Some Effects of Ionizing Radiations on Lipids. I. Monocarbonyl Production in Vegetable Oils [1]

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DURING THE PAST TEN YEARS the developments of radiation sources, both machine and isotopic, have led to increased activity in research on the utilization of the bactericidal effects of high energy radiations for the "cold sterilization" of foods and drugs.

The development of undesirable flavors in irradiated foods has been perhaps the most important single difficulty encountered by investigators attempting to refine the technique of radiation sterilization for commercial use. The production of compounds responsible for off-flavors may be attributed to the direct release of radiation energy to their precursors or, more probably, to an indirect effect involving reactive intermediates produced in the medium.

The theory that highly reactive free radicals and activated molecules are produced in the solvent medium has been extensively studied with the use of aqueous solutions. These studies have led to the reduction of flavor side-effects by irradiating *in vacuo* or at a reduced temperature or by the incorporation of protective substances that are believed to react harmlessly with the free radicals (1–7).

Until recently scant attention has been paid to the effects of radiations on lipoidal fractions of foods. Lipids include many unstable compounds easily altered by such agencies as heat, oxygen and light and have been shown to be involved in deteriorative changes in foods even when present in minute quantities. In addition, their reactivity in fats and oils during storage is inhibited by the same methods presently used to reduce flavor changes in irradiated foods.

In studies on oxidative rancidity and reversion in fats and oils, aldehydes and ketones have been found among the oxidation products (8–13). In some cases specific carbonyl compounds have been shown to be at least partially responsible for the off-flavors that developed (14, 15). Also the presence of peroxide compounds, the theoretical intermediaries in the atmospheric oxidation of fatty acids and esters, has been demonstrated in irradiated fats (16–18).

It was the purpose in part of the work reported here to determine the extent to which monocarbonyl compounds may be produced in irradiated oils and to study the methods by which their formation might be reduced.

Experimental Methods

Cottonseed, corn, and olive oils (purchased on the open market) were irradiated by cathode rays produced by a Van de Graaff Electrostatic Generator rated at three million electron volts (3 m.e.v.) constant potential (19). Except where otherwise noted, the samples were immersed in an ice-water bath for irradiation.

The dinitrophenylhydrazones of monocarbonyl compounds produced by the radiations were separated on alumina columns, and their absorption in alkaline solution at 425 mμ was determined in a Beckman Model DU Quartz Spectrophotometer (20). Quantitative estimations of the amount of monocarbonyl compounds in a sample were made by comparison with the absorption of a standard solution of heptaldehyde-dinitrophenylhydrazone. The error in measuring micromole quantities by this method was found to be less than 1%. The over-all error in the work, including variations in electron beam flux during irradiation, was estimated to be no greater than 5%.

Ultraviolet absorption measurements were made on solutions of the oils in absolute ethyl alcohol (United States Industrial Chemicals Company) with the use of the Beckman Model DU spectrophotometer.

Results and Discussion

Irradiation Yields. Olive, corn, and cottonseed oils were irradiated with a dose of 10^7 rep. (roentgen-equivalent-physical[3]). The average irradiation production of monocarbonyl compounds in the samples was found to be:

	$\mu M/g/10^6$ rep.
Olive oil	0.21
Corn oil	0.18
Cottonseed oil	0.21

The yields were substantially the same for doses of 10^6 and 5×10^6 rep. However, at 2.5×10^7 rep. and higher, the yield per unit dose (10^6 rep.) fell off, probably because of the decrease in the concentration of precursors and an increase in irradiation-induced reactions of the monocarbonyls which competed with the precursors for the free radicals produced by the radiations in the solvent.

It was believed that, since unsaturated molecules are important in oxidative rancidity and reversion in refined oils, the compounds, particularly if they contained conjugated systems, would be oxidized during irradiation and such oxidation would produce car-

bonyls. To test this hypothesis, ultraviolet absorption spectra were obtained on samples of cottonseed oil irradiated with doses of 10^6, 10^7, and 2.5×10^7 rep. The progressive effects of irradiation on the ultraviolet absorption curve of the oil and the absorption maxima found are shown in Table I.

TABLE I

Ultraviolet Extinction Maxima of Irradiated Cottonseed Oil

Control		10^6 rep.		10^7 rep.		2.5×10^7 rep.	
Wave-length (mμ)	E[a]	Wave-length (mμ)	E[a]	Wave-length (mμ)	E[a]	Wave-length (mμ)	E[a]
230	0.757	231	0.784	232	0.885	233	0.970
259	0.329	259	0.328	......	......	......	......
269	0.415	269	0.405	269	0.248	269	0.114
280	0.342	280	0.336	280	0.198	280	0.090

[a] Expressed in units liter g^{-1} cm^{-1}.

