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Effects of Ionizing Radiations on Fats. II. Accumulation of Peroxides and Other Chemical Changes[1,2]

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Abstract

The accumulation of peroxides, carbonyl compounds and reducing substances during irradiation and post-irradiation storage of pure fatty acid methyl esters has been studied.

Irradiation and storage of irradiated methyl myristate under vacuum results in formation of small quantities of these compounds. Irradiation under oxygen gives peroxides and carbonyl compounds in yields indicating that every ionization results in the formation of one molecule of each group, and antioxidants have no effect on the formation of these compounds during irradiation.

Irradiation of methyl linoleate under vacuum results in destruction of pre-formed hydroperoxides. During irradiation in oxygen, approximately one-eighth of the peroxides formed arises from the direct reaction of irradiation-induced free radicals with oxygen, while the rest is formed through a chain mechanism with an average chain length of 7.

Peroxides continue to accumulate in irradiated methyl linoleate stored under oxygen at a rate increasing with initial irradiation dose.

Antioxidants have some effect in retarding the formation of peroxides during irradiation of methyl linoleate and during post-irradiation storage, but the effect is small compared to their antioxygenic activity toward simple autoxidation. The effect varies with the nature of the antioxidant and with irradiation dose. Propyl gallate is much less effective than butylated hydroxyanisole and appears to be easily destroyed during irradiation.

Introduction

THE TREATMENT of fats with high energy radiations results in the formation of free radicals which may participate in a number of reactions in the irradiated medium. When molecular oxygen is available, the free radicals readily react with this substance to form hydroperoxides. Decomposition of these peroxides yields new free radicals and a chain oxidation process identical to that prevailing in autoxidation is initiated.

Most studies on the effects of radiations on lipids have been performed on unsaturated fats and it has

been impossible to differentiate between the products formed directly as the result of irradiation and those produced through the usual autoxidative chain process. Furthermore, irradiation produces a number of other chemical changes such as formation of carbonyl compounds, polymers, short-chain acids and hydrocarbons, but the formation of these compounds during irradiation and subsequent storage has not been fully investigated.

The results reported here are part of a broader study initiated to obtain additional information on these problems.

Experimental

Preparation and Irradiation of Samples. Methyl myristate was prepared by esterifying myristic acid (Eastman Chemicals) with methanol in presence of H_2SO_4, followed by the usual extraction, washing and drying procedures. After removal of the solvent under vacuum, the residual ester was nearly colorless, had an iodine value (I.V.) of less than 0.1, and was used without further purification.

Methyl linoleate was prepared by urea fractionation of safflower oil fatty acids (1), methylation of the most unsaturated fraction and vacuum fractional distillation of the methyl esters. The I.V. of the fraction used for these studies was 170.2, and purity was better than 95%.

Samples of methyl myristate or methyl linoleate containing 0.01% propyl gallate (PG), α-tocopherol (αT), butylated hydroxyanisole (BHA) and 0.01% each PG and citric acid (CA) were prepared by adding suitable amt of alcoholic solutions of the antioxidants to the methyl esters and removing the alcohol by evaporation under vacuum. In one experiment, methyl linoleate containing 0.1% propyl gallate was used also.

For irradiation under vacuum, one-ml aliquots of the samples were evacuated and sealed in 15-ml pyrex bulbs. For the experiments in an atmosphere of oxygen, 2 ml portions were sealed under oxygen in 40-ml tubes. All samples were irradiated at Materials Testing Reactor, Arco, Idaho, with doses of approximately 2,5 and 8 x 10^6 rads.

The samples were packed in dry ice during shipment to and from the irradiation facility, and they were usually received at our laboratory 2–4 days after irradiation. Controls were shipped with the samples and, with the exception of irradiation, their handling was identical to that of the irradiated samples.

