A Comparative Study on the Susceptibilities of Soybean, Sunflower and Peanut Oils to Singlet Molecular Oxygen Photooxidation

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The susceptibilities of crude soybean, sunflower and peanut oils to singlet oxygen photooxidation were determined in a kinetic study. The accumulation of photosensitized hydroperoxides, determined spectroscopically, and the quenching of singlet molecular oxygen phosphorescence by the crude oils and their fatty acid methyl esters were compared.

The relative tendency to photooxidation for the oils and the methyl esters was soybean >> sunflower > peanut. This trend was independent of the method employed in the determination of initial photodamaging.

Soybean oil was demonstrated to be the most unstable product, not only due to the presence of highly unsaturated fatty acids, but also due to the absence of natural constituents, capable of providing a protective antioxidant effect. This protection was more effective in sunflower and peanut oils.

KEY WORDS: Peanut oil, photodecomposition, photooxidation, singlet oxygen, soybean oil, sunflower oil.

A major pathway for the production of flavor components from edible oils is through the oxidation of the unsaturated fatty acids (1,2). This deterioration, generally called rancidity, is of great economic concern to the food industry and has received considerable attention.

Autoxidation, that is, the reaction with ground-state molecular oxygen, is the main process involved in the oxidative deterioration of fats. In general, exposure to daylight is known to cause a marked acceleration in the oxidative deterioration of oils. For several years (1,2), the role of photosensitized oxidations, singlet molecular oxygen [O_2 ($^1\Delta_g$)] interactions and their connection with autoxidation has been understood. The role of O_2 ($^1\Delta_g$) in the oxidation of lipid species has been the subject of considerable research in recent years, considering the involvement of cell constituents and other biological materials, in order to elucidate the problem of the so-called photodynamic effect (3,4).

Oxidative deterioration of edible oils, when initiated by an O_2 ($^1\Delta_g$) mechanism (5–8), is sensitized by chromophoric components in the oils, such as residual natural dyes and pigments. At the same time, even when the effect of light cannot be eliminated, some degree of inhibition of photooxidative deterioration is observed due to the presence of natural constituents in the crude oils, which can quench the oxidative process (5). This effect is totally absent in the fatty acid methyl esters (FAME) prepared from the oils. The inhibition is variable in quality and quantity, and depends on the O_2 ($^1\Delta_g$) quenching ability of the given constituent.

In this paper we have investigated, through a comparative study, the relative susceptibilities to O_2 ($^1\Delta_g$) photooxidation of soybean, sunflower and peanut oils and their FAME, as well as the degree of protection against photoinduced deterioration provided by the constituents present in their crude products.

EXPERIMENTAL PROCEDURES

Materials. Soybean, sunflower and peanut oils were obtained in our laboratory by Soxhlet extraction with hexane.

Fatty acid methyl esters were prepared from extracted lipids by addition of 10% BF_3/MeOH (Fluka, Ronkonkoma, NY) according to the AOAC (8) method.

The sensitizers rose bengal (RB) and zinc tetraphenyl porphyrin (ZnTPP) (Aldrich Chemical Co., Milwaukee, WI) were used as received. All solvents employed were high-performance liquid chromatography (HPLC) quality.

Methods. Gas chromatographic analysis of the FAME composition was carried out with a Shimadzu GC-6A instrument (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID), on a stainless steel column 3.0 m × 1.5 mm, packed with 10% EGA (Merck, Darmstadt, Germany) on 60–80 mesh Chromosorb WAW (Merck). The carrier gas was N_2 at a flow rate of 30 mL/min. The column was operated isothermally at 190°C, and the injector and detector temperatures were 220°C.

Relative percentage of the FAME determined by gas-liquid chromatography (GLC), considering an average molecular weight of the fatty acids, was as follows: i) soybean oil: linoleic acid 60.3%, oleic acid 17%, linolenic acid 11.2%, palmitic acid 8.7% and stearic acid 2.6%; ii) sunflower oil: linoleic acid 64.2%, oleic acid 27.7%, palmitic acid 4.2% and stearic acid 3.7%; and iii) peanut oil: linoleic acid 40.7%, oleic acid 43.5%, palmitic acid 5.9%, stearic acid 1.1% and others 8%.

