0.984	(0–120)
2710	−0.040	4.637	0.995	(0–72)	−0.034	4.555	0.992	(0–72)
5340	−0.045	4.501	0.987	(0–48)	−0.096	4.652	0.998	(0–24)

[a]Equation $y = e^{(ax+b)}$, where y = percent remaining pigment; x = time (h).

dation can be explained by its high reactivity toward singlet oxygen. According to Stevens *et al.*, the rate constant for the singlet oxygen reaction with α-T is 10 to 100 times less than the rate constant for deactivation (27). However, part of the destruction of α-T may be due to its free-radical scavenging properties in the above systems.

In VOO samples exposed to identical FLI, the rates of destruction of total polyphenols at 2 and 40°C were also similar (Fig. 6). It can therefore be concluded that light accounts for the major part of the destruction of these compounds.

Based on these results, PHE A promoted rapid photooxidation of VOO that resulted in destruction of β-C, α-T, and polyphenols. This photosensitizing effect of PHE A is consistent with results previously reported by other investigators (1–3,5). Gutierrez-Rosales and coworkers were the only investigators who did not find an increase of photooxidation due to added chlorophylls in VOO (28). They attributed the lack of increased photosensitized oxidation to the rapid disappearance of chlorophylls because of their photolability. The amounts of β-C, tocopherols, chlorophylls, and polyphenols in VOO largely depend on the cultivar, the degree of maturity of the olives, and the method used for oil extraction (29,30). Therefore, careful selection of the ripening stage of the olive, removal of leaves, which contain chlorophylls, prior to pro-

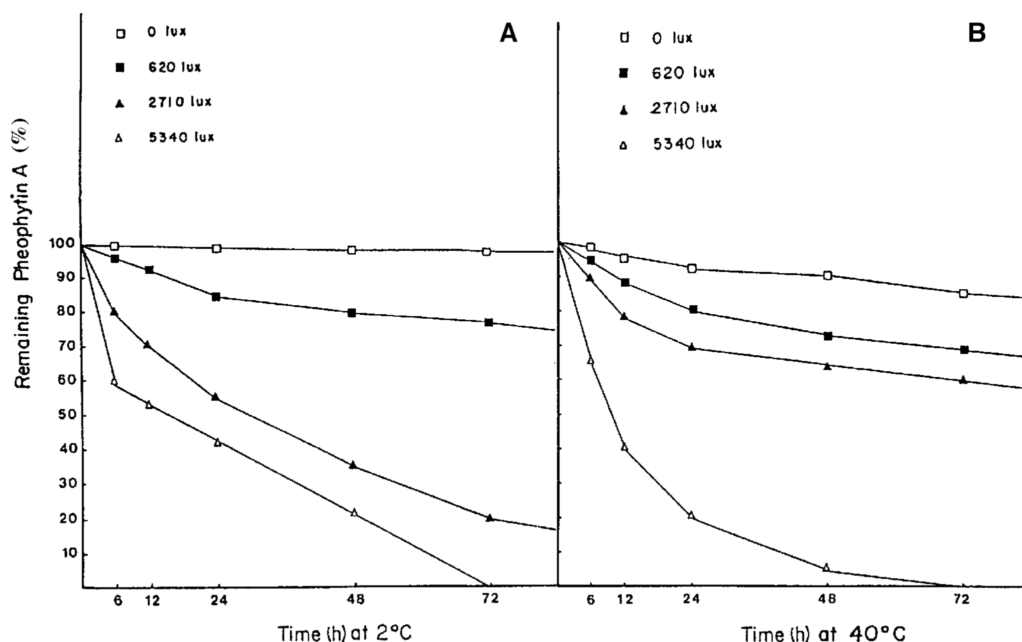

FIG. 3. Remaining pheophytin A in virgin olive oil exposed to various fluorescent light intensities at 2°C (A) and 40°C (B). Variations between duplicate determinations were less than 0.1 μg pheophytin A/g oil.