TABLE I
Chemical Changes in Methyl Myristate Irradiated and Stored under Vacuum

Change measured	Dose (10^6 rads)	Before storage	Storage temp and time					
			24C			−20C		
			2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk
Peroxide value (m.e./kg)	0	0	....	....	0	....	....	....
	1.86	0	....	....	0	....	....	....
	4.65	0	....	....	0	....	....	....
	7.44	0	3.6	1.2	0.6	5.5	....	0
Reducing compounds (m.e./kg)	0	0.01	....	....	....	....	....	....
	1.86	0.03	....	....	0.02	....	....	....
	4.65	0.04	....	....	0.03	....	....	....
	7.44	0.06	0.04	0.04	0.04	0.05	....	0.04
Unsaturated carbonyl (mmole/kg)	0	1.8	....	....	....	....	....	....
	1.86	1.1	....	....	0.8	....	....	....
	4.65	1.2	....	....	1.1	....	....	....
	7.44	1.5	1.5	1.0	1.2	1.1	....	1.5
Saturated carbonyl (mmole/kg)	0	4.2	....	....	....	....	....	....
	1.86	5.3	....	....	6.7	....	....	....
	4.65	7.2	....	....	8.4	....	....	....
	7.44	8.2	8.0	8.9	10.0	9.3	....	8.5
Total carbonyl (mmole/kg)	0	6.0	....	....	....	....	....	....
	1.86	6.4	....	....	7.5	....	....	....
	4.65	8.4	....	....	9.5	....	....	....
	7.44	9.7	9.5	9.9	11.2	10.4	....	10.0

Because of possible variations in the shipping, irradiation and storage conditions while the samples were away from our laboratory, experiments were planned so that specific information would be obtained from one series of samples shipped and irradiated at the same time, and presumably, subjected to identical conditions.

For similar reasons, in the case of storage studies, zero time has been taken as the time at which the irradiated samples were received in our laboratory and placed at the various storage temp. In this work, it has been assumed that no significant change occurred in the irradiated or control samples kept in dry ice during the relatively short time required to return them to our laboratory after irradiation.

Methyl linoleate irradiated under oxygen was expected to undergo rapid oxidative changes during storage in air at room temp. In order to avoid possible variations between individual duplicate samples, due to nonuniformity of flux density of the irradiation source, the contents of all the tubes irradiated with the same dose were pooled to give a more uniform sample from which aliquots were removed for storage at the different temp. This was not done in the case of methyl linoleate stored under vacuum or in the case of methyl myristate samples which were expected to show little change during storage.

Analytical Procedures. Peroxide values were determined iodometrically by the Wheeler method (2). For the determination of the carbonyl compounds, the method of Henick, et al. (3) was modified as described by Chipault, et al. (4).

Reducing compounds were measured by using the ferric chloride and a,a'-bipyridyl reagents of Emmerie and Engel (5). The method described by Mahon and Chapman (6) was modified to eliminate extraction of the reducing compounds from the lipid substrate since, in this case, the methyl esters used would be too soluble in the extracting solvents. The procedure adopted was as follows:

A portion of the methyl ester (200 mg) is weighed in a 5-ml volumetric flask and dissolved in 2 ml 0.1% a,a'-bipyridyl in absolute ethanol. Two ml 0.0832% ferric chloride hexahydrate dissolved in absolute ethanol is then added and the solution is made up to 5 ml with absolute ethanol. The color is allowed to develop at room temp in the dark for 40 min and its intensity is read in a Beckman spectrophotometer at 522 mμ. A correction is applied for a reagent blank prepared in an identical manner. The reaction is quite sensitive to light and it was found that more

reproducible results were obtained if addition of the reagents and handling prior to the spectrophotometric measurement were performed in an amber light of less than 1 ft-c intensity. The results of this analysis were measured from a standard curve obtained with pure propyl gallate and are expressed as milliequivalent of reducing compounds.

Results and Discussion

Chemical Changes and Accumulation of Peroxides in Irradiated Methyl Myristate. Methyl myristate is a saturated methyl ester which is liquid at irradiation temp and which undergoes no significant autoxidation under the conditions of time and temp used here. The experiments with this compound were designed to measure some of the chemical changes which occur as a direct result of irradiation.

Peroxides, reducing compounds and carbonyls formed during irradiation and storage of methyl myristate under vacuum show in Table I and similar data for irradiation and storage under oxygen appear in Table II.

