Quantification of Carbonyls Produced by the Decomposition of Hydroperoxides

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Carbonyls produced by the decomposition of cyclohexene hydroperoxide and various hydroperoxides of linoleic and linolenic acids and their methyl esters were determined by gas chromatography of the 2,4,6-trichlorophenylhydrazones. The effect of temperature, iron and copper ions, ethanol and several antioxidants on the rate of decomposition, the nature of the products and their yield was observed. The hydroperoxides of methyl esters decomposed more slowly than those of free fatty acids. Ethanol slowed, and metal ions accelerated the rates of decomposition. Metal ions, especially copper, increased the yield and complexity of the carbonyls formed, but ethanol decreased carbonyl yields.

Antioxidants and decomposition temperatures changed the relative yields of carbonyls produced. The 9- and 13-hydroperoxides of linoleic acid gave similar carbonyls, but those of linolenic acid did not. The carbonyl mixtures produced from autoxidized fatty acid methyl esters were more complex than those produced from lipoxygenase-treated fatty acids.

A number of studies have attempted to measure the amounts of carbonyls produced by the decomposition of fatty acid hydroperoxides. Several studies were based on the formation of 2,4-dinitrophenylhydrazones of the carbonyls and the separation of these by column and thin-layer chromatography procedures (1–5). Other studies were based on gas chromatography of volatiles produced by distillation (6), purging (7) or decomposition of the hydroperoxides in the injection ports of gas chromatographs (8–11). Some attempts have been made to study the effect of various factors on the yields of carbonyls, but the conditions that could be tested have been limited by experimental difficulties. The analysis of carbonyls as 2,4,6-trichlorophenylhydrazones (TCPH) by the method of White and Hammond (12) has the advantage of allowing the measurement of carbonyls in small samples with minimum artifact formation because of the decomposition of hydroperoxides during the analysis. This method facilitates studies of solvent and antioxidant effects on carbonyl yield.

This paper gives a quantitative analysis of the carbonyls produced by the decomposition of several hydroperoxides diluted in hydrocarbon solvents and the effect of temperature, metal ions, antioxidants and alcohol on the results.

METHODS

Tricaprin was synthesized by interesterification of methyl caprate and triacetin with 5% sodium methoxide catalyst. The tricaprin was purified by chromatography through alumina (13).

Hexane was purified by distillation and Florisil treatment following the method used for cyclohexane by White and Hammond (12). Ether was reacted overnight with lithium aluminum hydride at ambient temperature and freshly distilled.

Cyclohexene hydroperoxide was produced by the method of Gunstone *et al.* (14). 13-Linoleic and 13-linolenic acid hydroperoxides were made by the method of Gardner (15), and the 9-linoleic and 9-linolenic acid hydroperoxides were made by the method of Galliard and Phillips (16) as adapted by Chan *et al.* (8). The hydroperoxides of methyl linoleate and methyl linolenate were produced by oxidizing 1-g samples of the esters at 37°C in a 40-ml beaker in the dark. When the peroxide values reached 1000–1500, the hydroperoxides were isolated by thin-layer chromatography on 0.5-mm silica gel GF plates developed with hexane/diethyl ether (70:30, v/v). The hydroperoxides were detected with ferrous thiocyanate (14), eluted with methanol and stored at −20°C. The methanol was removed under a stream of nitrogen at ambient temperature before use. Cyclopentene carboxaldehyde was prepared by periodate-osmium tetroxide oxidation of cyclohexene according to Pappo *et al.* (17) and purified by thin-layer chromatography on silica gel by using ether/hexane (45:55, v/v) as a developing solvent. Other materials were purchased and used as received.

Peroxide values were determined by the method of Hamm *et al.* (18) except that the tetrachloroethane was purified as described by White and Hammond (12).

Excess cyclohexene hydroperoxide (70 mg) was dissolved in tricaprin (15 g) held in a water bath at 31–33°C to keep the tricaprin liquid. The solution was centrifuged, and the supernatant, which contained about 3 mg of hydroperoxide/g of tricaprin, was distributed into 28 Pyrex glass test tubes (10 mm, 80 mm). The test tubes were prepared from glass tubing that was soaked in alcoholic potassium hydroxide overnight and washed thoroughly with distilled water. The tubes were flushed with nitrogen and sealed under vacuum with a torch. The tubes were incubated in the dark at various temperatures. Duplicate tubes were taken for analysis. To test the effect of caprylic acid, monoolein and copper acetate on the decomposition of cyclohexene hydroperoxide, these substances were added at the rate of 1% of the weight of the tricaprin.

