

Kinetics and Mechanism of the Light-Induced Deterioration of Lemon Oil

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The kinetics and mechanisms involved in the light-induced photodegradation of lemon oil (LO) have been investigated using singlet molecular oxygen [$O_2(^1\Delta_g)$] time-resolved phosphorescence detection and steady-state irradiation, in organic and micellar aqueous solutions. A parallel study on the kinetics of limonene (L) photooxidation was carried out, for comparative purposes. An $O_2(^1\Delta_g)$ -mediated photooxidative process takes place in LO both in the absence and in the presence of external sensitizers. In the former case the pigments present in LO itself efficiently sensitize the generation of $O_2(^1\Delta_g)$ with a quantum yield of 0.14. L and other minor components of LO [especially the terpenes, efficient $O_2(^1\Delta_g)$ quenchers] are responsible for the photodegradation. Upon direct irradiation of LO in the wavelength range 300–350 nm an additional photochemical process operates: the decomposition proceeds from electronically excited states of LO susceptible components, which prevails over the competitive $O_2(^1\Delta_g)$ mechanism. The results should be carefully considered in a practical sense, when LO or LO-containing foods are stored under daylight conditions. Direct and sensitized light exposures cause irreversible transformations of LO.

The effect of light has long been known to play a role in the flavor stability of vegetable oils and different types of edible oil-containing products (Rawls and Van Santen, 1970; Nawar, 1985). Frequently the food pigments serve as the main promoters of photochemical reactions due to their light-absorbing capabilities. A recognized mechanism for the initiation of autoxidation in fatty acids is that which involves singlet molecular oxygen, $O_2(^1\Delta_g)$, formed through autosensitization reaction (Rawls and Van Santen, 1970; Palumbo et al., 1988; Neumann et al., 1991a,b). Besides, light exposure causes an increase in the rate of deterioration, not just of fatty acid-containing oils. A number of essential products, such as lemon oil (LO), experience a dramatic acceleration of damage in their flavor properties when stored in the presence of light (Schieberle and Grosch, 1988).

LO consists of more than 130 volatile compounds (Schieberle and Grosch, 1988; Van Straten and Maarse, 1983), of which limonene (L) (70% by weight) and several terpenes (T) (7% by weight) are the most abundant components. It has been demonstrated in several works that photochemically generated $O_2(^1\Delta_g)$, in solution, reacts with the endocyclic double bond of the terpenes (Schenck et al., 1964, 1965; Foote et al., 1965; Clark et al., 1981; Schieberle et al., 1987). As a result, several important off-flavor substances are formed.

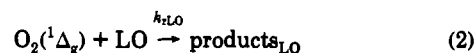
To model more closely the photodegradative process for LO under normal storage conditions (simultaneous presence of oxygen and visible light, in a fluid medium), information on the kinetics and mechanism governing the photodegradative event is needed.

In this paper we present a study on the direct and sensitized photooxidation of LO, with special emphasis on $O_2(^1\Delta_g)$ -mediated processes. The interaction between LO and photochemically produced $O_2(^1\Delta_g)$ and the efficiency of the photogeneration of the reactive intermediate by the oil itself were the points analyzed. A parallel study on the photooxidative properties of L was carried out for comparative purposes.

EXPERIMENTAL PROCEDURES

Materials. Cold-pressed LO (citrus lemon) was a gift from Prof. W. Grosch and Dr. P. Schieberle (origin: Sicily/Italy). It was stored in sealed flasks at -60°C and under nitrogen before use. (+)-Limonene (L), from Aldrich, was used as received. Solvents employed were of the highest quality available. Water was triply distilled. Sodium dodecyl sulfate (SDS), from Berna, was purified by recrystallization. Sodium azide (NaN_3), from Fluka, and Rose Bengal (RB) and anthracene, from Aldrich, were employed as received.

Methods. (a) *Determination of the Photooxidation Rate Constants, k_r .*



The $O_2(^1\Delta_g)$ time-resolved phosphorescence detection method (TRPD) was employed. Both apparatus and methodology have been described elsewhere (Neumann et al., 1991; Palumbo et al., 1991). In the present case, anthracene ($\text{Abs}_{337\text{nm}} = 0.2$) was employed as a sensitizer. The absorption at 337 nm by either LO or L in the experiments on $O_2(^1\Delta_g)$ quenching was negligible.