Before storage, no peroxides were detected in the samples irradiated under vacuum, but small amt of peroxides were found after two weeks at room temp or at −20C. These peroxide contents decreased on longer storage and were again nil at the end of 20 weeks. The formation of peroxides in methyl myristate irradiated under vacuum was unexpected. However, the analytical procedure used is capable of detecting peroxide concn less than 1 m.e./kg, and it is felt that the values obtained definitely indicate the presence of compounds capable of oxidizing potassium iodide to free iodine. It is possible that these compounds were produced during the peroxide determination when highly active free radicals could react with oxygen. The fact that no peroxides were detected immediately after irradiation or after longer storage suggests that these active molecules were not present at first, but developed during the first storage period and later disappeared. Bradshaw and Truby (7) have shown that free radicals produced in various fats by irradiation decayed into other free radical forms with different reactivities. However, these authors could detect no free radicals in lipids that were either irradiated in the liquid state or allowed to become liquid for only a few min after irradiation. The results reported here were obtained with methyl myristate irradiated in the liquid state and stored as a liquid for several weeks.

TABLE II

Chemical Changes in Methyl Myristate Irradiated and Stored under Oxygen

Change measured	Dose (10^6 rads)	Before storage	24C			2C			−20C		
			2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk
Peroxide value (m.e./kg)	0	0	....	....	....	....	....	....	....	....	....
	1.86	14	12	12	12	12	12	12	13	13	13
	4.65	30	25	22	26	24	26	24	29	30	28
	7.44	41	35	37	34	38	38	37	36	38	39
Reducing compounds (m.e./kg)	0	0.01	....	....	....	....	....	....	....	....	....
	1.86	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03
	4.65	0.07	0.06	0.03	0.05	0.05	0.04	0.05	0.06	0.06	0.06
	7.44	0.07	0.06	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.07
Unsaturated carbonyl (mmole/kg)	0	1.8	....	....	....	....	....	....	....	....	....
	1.86	2.1	2.7	2.0	1.8	2.5	1.9	1.7	2.9	1.7	1.7
	4.65	3.5	3.3	2.9	2.9	3.2	2.9	2.6	5.2	3.1	3.3
	7.44	4.2	4.6	4.1	3.7	7.0	3.5	4.0	6.0	6.4	4.9
Saturated carbonyl (mmole/kg)	0	4.2	....	....	....	....	....	....	....	....	....
	1.86	10.3	12.4	11.1	10.4	12.0	11.9	11.1	12.2	12.3	10.4
	4.65	18.3	18.6	19.9	17.6	17.8	21.9	19.5	24.3	21.0	17.3
	7.44	24.0	25.9	27.1	24.1	30.9	25.0	26.1	29.8	23.6	25.1
Total carbonyl (mmole/kg)	0	6.0	....	....	....	....	....	....	....	....	....
	1.86	12.4	15.1	13.1	12.2	14.5	13.8	12.8	15.1	14.0	12.1
	4.65	21.8	21.9	22.8	20.5	21.0	24.8	22.1	29.5	24.1	20.6
	7.44	28.2	30.5	31.2	27.8	37.9	28.5	30.1	35.8	30.0	30.0

The identity of these compounds as hydroperoxides has not been established; but, if they are, their structure, their formation mechanism and the effects of irradiation conditions and post-irradiation storage on their production remain largely unknown.

Small quantities of reducing compounds were formed as a result of irradiation under vacuum and the amt present increased with the dose. The nature of these compounds is unknown, but in separate experiments it was found that commercially available samples of high grade acrolein and crotonaldehyde reacted with the Emmerie and Engel reagents. It is possible, therefore, that these reducing substances are part of the unsaturated carbonyls found in the irradiated esters, although their development does not parallel changes in unsaturated carbonyl content.

The original methyl myristate used contained small amt of carbonyl compounds and irradiation resulted in an increase in carbonyl content approx proportional to irradiation dose. These changes reflect mostly increases in saturated carbonyls while the unsaturated compounds remain unchanged or show a slight decrease. The increase in carbonyl content was the greatest change observed as a result of irradiation under vacuum; in contrast to the peroxides, the carbonyls were detected immediately after irradiation and storage had no appreciable effect on their concn. It is not likely that these compounds are formed by reaction with oxygen during the carbonyl determination; it is more probable that they are the result of scission of the carbomethoxy group as suggested by the appreciable amt of methanol found in the volatiles of irradiated methyl oleate (8).

