Fluorescent Light Catalyzed Autoxidation of β-Carotene

Discussion hitherto on the cooxidation of β-carotene in fat solution has generally emphasized the role of unsaturated fatty acids in promoting loss of pigment. However, it is now shown on the contrary that in fluorescent light photocatalyzed autoxidation the rate of loss from solution in fatty acid esters falls in the order laurate > oleate > linoleate, thus leading to the view that protection against autoxidation is built into the system by the unsaturation. Loss is also retarded by inclusion of the antioxidant, ethoxyquin. Results have relevance to the stability of β-carotene and other carotenoids exposed under oxidative conditions to fluorescent light.

Retention of the color appeal of a food product which is due to its content of carotenoids poses a particular problem because of the relative ease with which these pigments may undergo degradation.

It was earlier proposed that bleaching of β-carotene incidental with lipoxidase-catalyzed autoxidation of oils was due to attack by intermediates of the oxidation process (Sumner and Sumner, 1940). This result placed emphasis on the role of unsaturated fatty acids to which attention has continued to be given. Thus it has been claimed that addition of unsaturated fat or a mixture of methyl esters of fatty acids to solutions of β-carotene in liquid paraffin resulted in shorter induction periods and higher rates of autoxidation (Budowski and Bondi, 1960).

By contrast recent studies on the photostability of oils have directed attention to protective properties of the pigment in retarding increase in peroxide value. At the same time, however, it was noted that the rate of photochemical oxidation of the oils did not correlate well with the degree of unsaturation or the unsaturation ratio (Sattar et al., 1976).

The continued emphasis on the association of unsaturated fatty acids in photocatalyzed and photosensitized reactions (Sattar et al., 1976; Clements et al., 1973; Carlsson et al., 1976; Chan, 1977) has drawn attention to the intrusion of singlet oxygen into the process. It has been proposed that β-carotene may enter into different reactions in photochemical processes, functioning either as a quenching agent and inhibitor of spontaneous fat oxidation (Herrisset, 1948) or as a substrate for degradation (Schenck and Schade, 1970).

As a contribution to dissection of degradation processes involving the pigment a direct comparison has now been made of the stability of β-carotene in solutions of saturated and unsaturated fatty acid esters in reactions with oxygen catalyzed by fluorescent light.

EXPERIMENTAL SECTION

β-Carotene (Roche) was purified to give only a single spot by thin-layer chromatography on silica gel G plates (0.5 mm) using acetone/light petroleum (bp 40/60 °C, 1:99) as developing solvent and to satisfactory spectroscopic standard. Methyl esters of the fatty acids prepared by conventional methods and separated by vacuum fractional distillation had 98–99% purity by gas–liquid chromatography. Ethoxyquin, purified by vacuum distillation [bp 123–5 °C (2 mm)] gave only one peak on gas–liquid chromatography.

Solutions of β-carotene (2.5 mL) in the fatty acid esters (0.43 mg/g of laurate, 0.42 mg/g of oleate, and 0.378 mg/g of linoleate) in small petri dishes (50 mm diameter), giving a layer about 2 mm deep, were placed in a tray (110 × 12 × 3 cm) provided with a sealable glass cover and gas inlet/outlet to maintain a slow flow of oxygen over the samples. Duplicate sets of dishes for each solution were placed in the tray at the same time. With threefold repetition of exposure in sets, the rate of loss from each solution was therefore measured six times. Moreover the comparisons of pigment loss had the built-in control of simultaneous exposure. Light from two parallel 40-W cool white fluorescent tubes gave an intensity of 6030 lux at the surface of the samples from a distance of 17.5 cm.