To prolong the $O_2(^1\Delta_g)$ lifetime into a measurable range (higher than 8–10 μs), chloroform-methanol (1:1 by volume) was employed as a solvent.

(b) *Determination of the Quantum Yield for $O_2(^1\Delta_g)$ Generation ($\phi_{\Delta LO}$) by LO.* The initial intensity of the $O_2(^1\Delta_g)$ phosphorescence signal (I_r) (Figure 1) was used as a relative measure of $O_2(^1\Delta_g)$ concentration provided that the light absorptions by the sensitizer, solvent, and apparatus geometry are kept constant. If a sensitizer of known ϕ_{Δ} is used as a reference (Ref), the absolute value of ϕ_{Δ} for a given sensitizer or combination of absorbing species can be calculated through eq 3 (Nonell i

$$I_{r\text{Ref}}/I_{rLO} = \phi_{\Delta\text{Ref}}/\phi_{\Delta LO} \quad (3)$$

Marrugat, 1989). Methanol-benzene (1:5 by volume) was the solvent, and ZnTPP, $\phi_{\Delta} = 0.73$, $\text{Abs}_{337\text{nm}} = 0.1$, was employed as a reference compound. The same absorbance was utilized for the solution of LO.

(c) *Nonsensitized Photolysis.* The sample (LO in methanol, $\text{Abs}_{314\text{nm}} = 1.5$) was photolyzed at 314 ± 2 nm in a 1-cm² quartz cuvette, under air-saturated conditions and continuous stirring.

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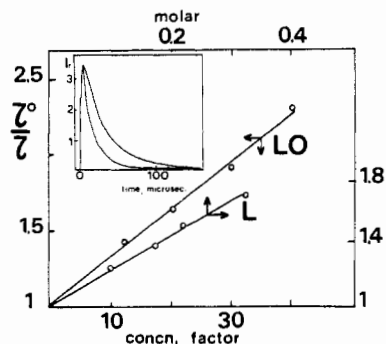


Figure 1. Stern-Volmer plot for L and LO $O_2(^1\Delta_g)$ luminescence quenching. (Inset) Decay curves for $O_2(^1\Delta_g)$ emission at 1270 nm, generated photochemically by anthracene, in chloroform-methanol (1:1); (upper curve) in the absence of any added quencher; (lower curve) in the presence of LO (concentration factor = 30; see text).

A PTI standard photolyzer was employed (double diffraction grating, 150-W xenon lamp). At different intervals the absorption spectrum was recorded, employing a Hewlett-Packard HP 8452 diode array spectrophotometer.

(d) *Sensitized Photolysis of L and LO in Micellar (SDS) Media.* In the presence of RB ($\phi_{\Delta} = 0.75$; Neckers, 1989), $Abs_{530nm} = 0.5$, the solutions of either LO or L were irradiated by employing the already described apparatus (Palumbo et al., 1988) (cutoff filter >400 nm), under constant stirring of the sample. The SDS concentration was 0.05 M. Difference spectra at several intervals of irradiation were recorded.

RESULTS

Due to the known presence in LO (Schieberle and Grosch, 1988) of different compounds able to quench photochemically generated $O_2(^1\Delta_g)$, experiments for the determination of the $O_2(^1\Delta_g)$ quenching efficiency of the oil as a whole were carried out. Simultaneously, the same was done, for comparative purposes, with pure L.

In Figure 1 (inset), the decay traces of $O_2(^1\Delta_g)$ photochemically generated by anthracene, in the absence and in the presence of LO, can be seen. From these curves, at different quencher concentrations [Q], the $O_2(^1\Delta_g)$ lifetimes were extracted. Through a simple Stern-Volmer treatment (eq 4) the k_r values could be calculated. The

$$\tau^0/\tau = 1 + k_r\tau^0[Q] \quad (4)$$

k_r values for L and LO can be associated with their respective abilities to quench photochemically generated $O_2(^1\Delta_g)$, i.e., their respective susceptibilities to $O_2(^1\Delta_g)$ -mediated photooxidation. τ^0 and τ are the $O_2(^1\Delta_g)$ lifetimes in the absence and in the presence of Q (either LO or L), respectively. τ^0 in chloroform-methanol (1:1) was 39 μ s, in agreement with expected values for the mentioned solvent composition (Rossbroich et al., 1985). In Figure 1, experimental data according to eq 4 for both quenchers are represented. In the case of LO, due to the impossibility to properly define its molar concentration, [Q] was replaced by an arbitrary concentration factor (expressed as microliters of pure LO in 2 mL of solution). If a 70% L content in the LO composition is considered (Schieberle and Grosch, 1988; Van Santen and Maarse, 1983) and assuming a density of LO = 0.85 g/mL, similar to that of L (Hodgman, 1963), a value of $1.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for the rate constant of $O_2(^1\Delta_g)$ quenching by LO (k_{rLO}) can be calculated. It resulted in a 3-fold higher value than the k_r value obtained (through data on Figure 1) for L ($k_{rL} = 5.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$), the latter being in excellent agreement with that reported by Gollnick (1962) and Koch (1968) for the same rate constant in MeOH.