Irradiation of methyl myristate under oxygen (Table II) resulted in formation of peroxides in appreciable quantities approx proportional to dose. Post-irradiation storage had little effect on the peroxide contents of the irradiated samples. Reducing substances were formed also, but the amt is not greater than that resulting from irradiation under vacuum.

The amt of unsaturated carbonyl originally present in the methyl myristate showed a small increase during irradiation, but the increase in saturated carbonyl content was much greater. The data indicate also that the effect, if any, of post-irradiation storage on carbonyl content was a slight decrease.

The efficiency of high energy radiations in producing chemical changes is often expressed as G value. This is defined as the number of molecules of a compound formed or destroyed for each 100 ev energy absorbed. A G value of 3.3 means that every ionization results in a change in one molecule, while a G value of 10 or more indicates definitely that the change measured takes placed through a chain mechanism. G values for the formation of peroxides and carbonyls in methyl myristate irradiated under vacuum and in oxygen show in Table III.

Under vacuum, carbonyls are formed with a G value of 0.5 or less indicating that only ca. 1 out of 7 ionizations results in the production of a carbonyl group. In an atmosphere of oxygen, the average G value for peroxide accumulation is 3.1. This indicates that virtually every free radical formed as a result of irradiation reacts with oxygen to give a peroxide, and that the peroxides are not formed through a chain mechanism. This is not unexpected since saturated methyl esters contain no labile hydrogen which may be abstracted by a peroxide free radical, and do not undergo rapid autoxidation. It will be noticed that the G value for peroxides decreases with irradiation dose. Two explanations are possible for this observation: first, as will be shown later, pre-formed peroxides are partly destroyed during irradiation and, therefore, less peroxides would accumulate because of increased destruction at higher doses; and secondly, at higher doses the oxygen available within the sample may become depleted and the formation of peroxides then is limited by the rate of diffusion of oxygen into the substrate. Under these conditions, a portion of the free radicals resulting from irradiation would become inactivated through recombination and polymerization reactions.

The yield of carbonyl compounds during irradiation in an atmosphere of oxygen is strikingly similar to that of peroxides. The average G value of approx 3.2 again indicates the formation of a carbonyl group from each ionization. The suggestion that these carbonyl compounds may result partly from irradiation-induced peroxide decomposition is unlikely, since, if

TABLE III

G Values for Formation of Peroxides and Carbonyls in Irradiated Methyl Myristate

Irradiation atmosphere	Dose (10^6 rads)	G Values for formation of:			
		Peroxides	Unsat. carbonyls	Sat. carbonyls	Total carbonyls
Vacuum	1.86	0	−0.4	0.6	0.2
	4.65	0	−0.1	0.6	0.5
	7.44	0	0.0	0.5	0.5
Oxygen	1.86	3.6	0.1	3.2	3.3
	4.65	3.1	0.4	2.9	3.3
	7.44	2.7	0.3	2.6	2.9

TABLE IV

Effect of Antioxidants on Accumulation of Peroxides in Methyl Myristate Irradiated and Stored under Oxygen
(Peroxide Values in m.e./kg)

Antioxidant (0.01%)	Dose (10⁶ rads)	Before storage	24C			2C			−20C		
			2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk
None	0	0	....	....	....	....	....	....	....	....	....
	8	29	....	....	....	....	....	....	....	....	....
Propyl gallate	0	0	0	2	....	0	0	0	0.7	0	0
	2	9	9	8	7	13	9	8	10	12	6
	5	24	19	18	19	20	19	20	24	22	22
	8	30	25	26	26	18	25	28	35	31	27
a-Tocopherol	0	0	....	....	....	0	0	0	....	....	....
	2	7	....	....	....	12	10	14	....	....	....
	5	21	....	23	21	23	22	20	25	24	25
	8	25	....	....	....	29	27	26	....	....	....
Butylated hydroxyanisole	0	0	....	....	....	0	0	0	....	....	....
	2	11	....	....	....	11	10	....	....	....	....
	5	24	24	22	20	26	20	23	21	22	23
	8	36	....	....	....	33	32	28	....	....	....
Propyl gallate and citric acid	0	0	....	....	....	0	0	0	....	....	....
	2	10	....	....	....	9	10	10	....	....	....
	5	23	25	21	22	21	20	22	21	23	23
	8	31	....	....	....	31	33	31	....	....	....