In the photooxidation experiments, 3 mL of solutions of oil or FAME (0.1% by volume) were exposed, with the sensitizer (RB Abs. at 560 nm = 0.7) in air-saturated conditions, to light in 1-cm absorption cells in the previously described (9) apparatus. Light was cut off in order to transmit only above 400 nm. Hydroperoxide build-up was monitored at 234 nm in a Hewlett-Packard 8452A (Hewlett-Packard, Palo Alto, CA) or a Shimadzu UV-140-02 apparatus.

The quenching of O_2 ($^1\Delta_g$) by the oils and their FAME was carried out by means of the time-resolved O_2 ($^1\Delta_g$) phosphorescence method. This method has been described elsewhere, together with the sensitizing conditions (10). Briefly, it consisted of a N_2 laser as an excitation source, gated at 50 Hz (FWHM 3.5 ns) at 337 nm. The 1-cm^2 fluorescence cuvette, containing the air-equilibrated sensitizer solution, was placed into a block, which also includes the germanium detector (Judson J16, Judson Infrared Inc., Montgomery, PA). The amplified phosphorescence signal was fed into a Hewlett-Packard digital oscilloscope, and interfaced to an IBM microcomputer that was employed to monitor the signal. Although the S/N ratio was sufficiently good, 16 signals were typically averaged to calculate the decay times. In the absence of quencher, the lifetime of O_2 ($^1\Delta_g$), employing ZnTPP as a

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sensitizer (Abs. at 337 nm = 0.8) in methanol/chloroform (4:1), was 18 microseconds. Increasing volumes of sensitizer-substrate solution were added to the initial volume (1.5 mL) of sensitizer solution contained in the fluorescence cell, and the O_2 ($^1\Delta_g$) phosphorescence lifetimes were determined.

RESULTS

It is well known (11,12) that long exposure to irradiation of either FAME or the crude oils (soybean, sunflower or peanut) in the presence of a sensitizer produces oxidation of the fatty components. This can be seen in the chromatograms shown in Figure 1 for sunflower oil, before and after 10 hr of irradiation at wavelengths higher than 400 nm with RB as a sensitizer. Similar changes were obtained with soybean and peanut FAME.

Much evidence exists (11,12) that during prolonged irradiation, both singlet molecular oxygen and radical mechanisms operate simultaneously in the photooxidative process. Hydroperoxides, the primary initial products of lipid oxidation enter into numerous and complex breakdown and interaction mechanisms. It is practically impossible to evaluate the relative importance of the mechanisms involved in the photodamage under conditions in which peroxidation of initial products takes place.

Because our main interest was to investigate the susceptibility of the different oils to the photooxidative process, the early stages of photooxidation were analyzed. These initial contributions constitute a measure of the potential tendency of the different substrates to undergo photosensitized oxidation, and were evaluated as indicated in the following sections.

Singlet molecular oxygen lifetime and substrate quenching studies. Singlet molecular oxygen decay mechanisms can be studied independently by means of the time-resolved quenching of O_2 ($^1\Delta_g$) phosphorescence. The sensitized photooxidation steps can be summarized as follows:

$$\text{Sensitizer} \xrightarrow{h\nu} \text{Sensitizer}^* \quad [1]$$

$$\text{Sensitizer}^* + {}^3O_2 \rightarrow \text{Sensitizer} + O_2\,(^1\Delta_g) \quad [2]$$