The decrease in absorption at 259, 269, and 280 mμ is indicative that conjugated trienes were under attack during irradiation. This decreased absorption may have been the result of a scission of the chain or the formation of peroxido groups with or without subsequent breaking of the chain. The effect was approximately proportional to dose up to 10^7 rep., but, as had been shown by measurements of monocarbonyl production, the rate decreased at a dose of 2.5×10^7 rep.

The products from the triene reactions resulted in a simultaneous increase in absorption in the conjugated diene region (230 mμ), but the yield was not proportional to the destruction of conjugated trienes. This may be expected since the compounds absorbing at the lower wavelengths may be produced by reactions involving other compounds. Conjugated dienes, peroxido types, and carbonyls absorbing at 220 to 240 mμ are likely products from oxidations and shifting of double-bond in unconjugated systems. Moreover the reactivity of the products themselves has an adverse effect on the yield, which quantitatively depends on the relative concentrations and radio-sensitivities of all the reactants and products involved.

The progressive bathochromic shift of the maximum absorption in the diene region was found to be a consistent effect in the irradiation of oils. Subsequent tests showed that compounds absorbing at higher wavelengths were produced at the same time that those at lower wavelengths were destroyed.

Effect of Temperature. Paired samples of olive, corn, and cottonseed oils were irradiated with a dose of 10^7 rep. in a room-temperature bath and in an ice-water bath. A number of replicate runs were made. The average monocarbonyl yields (μM/g/10^6 rep.) in the irradiated oils were as follows:

Irradiated oil	Ice-water bath	Room temp. bath
Olive	0.21	0.19
Corn	0.18	0.17
Cottonseed	0.21	0.19

In all the replicate runs the yield was lower for each pair of samples with a room-temperature bath than with an ice-water bath. However it should not be inferred that irradiation at reduced temperature increased the production of the monocarbonyls. Subsequent research by the authors with pure compounds has demonstrated that the results obtained were caused by a greater reactivity of the products,

particularly unsaturated ones, at the higher temperature. Thus it is probably true that, although more carbonyls were produced at the higher temperature, a larger number of them reacted still further and were not measured in the test. These results and others reported below show that modifications in irradiation conditions affect the stabilities of the reaction products.

Irradiation in Vacuum. To test the effect of the removal of oxygen, samples of cottonseed oil were irradiated at one atmosphere, 25 mm. Hg and 1 mm. Hg, with doses of 10- and 25-million rep. The average yields of monocarbonyls in these samples were as follows:

Dose (rep.)	Pressure	Monocarbonyls (μM/g/10^6 rep.)
10^7	1 atm.	0.21
10^7	25 mm. Hg	0.20
10^7	1 mm. Hg	0.20
2.5×10^7	1 atm.	0.18
2.5×10^7	25 mm. Hg	0.18

The ultraviolet absorption maxima of these samples are reported in Table II.

TABLE II

Effect of Irradiation in Vacuum on Ultraviolet Absorption Spectrum

Wave-length (mμ)	Extinction Maxima (liter g^{-1} cm^{-1})				
	Control	10^7 rep.		2.5×10^7 rep.	
		25 mm. Hg	1 atm.	25 mm. Hg	1 atm.
230	0.781	......	......	......	......
231	......	0.922	......	......	......
232	......	......	0.894	1.229	1.156
259	0.331	......	......	......	......
269	0.428	0.254	0.253	0.139	0.119
280	0.352	0.202	0.201	0.115	0.097

No differences greater than the experimental error occurred in the monocarbonyl yields at the different pressures for a given dose level. However the yield efficiency was again less at 25-million rep. than at 10-million rep. at both one atmosphere and 25 mm. Hg. These data indicate that the removal of oxygen was of no practical benefit in reducing the yield of monocarbonyl compounds.