this were the case, carbonyl content should increase at the higher irradiation levels. The extent to which hydroperoxides interfere with the determination of carbonyls by the procedure of Henick, et al. (3) is not known. It has been reported that, under acidic conditions, hydroperoxides are decomposed to hydroxyl rather than carbonyl groups (9), and Schwartz (10) has shown that methyl linoleate hydroperoxides give no hydrazone when passed on a chromatographic column coated with 2,4-dinitrophenylhydrazine and phosphoric acid. On the other hand, Gaddis et al. (11) have reported that carbonyl precursors (probably hydroperoxides) are decomposed to yield carbonyl compounds during the Henick determination, and Keith and Day (12) have shown that the Henick method gives poor results with complex mixtures of carbonyl compounds. We have observed that careful reduction of autoxidized methyl linoleate with stannous chloride has no effect on the Henick carbonyl content. This suggests that methyl linoleate hydroperoxides do not yield carbonyls during the Henick procedure and is in agreement with the findings of Schwartz. However, we have also found that with other autoxidized methyl esters, reduction of peroxides does decrease carbonyl value (13). Therefore, the possibility exists that these carbonyl compounds are derived from the peroxides during the carbonyl determination.

The data indicate that, if this is true, the transition from peroxide to carbonyl is quantitative and the myristate peroxides formed here differ in this respect from linoleate hydroperoxide.

In Table IV, samples of methyl myristate containing various antioxidants have been irradiated and stored in an atomsphere of oxygen. The results show that none of the common antioxidants studied had

any effect on the formation of peroxides during irradiation, again indicating the absence of a chain mechanism.

Since a chain mechanism is not involved in the formation of peroxides from methyl myristate during irradiation, the peroxides present must represent the primary products of the reaction, with oxygen, of the free radicals resulting directly from irradiation. However, the nature of these peroxide groups and their location on the fatty acid chain are not known.

Table V shows the peroxide content of irradiated methyl linoleate samples. In vacuum the pre-formed peroxides are destroyed with a G value of approx 1, and there is some evidence that the peroxides remaining in the samples continue to decompose during storage at the higher temp.

The decrease in peroxide content during storage at 24C is approx 1 m.e./kg/wk, and is nil at −20C for up to 20 wk. Consequently, no change is to be expected from normal peroxide decomposition reactions, either during irradiation for less than 3 hr at 21C, or during the 2–4 days at dry ice temp required for return shipment of the irradiated samples. The decrease in peroxide value observed after irradiation, therefore, must be induced by irradiation. However, the peroxide concn on a molar basis is only ca. 1 mole peroxide in 300 moles linoleate. Clearly, the destruction of peroxides is 100 times greater than can be accounted for by direct ionization of peroxide molecules, and must be the result mostly of secondary reactions between the peroxides and other activated linoleate molecules. The nature, mechanism and products of this irradiation-induced peroxide decomposition are not known.

In an atmosphere of oxygen, the peroxide content of the linoleate sample increased by 330 m.e./kg dur-

TABLE V

Accumulation of Peroxides in Irradiated Methyl Linoleate
(Peroxide Values in m.e./kg)