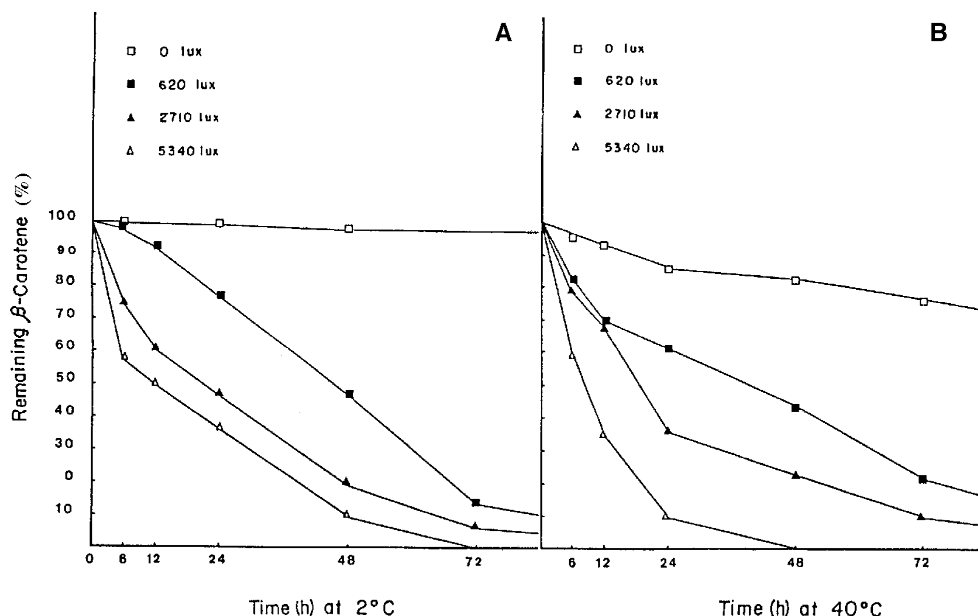

FIG. 4. Remaining β-carotene in virgin olive oil exposed to various fluorescent light intensities at 2 (A) and 40°C (B). Variations between duplicate determinations were less than 0.1 μg β-carotene/g oil.

TABLE 3
Regression Equation and Coefficients of Correlation Between Peroxide Values and Remaining Percent of β-Carotene (*C*) in Virgin Olive Oil Photooxidized at 2°C

Fluorescent light intensity at surface (lux)	Regression equation	Correlation coefficient (r)
0	PV = 38.620 – 0.237[a] (C)	–0.961
620	PV = 115.419 – 0.992[a] (C)	–0.999
2710	PV = 148.586 – 1.516[a] (C)	–0.942
5340	PV = 182.583 – 1.900[a] (C)	–0.934

Peroxide value = PV (meq/kg); PV = $b - a$ (x); x = (*C*) remaining percent β-carotene.
[a]Coefficient A.