At 10 million rep. the destruction of conjugated trienes in vacuum-packed samples was not lessened, but the decrease in oxygen tended to reduce the shift in the wavelength of maximum absorption caused by the products formed. At about 25 million rep. the triene destruction was decreased slightly, and the dose was sufficient to eliminate the effect of oxygen on the extent of the shift. At both dose levels the absorption of the products was less at one atmosphere than at 25 mm. Hg. Evidently the yield of compounds causing the shift was increased by presence of oxygen as well as by the extent of the dose. At the higher dose level the concentration of trienes was considerably reduced, and the spectrum of reactants was altered by the radiations. Under these conditions mechanisms that caused the shift in wavelength were favored even under reduced oxygen.

The lower absorbancy of compounds produced by both dose levels at one atmosphere compared with that at 25 mm. Hg is not attributed to a decreased production of the primary products. Subsequent observations with purified fatty acids and esters indicate that the effect was caused by further reactions of these products in which oxygen plays a role.

Addition of a Protector. The addition of appropriate substances to aqueous media has been shown to reduce the chemical side-effects of irradiation (3, 4). The added compounds, reacting with the free radicals produced, form innocuous products. Thus, by a process of competitive inhibition, they reduce the indirect conversion of sensitive compounds into undesirable end-products.

Samples of cottonseed oil containing added amounts of the antioxidants, 6-palmityl ascorbic acid and Tenox II (20% butylated hydroxyanisole, 4% anhydrous citric acid, and 6% propyl gallate in propylene glycol), were irradiated at doses of 1- and 10-million rep. Irradiation effects were compared with those in paired samples not containing the antioxidants.

These additives are commonly used to suppress oxidative changes and, in particular, those reactions involving unsaturated components in refined oils. Ultraviolet absorption measurements showed that the irradiation attack on the conjugated triene systems was depressed by about 20% through the protective action of the antioxidants. Thus at least part of the destruction of the trienes was due to an indirect effect. However the additives had no inhibiting effect on the wavelength shift in the 230 mμ region or on the absorbancy increase caused by the products of irradiation. Moreover, as shown in Table III, the yield of monocarbonyl compounds was greater, rather than less, than in the samples not containing the antioxidants.

TABLE III

Effect of Addition of Antioxidants on Yield of Monocarbonyl Compounds in Cottonseed Oil

Added antioxidant Type	Amount (% by wt.)	Percentage increase in monocarbonyl compounds [a]	
		10⁶ rep.	10⁷ rep.
Tenox II	0.1	3	2
Tenox II	0.5	10	14
Tenox II	1.0	24	28
6-Palmityl ascorbic acid	0.05	13	17
6-Palmityl ascorbic acid	0.10	24	24

[a] Average percentage increase in yield (μM/g/10⁶ rep.) of samples with antioxidant over yield of samples without antioxidant.

The increase in yield in those samples containing the antioxidant over that obtained from irradiated controls without added antioxidant was probably the result of reactions of these additives themselves with free radicals on activated molecules and, as a likely consequence, the "protection" of the carbonyls produced from further reaction. It has been subsequently shown by the authors that the unsaturated members of the carbonyl group measured may be expected to undergo further attack, reducing the yield. Since the antioxidant did decrease the reaction of the highly sensitive conjugated trienes, it is probable that it similarly inhibited the "destruction" of some of the monocarbonyls. Thus its most important action in the system appears to have been one of competitive inhibition even though the net yield of monocarbonyls was increased. (These results emphasize the complexity of the irradiation-induced reactions in the medium.)

Summary and Conclusions

Irradiation of refined vegetable oils by high-energy cathode rays resulted in the production of monocarbonyl compounds. The yield was about 0.2 μM per gram per 10⁶ rep. for doses up to about 10⁷ rep. Some of the products were themselves sensitive to the radiations, and the net yield was dependent upon a function of the concentrations and reactivities of the precursors and of the end-products.

Concurrently with the formation of the monocarbonyls there was an attack on the conjugated triene systems in the media and an increase in ultraviolet absorption in the 225–240 mμ region. However the irradiation products resulted from other mechanisms in addition to those involving the trienes.

Irradiation under reduced temperature or in vacuum or with the addition of antioxidants did not decrease the yield of monocarbonyl compounds. However the use of a vacuum and the inclusion of antioxidants were partially effective in reducing the irradiation attack on triene groups.

The net yields of monocarbonyls were dependent upon the concentrations and sensitivities of the products as well as of their precursors. Thus, although methods commonly successful in inhibiting oxidations of oils did not reduce these yields, they did suppress some irradiation-induced reactions, particularly those involving unsaturated compounds. In this respect their action was similar to that which has been demonstrated in inhibition studies of antioxidations in oils.

Acknowledgment

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