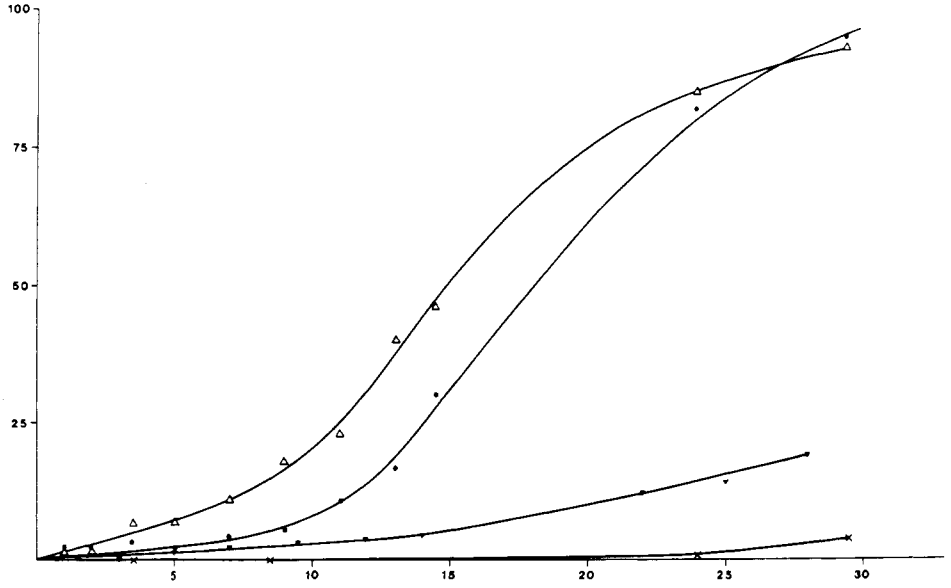

Figure 1. Rate of loss of β-carotene from fatty acid ester solutions exposed to fluorescent light (6030-lux intensity) plus oxygen. Exposure time, h. Methyl ester solutions: (△) laurate; (●) oleate; (▼) linoleate; (×) laurate in dark. No loss occurred with either oleate in dark or with oleate plus 0.02% ethoxyquin exposed to light.

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Loss of β-carotene was monitored from spectra over the range 350–520 nm of solutions of aliquots (about 100 mg) in light petroleum (10 mL). Pigment concentration was calculated from the absorbance at 450 nm assuming $E_{1cm}^{1\%}$ = 2500. Control experiments were carried on simultaneously by storing trays in the dark. Antioxidant effect of ethoxyquin (0.02%) was determined in methyl oleate solutions exposed to the light.

RESULTS AND DISCUSSION

The rates of loss of β-carotene, presented in Figure 1 as averages of the duplicate experiments, reveal several significant points regarding the nature of the autoxidation process. It is clear that, with the superimposed effect of the fluorescent light, unsaturation in the fatty acid solvents is not a necessity for oxidative loss of pigment. Indeed the distinct and consistently slower rates of loss show that a protective effect is being introduced with greater efficiency as unsaturation is increased.

The autocatalytic nature of the curves with marked induction periods and the inhibition given by the antioxidant suggest that free radical reactions are involved in loss of the pigment. In this respect results are in accord with those previously obtained from oxidations in toluene solution at higher temperatures with azoisobutyronitrile as added initiator (El-Tinay and Chichester, 1970), but present results differ from those at the higher temperature which show no lag phase.

In the present instances sensitizers are absent so that the involvement of singlet oxygen must be ruled out and the effect of the light must be attributed to absorption by the β-carotene itself. The control experiments in the absence of light gave no measurable loss of pigment. It is important, therefore, to realize that reaction with oxygen is probably being promoted by absorption of light in the visible region as distinct from the more usual ultraviolet source of activation particularly in view of the screening given by the plate glass cover to the sample holder. The site of attack of oxygen has been open to much discussion although it has been claimed that β-ionone has been identified as a product of photooxygenation without sensitizer (Isoe et al., 1969).

In the event that degradation occurs as a free radical process, the protective effect of the unsaturated fatty acid esters is explained in providing substrates for diversion of sequence in a chain process, with the tendency to diversion enhanced as unsaturation increases.

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Toxicity of *O,O,S*-Trimethyl and Triethyl Phosphorothioate to the Rat

O,O,S-Trimethyl phosphorothioate administered orally to rats caused mortality at doses as low as 15 mg/kg. At the lower doses of 15–80 mg/kg death occurred 4–22 days following treatment. The animals which died after treatment at the low doses appeared to be physically normal before death except for loss of weight. *O,O,S*-Triethyl phosphorothioate was slightly less toxic than the trimethyl analogue.

In a recent study concerned with the identification of impurities present in technical malathion and the effect of the impurities on the toxicological properties of malathion, we reported the presence of *O,O,S*-trimethyl phosphorothioate as an impurity, along with other sulfur-containing trimethyl esters (Umetsu et al., 1977). The acute oral LD_{50} of this compound was reported as 260 mg/kg based on 24–48 h mortality. In continuing studies on the potentiating and toxicological properties of these impurities in rats and mice, we have discovered that *O,O,S*-trimethyl phosphorothioate is a far more hazardous material than estimated from 48-h mortality data.

EXPERIMENTAL SECTION

Rat oral LD_{50} determinations were made with 95–120-g female albino rats (Sprague-Dawley derived) obtained from Simonsen's Laboratories, Gilroy, CA. Solutions of the toxicants in corn oil were administered orally at 0.2 mL/100 g rat. The animals were fasted for 6 h before treatment and kept under observation for 25 days. The ranges given for the LD_{50} values were estimated from the mortality data given in Tables I and II.

Malathion carboxylesterase activity in rat serum was assayed by coupling the hydrolysis of malathion to the reduction of a tetrazolium dye according to Talcott (1979). Groups of rats received graded oral doses of test compound, and blood samples were obtained from each rat at different time intervals after dosing. Aliquots of the sera were assayed for malathion carboxylesterase and cholinesterase activities (Talcott et al., 1979) and the results of each time point were expressed as percentages of the control activity (sera from untreated animals). Rat serum

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