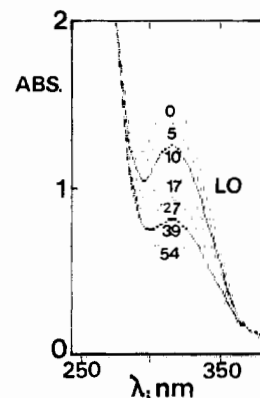


Figure 2. Absorption spectra of photolyzed LO in MeOH, irradiated at 314 nm. Numbers correspond to irradiation time in minutes.

Concentrated solutions of LO are colored (yellowish). This property prompted us to determine whether light absorption by the oil leads to $O_2(^1\Delta_g)$, which then interacts with reactive oil constituents. A realistic measure of the efficiency of $O_2(^1\Delta_g)$ generation by a given compound is represented by its ϕ_{Δ} value. For the determination of the absolute ϕ_{Δ} value for the visible light-absorbing species in cold-pressed lemon oil, the initial $O_2(^1\Delta_g)$ emission intensities of reference and LO were compared, under identical experimental conditions (see Experimental Procedures).

The $\phi_{\Delta LO}$ gave 0.14 as an average value of four determinations (dispersion lower than 10%).

To investigate the effect of direct and indirect (sensitized) light irradiation on LO stability, experiments in the absence and in the presence of an external added sensitizer were carried out.

Figure 2 shows the spectral changes produced in the absorption spectrum of a methanolic solution of LO upon direct irradiation at 314 nm, under air-saturated conditions. The spectral behavior was exactly the same when run in the presence of NaN_3 ($2 \times 10^{-2} \text{ M}$). At this concentration the azide is able to produce a considerable quenching of $O_2(^1\Delta_g)$ (Usui et al., 1978). This result clearly demonstrates that decomposition of LO upon irradiation in the band of 300–350 nm mainly proceeds by a mechanism different from $O_2(^1\Delta_g)$ -mediated photooxidation.

Sensitized photolysis of LO solutions was carried out by irradiation at wavelengths higher than 400 nm, employing RB as a sensitizer. The experiments were made in SDS micellar solutions to check the behavior of LO in an aqueous environment.

In Figure 3 the spectral changes upon sensitized irradiation of LO micellar solutions can be seen. Substrate consumption is clearly observed in the typical region of terpenic absorption, while the band at 314 nm remained unaltered. The same was true when LO concentrations 5-fold higher ($Abs_{314nm} = 0.4$) were employed.

Similar experiments were carried out for L. In Figure 4 the respective rates of L ($rate_L$) and LO ($rate_{LO}$) photooxidation are represented. They were obtained under identical experimental conditions (light dose, geometry, sensitizer, surfactant and substrates concentrations, the latter expressed as L content). The ratio $rate_{LO}/rate_L = 3$ shows a remarkable similarity with the ratio between the respective rate constants for $O_2(^1\Delta_g)$ quenching (being expressed as k_{rLO}) as a function of L content).

DISCUSSION

On the basis of the reported work three important points should be considered.

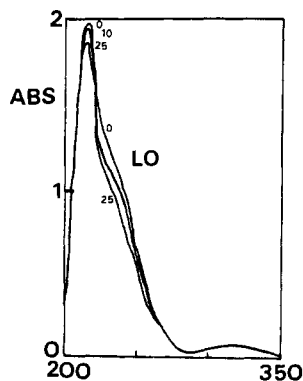


Figure 3. Absorption spectra of photolyzed LO, in SDS (0.05 M) micellar solution, irradiated at wavelengths higher than 400 nm, in the presence of RB, $Abs_{630nm} = 0.5$. Numbers correspond to irradiation time in minutes.

(i) LO Degradation by Photoexcitation of Susceptible Constituents (X).