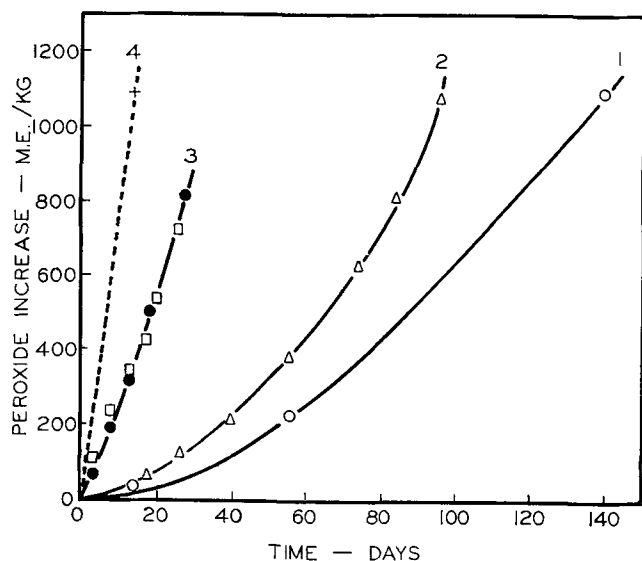| Antioxidant	Dose (10⁶ rads)	Before storage	24C			2C			−20C		
			2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk	2 Wk	8 Wk	20 Wk
Irradiation under vacuum 0.1% Propyl gallate	0	23	....	....	....	....	19	20	....	....	....
	2	17	....	....	....	20	18	16	....	....	....
	5	13	10	5	1	13	12	9	12	12	12
	8	8	....	....	....	13	....	6	....	....	....
Irradiation under oxygen None	0	25									
	8	356									
0.1% Propyl gallate	0	25	....	....	....	28	27	32	....	....	....
	2	43	....	....	....	50	45	56	....	....	....
	5	120	1232	1120	711	164	359	1231	104	97	140
	8	185	....	....	....	1222	1275	1150	....	....	....

Fig. 1. Accumulation of peroxides in methyl linoleate irradiated in oxygen and stored at 2C in air. Curve 1—5 x 10^6 rads, 0.1% propyl gallate; Curve 2—not irradiated, no propyl gallate (control); Curve 3—4.65 x 10^6 rads, ● no propyl gallate, □ 0.01% propyl gallate; Curve 4—8 x 10^6 rads, 0.1% propyl gallate.

Fig. 2. Accumulation of peroxides in methyl linoleate irradiated in oxygen and stored at room temp in air. Dose 4.65 x 10^6 rads. Curve 1—no antioxidant; Curve 2—0.01% propyl gallate; Curve 3—no antioxidant, not irradiated (control); Curve 4—0.01% butylated hydroxyanisole; Curve 5—0.01% butylated hydroxyanisole + 0.01% citric acid.

ing irradiation with 8 x 10^6 rads over a period of 3 hr at 21C. For comparison purposes, the same sample of methyl linoleate when autoxidized in air at room temp required 5 days to attain the same peroxide value. The G value for accumulation of peroxides during irradiation of methyl linoleate was 20. In another experiment, a G value of 22 was obtained for formation of peroxides in methyl linoleate irradiated with 4.65 x 10^6 rads. If we assume that the changes resulting directly from irradiation are the same in methyl linoleate as in methyl myristate (peroxides formed with a G value of approx 3.0), and that the additional peroxides found in irradiated methyl linoleate are formed through the usual chain mechanism prevalent in autoxidation, it can be estimated that under these conditions the average length of these chains is approximately 7. This is much smaller than the value of 100 estimated by Bolland and Gee (14) for the uncatalyzed autoxidation of ethyl linoleate. This chain length of 7, however, is in the range of that found by Bolland and Ten Have (15) for the autoxidation of ethyl linoleate in presence of hydroquinone. Hydroquinone and other phenolic antioxidants are effective because they are able to inactivate free radicals and interrupt autoxidation chains. Free radical inactivation occurs also when the concn of free radicals is high and the availability of oxygen is low. This situation exists when methyl linoleate is irradiated without agitation in an atmosphere of oxygen. Under these conditions, a large number of the free radicals produced by the high energy radiations are inactivated through recombination and other chain terminating reactions.