$$O_2\,(^1\Delta_g) \xrightarrow{k_d} {}^3O_2 \quad [3]$$

$$O_2\,(^1\Delta_g) + Q \xrightarrow{k_q} \text{oxidized products} \quad [4]$$

The electronically excited sensitizer, produced upon light absorption [1], transfers energy to ground-state oxygen [2] and O_2 ($^1\Delta_g$) is produced. In our case, the O_2 ($^1\Delta_g$) luminescence generated by low concentrations of ZnTPP has a radiative lifetime ($\tau^\circ = 1/k_d$) of 18 microseconds, in agreement with the expected values for the solvent mixture methanol/chloroform (4:1) (13). Addition of increasing concentrations of crude oils or FAME, (Q), diminished the lifetime of O_2 ($^1\Delta_g$) [$Z = 1/k_d + k_q$ (Q)]. Experimental data were treated through the Stern-Volmer equation:

$$\tau^\circ/\tau = 1 + k_q\tau^\circ(Q) \quad [5]$$

from which the rate constant, k_q can be obtained graphically. In Figure 2, typical traces of O_2 ($^1\Delta_g$) luminescence decay, in the absence and presence of a given

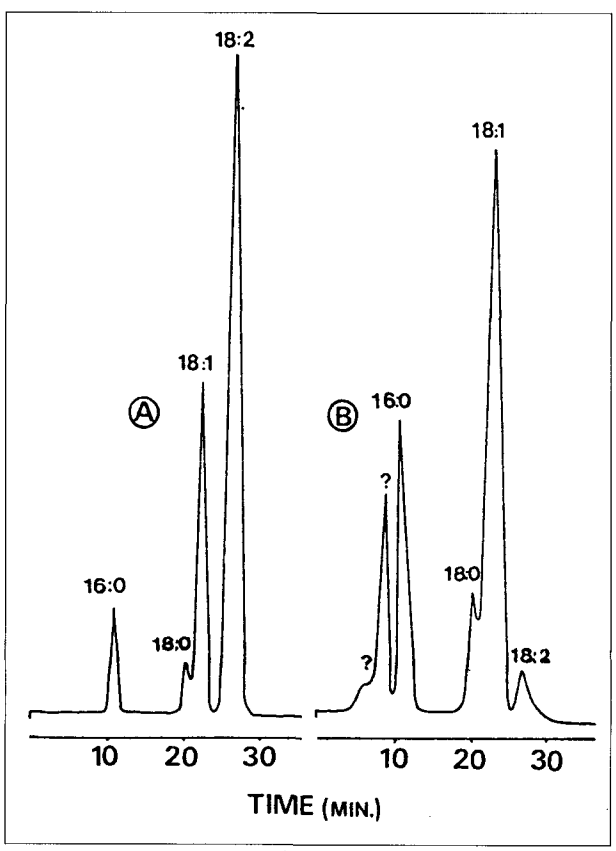

FIG. 1. Gas chromatograms of methyl esters from sunflower oil fatty acids, before (A) and after (B) irradiation in the presence of RB; $Abs_{560\ nm}$ = 0.7. Abbreviations for the FAME: 16:0 palmitic; 18:0 stearic; 18:1 oleic; 18:2 linoleic.

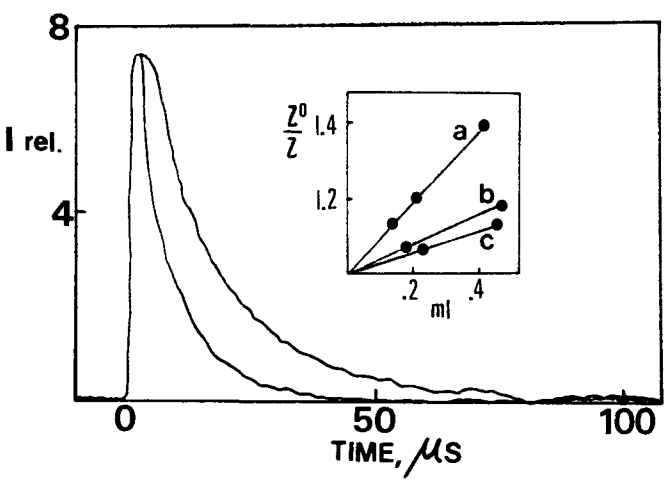

FIG. 2. Time-resolved emission of O_2 ($^1\Delta_g$) in aerated MeOH/chloroform (4:1) solutions. Sensitizer ZnTPP; $Abs_{337\ nm}$ = 0.3. Upper trace: without quencher. Lower trace in the presence of soybean FAME at approximately 0.12 M. Inset: Stern-Volmer plot for the O_2 ($^1\Delta_g$) phosphorescence quenching by FAME of a, soybean oil; b, sunflower oil; c, peanut oil.