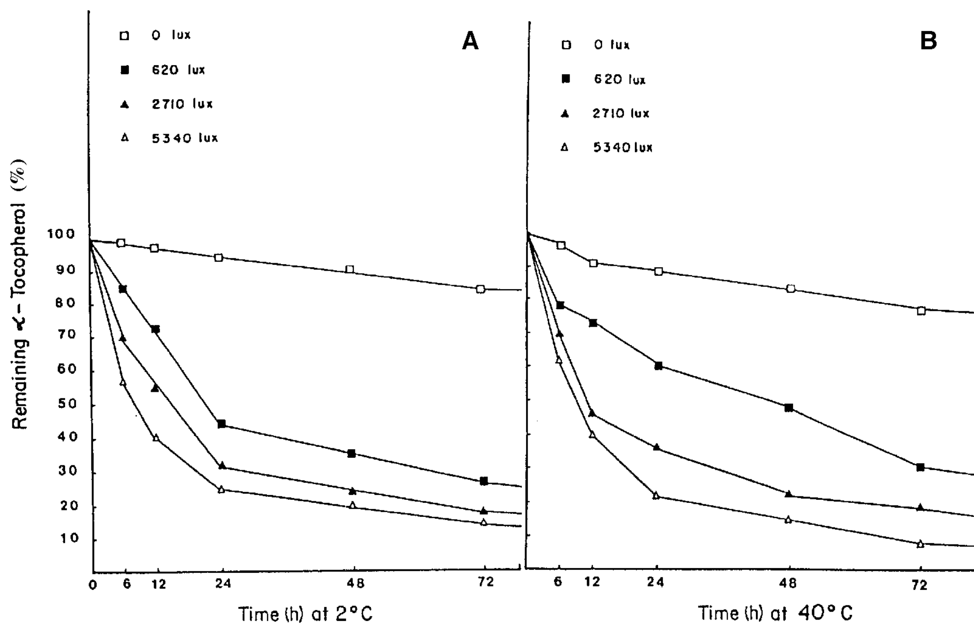

FIG. 5. Remaining α-tocopherol in virgin olive oil exposed to various fluorescent light intensities at 2 (A) and 40°C (B). Variations between duplicate determinations were less than 1.2 μg α-tocopherol/g oil.

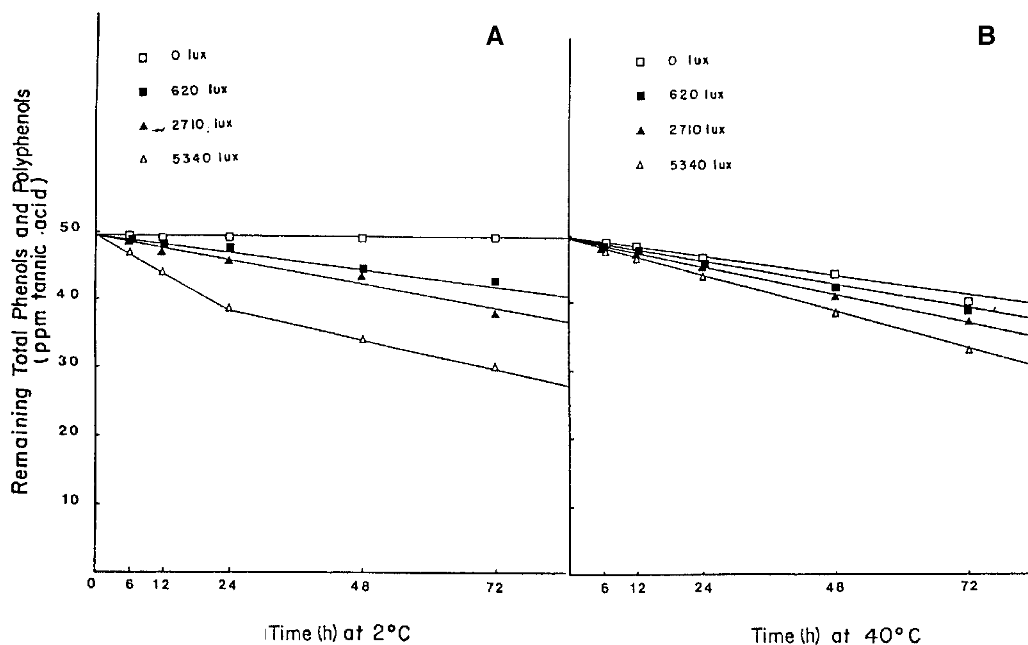

FIG. 6. Remaining total phenols and polyphenols, expressed in tannic acid equivalents, in virgin olive oil exposed to fluorescent light intensities at 2°C (A) and 40°C (B). Variations between duplicate determinations were less than 1.4 μg total phenols and polyphenols/g oil.

cessing, and use of the least amount of water during oil extraction are recommended to ensure the maximum protection of oil from photooxidation.

ACKNOWLEDGMENT

This study was published as paper No. 22,158 of the contribution series of the Minnesota Agricultural Experiment Station on research conducted under Project No. 18-084, supported by Hatch funds, and by the U.S. Agency for International Development, Contract No. 608-0160, IAV, Morocco Project, and a grant (E/1010-2) from the International Foundation for Science.

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[Received October 27, 1997; accepted February 2, 1998)