The fatty acid and methyl ester hydroperoxides were decomposed in saturated solutions in dodecane. About 180 mg of each peroxide was mixed with 20 ml of dodecane at room temperature and centrifuged to separate the excess peroxide. The upper phase was distributed to 35 test tubes (0.4 ml/tube), which were flushed with nitrogen and sealed under vacuum as before. The tubes were incubated at various temperatures, and two tubes were taken for analysis at each condition. To test the effect of propyl gallate (PG), tert-butylhydroquinone (TBHQ), copper acetate and ferrous acetate on the decomposition, an amount of each additive that was equimolar with the hydroperoxide was dissolved in a volume of ethanol equal to 5% of the volume of dodecane.

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After suitable incubation, the tubes were opened and 0.1-0.2 g of sample was weighed into a 50-ml round-bottom flask containing 11 mg of 2-octanone (2-heptanone in the case of cyclohexene hydroperoxide) as an internal standard, 0.1 g trichlorophenylhydrazine, 0.5 g Florisil and 15 ml ether. The ether was evaporated at 25°C with a rotary evaporator. The residue was slurried with hexane and transferred to a 11-mm × 33-cm column containing 9.5 g Florisil. The column was washed with 75 ml hexane/ether (99:1, v/v). The first 30 ml of eluate were discarded, and the remainder was concentrated to 3 ml in a rotary evaporator, transferred to a 15-ml centrifuge tube and evaporated to 60 μl at 30°C under a stream of nitrogen.

For identification purposes, the TCPHs of 2-ketones (C4-C10), aldehydes (C3-C10), 2-enals (C5-C10) and 2,4-dienals (C6-C10) were prepared in the same way as used for the carbonyls from hydroperoxides.

For the cyclohexene hydroperoxide samples, gas chromatography was done on a Varian 1520 fitted with a 30-m SE30 capillary column and a flame ionization detector. For fatty acid hydroperoxides, a Varian 3700 fitted with a 15-m SPB-1 column was used. Temperatures were programmed from 50° to 250°C at 12°C/min. Mass spectra were obtained on a Finnegan (Sunnyvale, CA) 400 gas chromatograph-mass spectrometer fitted with a SE30 capillary column.

RESULTS AND DISCUSSION

Under the gas chromatographic conditions used in these studies, n-aldehydes were observed to give double peaks. The first of the two peaks was about 5 times greater than the second. Only one peak was given by 2-ketones, 2-enals and 2,4-dienals. Mass spectra for the two peaks obtained from aldehydes were identical. Gas chromatography has been reported to give double peaks for 2,4-dinitrophenylhydrazones (19,20). Johnson and Hammond (21) reported double peaks with TCPHs when metal, but not glass, columns were used. White and Hammond (12) did not experience double peak formation with TCPHs. These double peaks have been attributed to *syn-* and *anti*-isomers, and their formation is said to vary with the solvent used for injection into the gas chromatograph (19). It may be that the use of hexane as a solvent in these studies rather than the cyclohexane used by White and Hammond encouraged the formation of double peaks. For these analyses, the areas of double peaks were summed.

Preliminary results indicated that, although the response of the gas chromatograph was linear with the amount of carbonyl in the sample, the response varied significantly for the various types of carbonyls and decreased with the chain length of the carbonyl. Attempts to increase the yields of carbonyl TCPHs by varying reaction and GC conditions were not effective. Thus, correction factors were needed for 2-cyclohexenone (9.1), nonadienal (2.22) and 2,4-decadienal (4.0). These factors were multiplied times the peak heights to relate these compounds to equal weights of internal standard. Other known compounds did not require correction factors. The 2,4-nonadienal factor was used to correct unknown peaks with retention times similar to 2,4-nonadienal.

White and Hammond (12) reported that the reaction of hydroperoxide with the reagent during the formation of the TCPHs resulted in the formation of a small additional amount of carbonyl. To avoid including any carbonyls formed from the hydroperoxides during derivatization in our analytical results, either the hydroperoxides were incubated long enough so that decomposition was complete before the samples were reacted to form derivatives, or the results were corrected. To make this correction, the amounts of carbonyls formed by the reaction of the initial amount of hydroperoxide with the reagent was determined, and the amount of hydroperoxide remaining in the reaction mixture was determined from the peroxide value. The amount of the carbonyl proportional to the residual hydroperoxide was subtracted from the amount determined in the samples. The chief carbonyl found in these blanks was hexanal, although in a few instances, shorter chain lengths also were present. Total yield was ~5.5 mol % of the residual hydroperoxide.