concentration of soybean FAME, permitted calculation of the respective values of singlet molecular oxygen lifetime. The Stern-Volmer plots for O_2 ($^1\Delta_g$) quenching soybean, sunflower and peanut FAME are shown in the inset of Figure 2.

The values of (Q) in Equation [5] were replaced by total volume of quencher added to the fluorescence cell (see Experimental Procedures), and it was converted to approximate molar concentrations with FAME. Due to the impossibility of adequately defining the molar composition of the substrates, especially the oils, the O_2 ($^1\Delta_g$) quenching ability of the oils and FAME was represented by the slopes of the respective Stern-Volmer plots. Their relative values are shown in Table 1. The rate constants for O_2 ($^1\Delta_g$) quenching, k_q, for the FAME are presented in Table 1. They are in agreement with mean values for individual fatty acids and esters reported in the literature (13,14). These comparative data represent a good test for the accuracy of the experiments.

Photosensitized hydroperoxide generation. It is well established (11) that a direct relationship can be obtained between the increase of absorbance at 234 nm (due to the appearance of hydroperoxides of the fatty acids) and the degree of oxidation in the early stages of dye-sensitized oxygenation of unsaturated oils (and FAME). It is important to emphasize that dye-sensitized photooxidation, monitored by this method, is interpreted (15) in terms of a dual mechanism involving both singlet oxygen and radical attack on the double bonds of the oxidizable substrates. The relative distribution varies with the nature of the sensitizers employed. In addition, the decomposition of the sensitizer may also affect that balance. In the present study, the same photosensitizer was employed for all the substrates; it can be assumed that a unique pattern of products distribution operates.

Results for hydroperoxide build-up for the FAME are shown in Figure 3. Similar qualitative behavior was found for the crude oils. The rates of the photooxidative process were expressed as the initial slopes of the curves, and have been collected in Table 1.

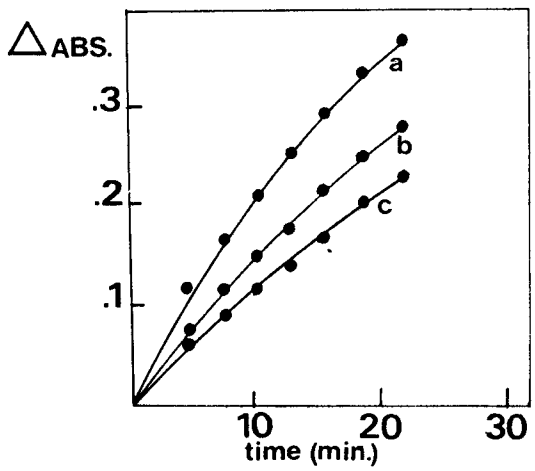

FIG. 3. Photooxidation of FAME 2 mM (a, soybean; b, sunflower; and c, peanut oils) as a function of irradiation time. Sensitizer RB; $Abs_{560\ nm}$ = 0.5 in MeOH.

DISCUSSION

The retardation of photooxidation of unsaturated oils could, in principle, arise from one or more causes—singlet molecular oxygen quenching by impurities and/or antioxidant activity of chain-breaking compounds that trap peroxy radicals.

Comparison of the relative O_2 ($^1\Delta_g$) quenching ability (Table 1) between the three crude oils indicates, as expected, predominancy of soybean oil. The presence of more than 11% of highly unsaturated linolenic acid in that oil justifies its behavior. Sunflower and peanut oils behave similarly in their reactivity towards O_2 ($^1\Delta_g$). This is in agreement with their similar distributions of unsaturated fatty acids. In both oils the sum of linoleic and oleic acids amounts to approximately 90%.