In the presence of propyl gallate, peroxides are formed with a G value of 10 and the accumulation of peroxides is reduced to half of that observed in the absence of antioxidants. The sample receiving 2 x 10^6 rads retained enough propyl gallate to prevent oxidation during post-irradiation storage. With 5 x 10^6 rads, oxidation proceeded rapidly at room temp so that a max peroxide value was obtained in less than 8 weeks, and longer storage showed lower peroxide contents. At 2C, autoxidation is considerably slower and at −20C, virtually no peroxidation occurs.
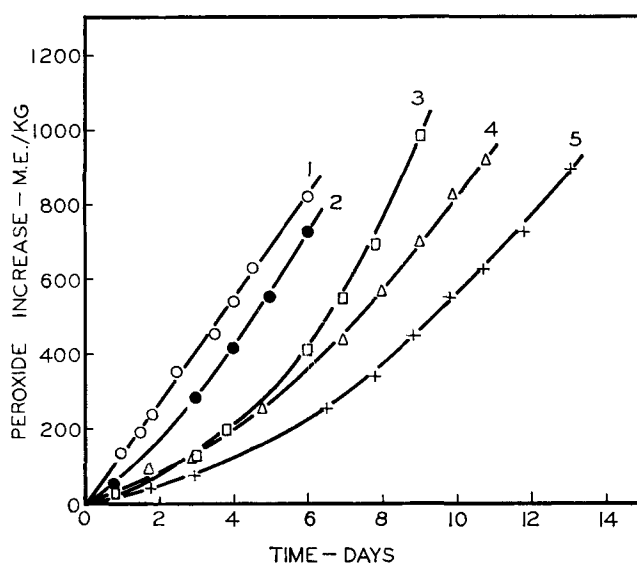
With 8 x 10^6 rads, oxidation during storage at 2C again is very rapid. It reaches a max peroxide content in ca. 2 weeks, but at this temp the peroxides are quite stable and only a small decrease is observed by the end of the 20 weeks storage period.

Figure 1 compares the rates of peroxide accumulation in irradiated methyl linoleate stored at 2C in air. Methyl linoleate containing 0.1% propyl gallate and irradiated with 5 x 10^6 rads (curve 1) accumulates peroxides at a rate approx half that of the non-irradiated, antioxidant-free control (curve 2) and approx one-fifth that shown by an irradiated linoleate sample containing no antioxidant (curve 3). Thus, with a dose of 5 x 10^6 rads and a high concn of propyl gallate (0.1%), enough antioxidant remains in the irradiated material to give it some protection against post-irradiation autoxidation. With 8 x 10^6 rads, however, (curve 4) all antioxidant effect is destroyed and this sample oxidizes more rapidly than linoleate free of antioxidant and exposed to only 4.65 x 10^6 rads (curve 3). Therefore, a dose between 5 and 8 x 10^6 rads is sufficient to counteract completely the antioxidant effect of propyl gallate used at a concn of 0.1% in methyl linoleate. A single line (curve 3) fits the two samples, with and without propyl gallate, that received 4.65 x 10^6 rads, showing that this dose is just sufficient to eliminate entirely the protective action of 0.01% propyl gallate.

Figure 2 shows the effect of several antioxidants on the accumulation of peroxides in irradiated linoleate stored in air at room temp. Again, the ineffectiveness of propyl gallate is shown by the almost identical peroxide curves given by irradiated linoleate without (curve 1) and with (curve 2) added propyl gallate. Similar results were obtained also at −20C.

BHA remains partially effective after irradiation and a concn of 0.01% of this material (curve 3) is more than adequate to counteract the prooxidant effect of a dose of 4.65 x 10^6 rads. Citric acid futher enhances this effect (curve 4).

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Gas-Liquid Chromatography of Fat-Soluble Vitamins[1]

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Abstract

Gas-liquid chromatography (GLC) was found to be useful for analysis of tocopherols and vitamin K_1. Vitamin A preparations showed evidence of alteration and ubiquinones gave no peaks on the chromatogram under the conditions used.

Introduction

A REPORT (8) on the application of GLC to separation of different members of the vitamin D group of compounds prompted us to investigate the possibility of separating other fat-soluble vitamins by this technique. Results obtained with vitamin A, tocopherols, vitamin K_1, and with ubiquinones on columns of SE-30 and QF-1-0065 are reported in this paper.