Cyclohexene hydroperoxide. We had hoped to use cyclohexene hydroperoxide to study the yield of the scission reaction, but when cyclohexene hydroperoxide was decomposed in tricaprin at 55°, 80° and 130°C for times varying from 12 to 170 hr, 2-cyclohexenone was the only product that could be detected. An expected scission product, cyclopentene carboxaldehyde, was synthesized, and the derivative was shown to separate from that of cyclohexenone. The results indicated that the yield of scission product had to be less than 1% to escape detection. The addition of caprylic acid, monolein and copper acetate did not affect the outcome. The molar yield of 2-cyclohexenone from the hydroperoxide was about 60%.

Linoleic acid hydroperoxides. The carbonyls produced by the decomposition of the 9- and 13-hydroperoxides of linoleic acid at various temperatures are shown in Tables 1, 2 and 3. Duplicate analyses such as these

TABLE 1

Yields (Mol %) of Carbonyls Produced from Linoleic Acid 13-Hydroperoxide[a] Decomposed at Various Temperatures

Temp. of incubation	Time of incubation	% Decomposition	Hexanal (Mol %)[b]	2,4-Decadienal (Mol %)[b]
40°C	6 days	50%	10.4 A	0.0 A
55°C	6 days	100%	11.7 A	1.3 A
80°C	96 hours	100%	8.5 A	4.3 B
160°C	6 hours	100%	7.1 B	4.1 B

[a] Concentration of the hydroperoxide in dodecane was 4.2 mg/g.
[b] Means that share the same letter are not significantly different ($p < 0.05$).

TABLE 2

Yields (Mol %) of Carbonyls Produced from Linoleic Acid 9-Hydroperoxide[a] Decomposed at Various Temperatures

Temp. of incubation	Time of incubation	% Decomposition	Hexanal (Mol %)[b]	2,4-Decadienal (Mol %)[b]
40°C	6 days	40%	11.7 A	0.0 A
55°C	6 days	80%	10.5 A	0.0 A
80°C	96 hours	100%	7.2 A	5.6 B
160°C	6 hours	100%	5.5 B	5.7 B

[a] The concentration of the hydroperoxide in dodecane was 3.1 mg/g.
[b] Means that share the same letter are not significantly different ($p < 0.05$).

TABLE 3

Yields (Mol %) of Unidentified Carbonyls Produced from Linoleic Acid Hydroperoxides Decomposed at 160°C for 6 hr

	Peak no.				
	3	4	5	6	
	Elution temperature				
	223°C	225°C	227°C	230°C	Total
13-Hydroperoxide	2.0	1.5	1.3	2.0	6.8
9-Hydroperoxide	2.6	1.7	1.7	3.5	9.5

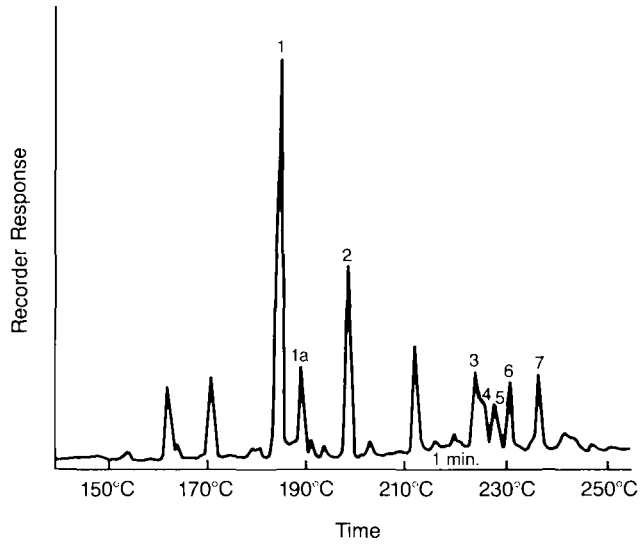

FIG. 1. Chromatogram of a typical TCPH separation, in this instance decomposition of linoleic acid hydroperoxide at 160°C. Peaks 1 and 1a are the two hexanal TCPHs. Peak 2 is 2-octanone TCPH. Peak 7 is 2,4-decadienal TCPH. Peaks 3-6 are unknown carbonyls formed at high temperatures or in the presence of metal ions. The unnumbered peaks near 163° and 170°C are carbonyls from the solvents. The peak near 212°C is an impurity from the TCPH.