The same type of analysis on the FAME shows a similar general trend. The soybean derivative is an even more effective O_2 ($^1\Delta_g$) quencher than the rest of the FAME, as compared with the values for the crude oils. The high degree of relative quenching effectiveness in the soybean FAME as compared with the respective crude oil should be attributed to the presence of efficient O_2 ($^1\Delta_g$) quenchers in the unsaponifiable fraction of peanut and sunflower oils. This fact may be interpreted as a degree of protection in sunflower and peanut oils against photo-promoted, O_2 ($^1\Delta_g$)-mediated degradation. Tocopherols, cholesterol and carotenoids [especially the first two, based on their rate constants of O_2 ($^1\Delta_g$) quenching (14) and their relative concentrations] can be responsible for the observed effect.

It should be pointed out that the impurities present in a crude oil could contribute a combined effect of singlet molecular oxygen generation (sensitization) and quenching. This is known of chlorophyll (1), which is able to generate O_2 ($^1\Delta_g$) with a quantum yield of 0.6 (16), and simultaneously it is an efficient O_2 ($^1\Delta_g$) quencher [k_q = 7.3×10^8 M^{-1} s^{-1} (16)].

The relative rates of hydroperoxide build-up in the oils (Table 1) indicate a prevailing photooxidability in soybean and sunflower oils, which is even higher for soybean when the FAME are considered.

Regarding the data in Table 2, the ratio of the Stern-Volmer slopes between the oils and the FAME can be interpreted as the relative degree of protection of the oils

TABLE 1

Relative Values for the Quenching of [O_2 ($^1\Delta_g$)] Phosphorescence Emission by Soybean, Sunflower and Peanut Oils and their FAME in Methanol/Chloroform (4:1)[a]

Quencher	Relative quenching	Relative rates	kt × 10^5
(I) Soybean oil	1	1	
(II) Sunflower oil	0.65	1	
(III) Peanut oil	0.62	0.75	
(I) FAME	1	1	3.6
(II) FAME	0.4	0.7	0.9
(III) FAME	0.35	0.55	0.8

[a]Relative rates of photooxidation of the oils and their FAME in diethyl ether/methanol (1:1) and methanol, respectively, and approximate rate constants kt (M^{-1} s^{-1}) for [O_2 ($^1\Delta_g$)] quenching in methanol/chloroform (4:1) for the FAME.

TABLE 2

Ratios of the Slopes of Stern-Volmer Plots and Hydroperoxide Build-Up Between Soybean, Sunflower and Peanut Oils and their Respective FAME

Substrate	Slope oil / Slope FAME[a]	Slope oil / Slope FAME[b]
Soybean	1.2	1.8
Sunflower	2	2.5
Peanut	2.2	2.1

[a]Stern-Volmer plots.

[b]Hydroperoxide build-up.

against pure singlet molecular oxygen photooxidation. A similar consideration is valid for the ratios of slopes of hydroperoxide build-up, as a protection against a combined O_2 ($^1\Delta_g$)-radical mechanism. All these considerations are only valid if the assumption of invariance holds in the fatty acid composition upon esterification of the oils.

From the comparison of the values for each substrate in both columns of Table 2, two aspects should be emphasized: i) the similarity for each substrate between O_2 ($^1\Delta_g$) and hydroperoxides data; and ii) the identical trend for increase of protection by impurities. These facts can be explained by taking into account a majority contribution of the singlet molecular oxygen mechanism [between 55 and 80%, depending on the sensitizer (15)] in the photosensitized production of hydroperoxides. Again, sunflower and peanut oils appear as the more effectively protected oils due to the presence of natural impurities.

Finally, an important point should be re-emphasized—the enhanced photooxidability of soybean oil, which exhibits a particular stability problem, is due not only to its pronounced unsaturation but also to the lack of protection of the oil as compared with peanut and sunflower oils, according to the data in Table 2.

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