Experimental

Analyses were carried out on a Barber-Colman Model 10 chromatograph equipped with a 1 cm diode detector containing 80 μc Radium[226]. The column packing consisted of Gas Chrom P, 100-140 mesh, siliconized according to the procedure of Sjovall et al. (6) and coated with 3% by wt of either SE-30 or QF-1-0065. Gas Chrom P was obtained from Applied Science Laboratories, State College, Pa.; SE-30 from Wilkins Instrument Co., Walnut Creek, Calif.; and QF-1-0065 from Dow Corning Corp., Midland, Mich. The coated support was packed in 6 ft x ⅛ in. glass columns and conditioned at 225C for 48 hr. Materials to be separated were dissolved in chloroform and injected from a Hamilton syringe in a volume of 1-5 μl. The vitamin A and tocopherol preparations used for these studies were supplied by Distillation Products Industries. The vitamin K_1 was obtained from the California Corp. for Biochemical Research, Los Angeles, Calif. and the ubiquinones were kindly provided by W. E. J. Phillips of the Science Branch, Canada Department of Agriculture, Ottawa, Ontario.

Results

Tocopherols. The initial experiments were carried out with tocopherols since at least eight isomers with different biological activities are known to occur in nature and since the best chemical methods of assay are either incomplete (2) or very tedious (1,3). It was found that the *d*-isomers of monomethyl-, dimethyl- and trimethyltocols emerged in that order and were readily separated at 205C on the SE-30 column (Fig. 1). The retention times of *d*-gamma- and *d*-beta-tocopherols were too similar to permit resolution on this column and another dimethyltocol, *dl*-zeta₂-to-

copherol was also eluted at approx the same time. The optical isomers of *dl*-zeta₂-tocopherol and of *dl*-alpha-tocopherol appeared to have identical retention times. Alpha-tocopherol acetate was eluted after alpha-tocopherol and these two compounds were cleanly separated. Retention times show in Table I.

The retention times of the tocopherols were also determined on a column of 3% QF-1-0065 on siliconized Gas Chrom P at 195C (Table I). Delta-, beta- and alpha-tocopherols were separated on this column but not as well as on SE-30. The order of elution of gamma- and beta-tocopherol was reversed but their retention times on QF-1-0065 were nearly identical. Alpha-tocopheryl acetate had a greater relative retention time on this column.

Vitamin K_1. The chromatographic behaviour of vitamin K_1 on these columns was also investigated. Two peaks were obtained on the SE-30 column with retention times of 41.7 and 50.3 min and peak areas bearing a ratio of 4:1. On QF-1-0065 the same preparation gave a single peak with a retention time of 27.8 min. It is not known whether the second peak observed on the SE-30 column was due to an impurity in the preparation or whether it resulted from alteration on the column.

Vitamin A. The chromatographic behaviour of all-*trans* retinol and its acetate ester was investigated with the SE-30 column. Since vitamin A has a shorter side-chain than the tocopherols, it was neces-

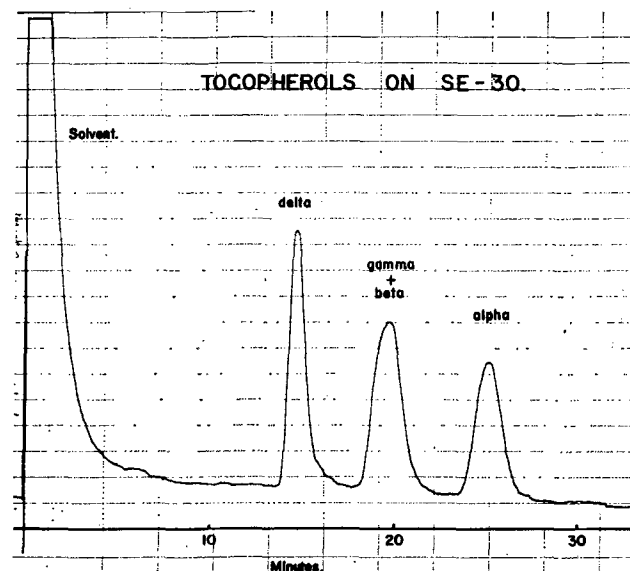
FIG. 1. Gas chromatographic separation of tocopherol isomers. Conditions used for analysis show in Table I. The column load was approx 0.5 μg of each isomer.