generally agreed within 10%. Figure 1 shows a typical chromatogram of the TCPH separation. Hexanal was the only carbonyl detected at an incubation temperature of 40°C. Produced at an incubation temperature of 55°, 80° and 160°C was 2,4-decadienal. At 160°C, four additional carbonyl peaks were detected that did not belong to any of the series for which we had standards. Hexanal and 2,4-decadienal are the expected products from the scission of the 13- and 9-hydroperoxides, respectively, but the results show that both carbonyls were produced from each of the hydroperoxides. This is in agreement with the results of Chan *et al.* (8), who decomposed these hydroperoxides in the injection port of a gas chromatograph at 160°C. They suggested that rapid isomerization between the two hydroperoxides at 160°C accounted for their giving the same scission products. They showed that such isomerization occurs readily at low temperatures (22). Probably isomerization of our hydroperoxides occurred during their preparation, purification and incubation. The almost exclusive formation of hexanal at low temperatures and the production of 2,4-decadienal at higher temperatures also has been reported by previous investigators (2,6).

The yields of carbonyl (5.5 to 11.7% for hexanal, 0 to 6% for 2,4-decadienal) were much lower than the 67 to 80% reported by Chan *et al.* (8) on decomposing these compounds in a gas chromatograph injection port at 160°C. Other reports of yields of 10 to 20% (1,2) and 5% (4) are closer to ours.

At 160°C, the 9- and 13-linoleic acid hydroperoxides produced considerable amounts of four additional carbonyls that were not produced at lower temperatures. These carbonyls had retention times that did not correspond to those of any of the homologous series of carbonyl standards, but their retention times were in the general vicinity of those of a nine-carbon carbonyl derivative. Since there was no standard for these compounds, in calculating their yield, we assumed that their yield was similar to that of 2,4-nonadienal.

The analyses of other researchers do not suggest compounds that account for these products (1-3,8,10). The ω-oxofatty acids produced from the chain scission evidently are not recovered in our procedure.

Linoleic acid hydroperoxides were decomposed at 55°C for 6 days in the presence of equimolar amounts of copper or ferrous acetate. The copper and iron acetates were added in ethanol solution, so an equal amount of ethanol was added to the control containing hydroperoxides without metal additives. The results in Table 4 show that the metal ions gave greater yields of hexanal and 2,4-decadienal than did the controls, and the metal ions also caused the production of the same four unknown carbonyls found with 160°C thermal decomposition. Copper gave greater yields of all these carbonyls than iron. Kimoto and Gaddis (2) obtained good yields of 2,4-decadienal when they decomposed trilinolein hydroperoxide in sealed tubes at 85°C with copper stearate.

Comparison of the results in Table 4 with those in Table 1 indicate that 5% ethanol in the reaction mixture reduced the yields of hexanal. Similar results are seen in Table 5.

Primary antioxidants were dissolved in either alcohol (PG and TBHQ) or dodecane (butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], alpha-tocopherol) in an amount equimolar with the 13-hydroperoxide of linoleic acid and heated to 55°C for 6 days. A comparison of the results in Table 5 and Table 1 shows that ethanol retarded the decomposition of the hydroperoxide. Presumably, this effect is brought about by hydrogen bonding between the ethanol and hydroperoxide. This result was surprising since Mistry and Min (23) reported that monolinolein accelerated the oxidation of soybean oil. Tocopherol and TBHQ were most effective in accelerating hydroperoxide decomposition. Such accelerated decomposition of peroxides by antioxidants in the absence of oxygen has been noted before (24-27). Hexanal and 2,4-decadienal were the only carbonyls that could be identifed. All the antioxidants tended to decrease the amount of hexanal formed, but this was statistically significant only for BHA and tocopherol. PG and TBHQ significantly increased the amounts of 2,4-decadienal. Other antioxidants decreased 2,4-decadienal but not significantly. Antioxidants might reduce the amount of carbonyls formed by radical addition of alkoxy radicals

TABLE 4

Yields (Mol %) of Carbonyls Produced from Linoleic Acid 13-Hydroperoxide[a] Decomposed in the Presence of Metal Ions at 55°C

Trial	Hexanal[b]	2,4-Decadienal[b]	Unknown carbonyls				
			223°C	225°C	227°C	230°C	Total
None[c]	6.3 B	0.9 A	—	—	—	—	1.5
Cu^{++} Acet.	9.1 A	4.7 B	8.9	6.3	7.6	10.6	33.4
Fe^{++} Acet.	7.6 B	2.0 C	3.0	1.9	2.7	5.6	3.2

[a]The concentration of the hydroperoxide in dodecane was 3.1 mg/g.
[b]Means that share the same letter are not significantly different ($p < 0.05$).
[c]5% ethanol was added.