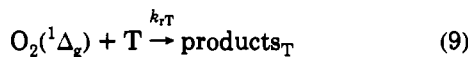


Under direct irradiation a complex scheme of competitive reactions takes place. An $O_2(^1\Delta_g)$ mechanism should operate (to be discussed latter) due to the significantly high ϕ_Δ value determined for LO and the already mentioned recognized reactivity of LO components toward singlet oxygen. On the other hand, this mechanism should contribute in a secondary fashion to LO decomposition: the dramatic spectral changes upon LO irradiation are insensitive to the addition of specific $O_2(^1\Delta_g)$ quenchers. As a consequence, the main contribution comes from decomposition of susceptible LO constituents from their electronically excited states (X^*).

(ii) LO Degradation through Interactions with $O_2(^1\Delta_g)$ Generated by Photoexcitation of Specific Added (RB) Sensitizers.



$$(k_{rL} = 5.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1})$$



$$(k_{rT} \sim 1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1})$$

Regarding the pure $O_2(^1\Delta_g)$ interactions, as measured by the TRPD method, results clearly show the importance of other efficient $O_2(^1\Delta_g)$ quenchers different from L. Concentration levels of certain terpenes are in the range 6–7% by weight (see introduction) (Schieberle and Grosch, 1988; Van Santen and Maarse, 1983). It is known that these LO components are relatively good $O_2(^1\Delta_g)$ quenchers, with reported rate constants (k_{rT}) for process 9 in the range $10^7 \text{ M}^{-1} \text{ s}^{-1}$ (Koch, 1968). On this basis it is easy to understand why the value of k_{rLO} is significantly higher than that calculated for L [the former on the assumption that L is the unique $O_2(^1\Delta_g)$ quencher present

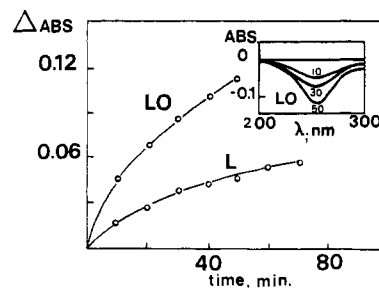


Figure 4. Rates of L and LO decomposition upon sensitized irradiation (wavelength higher than 400 nm) in the presence of RB ($Abs_{630nm} = 0.5$ and SDS 0.05 M) monitored by the decrease in absorbance at 250 nm as shown in the difference spectra (inset). Numbers correspond to irradiation time in minutes.

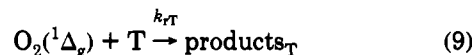
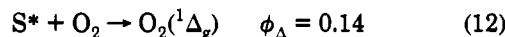
in LO]. The same was true for the ratio of rates of L and LO degradation in SDS when RB was employed as a sensitizer.

Furthermore, given that the effective rate constant for $O_2(^1\Delta_g)$ quenching by LO is a composite of several terms involving the product of quenching constants and molar concentrations of a particular quencher (eq 10) and being

$$k_{rLO}[LO] = k_{rL}[L] + k_{rT}[T] \quad (10)$$

70 and 7% by weight the concentrations of L and T in pure LO, respectively, it is possible to estimate the " $O_2(^1\Delta_g)$ quenching constant" of pure LO in units of s^{-1} as 4×10^6 (given the impossibility to properly define "LO concentration"). This result indicates that T, representing only a minor fraction of LO, is mainly responsible for the $O_2(^1\Delta_g)$ quenching efficiency of the oil.

(iii) LO Degradation through Interaction with $O_2(^1\Delta_g)$ Generated by Photoexcitation of Unspecified LO Constituents (S).



This work demonstrated the relatively high efficiency of $O_2(^1\Delta_g)$ by the oil itself. However, the $O_2(^1\Delta_g)$ interaction operates mainly in the presence of an external dye sensitizer (irradiation wavelength higher than 350 nm). In this condition LO decomposes (as shown in Figure 3), affecting the terpene-absorbing region of the spectrum. Upon LO irradiation in the range 300–350 nm, the mechanism depicted under point i mainly operates.

Besides the interest on the basic knowledge for the photoprocesses that operate upon LO and terpene irradiation, the preceding results should be carefully considered in a practical sense—when LO or foods containing LO are stored under daylight conditions. Solar irradiation contains, even when low, a considerable contribution of light of wavelength in the range 300 nm (Draper, 1985). Under these circumstances both direct and sensitized photolysis will produce a rapid photodamage of LO.

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