TABLE 5

Yields (Mol %) of Carbonyls Produced from Linoleic Acid 13-Hydroperoxide[a] Decomposed in the Presence of Various Antioxidants at 55°C

Trial	% Decomposition	Hexanal[b]	2,4-Decadienal[b]
None (dodecane)	100%	11.7 A	1.3 A
BHA (in dodecane)	73%	7.9 B	0.4 A
BHT (in dodecane)	63-67%	9.9 A	0.4 A
α-Toco.	100%	5.6 B	0.3 A
None (ethanol)[c]	30-35%	6.3 B	0.9 A
PG (in ethanol)	73%	5.9 B	3.2 B
TBHQ (in ethanol)	100%	4.5 B	2.5 B

[a]The concentration of the hydroperoxide in dodecane was 3.1 mg/g.
[b]Means that share the same letter are not significantly different ($p < 0.05$).
[c]5% ethanol was added to facilitate the solution of PG and TBHQ which were not very soluble in dodecane.

TABLE 6

Carbonyl Yields from the Decomposition of Methyl Linoleate[a]

Aldehydes	Carbonyl yields (mol %)	
Me 18:2-OOH	160°C (100% decomposition)	55°C (5% decomposition)
Pentanal	1.1	—
Hexanal	7.8	8.4
2-Heptenal	1.1	—
2,4-Decadienal	5.8	—

[a]Concentration of the hydroperoxide in dodecane = 4.4 mg/g.

TABLE 7

Carbonyl Yields from the Decomposition of Linolenic Acid 13-Hydroperoxide[a]

Aldehydes	Carbonyl yields (mol %)	
18:3-13-OOH	160°C (100% decomposition)	55°C (80% decomposition)
2-Pentenal	2.5	6.9
2/3-Hexenal	3.2	—

[a]Concentration in dodecane = 2.3 mg/g.

TABLE 8

Carbonyl Yields from the Decomposition of Methyl Linolenate[a] Hydroperoxides

Aldehydes	Carbonyl yields (mol %)	
Me 18:3-OOH	160°C (100% decomposition)	55°C (5% decomposition)
Propanal	4.0	3.6
Butanal	tr. (<1%)	tr. (<1%)
2-Butenal	1.0	—
2-Pentenal	1.3	—
2-Hexenal	tr. (<1%)	—
2,4-Heptadienal	3.7	4.1

[a]Concentration in dodecane = 5 mg/g.

to the antioxidants (28) or by reduction of the alkoxy radicals to alcohols. Seemingly antioxidants also influence the direction of cleavage of the alkoxy radicals. In addition to the ability of antioxidants to stop chain reactions, food technologists need to consider their effect on the kind and amounts of carbonyls produced.

The hydroperoxides isolated from autoxidized methyl linoleate also were decomposed at 55° and 160°C for 6 days and 6 hr, respectively. Table 6 shows that, compared with linoleic acid hydroperoxides, the methyl esters were much more stable, and only 5% were decomposed after 6 days. It is well-known that free fatty acids accelerate oxidation, probably by catalyzing the decomposition of hydroperoxides (29,30). The major decomposition products of the methyl esters were hexanal and 2,4-decadienal in yields comparable to those produced by the linoleic acid hydroperoxides. In addition, there were significant amounts of pentanal and 2-heptenal. Possibly 2-heptenal might arise from the decomposition of the 12-linoleic acid hydroperoxide formed by photoxidation of linoleic acid. Our autoxidation was carried out in the dark, but light was not rigorously excluded during the isolation of the hydroperoxides. The formation of pentanal cannot be explained by classical cleavage schemes. Frankel *et al.* (10) have reported all these products in the decomposition of autoxidized and photoxidized methyl linoleate in the injection port of a gas chromatograph. The four unknown carbonyls produced at 160°C from the decomposition of linoleic acid hydroperoxides and reported in Table 3 were not produced from the methyl ester hydroperoxides decomposed at this temperature.

Linolenic acid hydroperoxide. Linolenic acid 9- and 13-hydroperoxides were decomposed at 55° and 160°C for 6 days and 6 hr, respectively. The results are shown in Table 7. No detectable carbonyls were obtained from the 9-isomer. Peers *et al.* (11) identified 2,4,7-decatrienal from the thermal decomposition of this isomer. There is no reason to think that our method would not detect decatrienal, but we had no pure carbonyl with which to confirm this.

The 13-hydroperoxide yielded 2-pentenal and 2-hexenal at 160°C and only 2-pentenal at 55°C. The expected product from classical scission is 2-hexenal. Seemingly, the equilibration found between the linoleic acid hydroperoxide isomers does not occur with the linolenic acid hydroperoxide isomers. This agrees with the results of Peers *et al.* (11), although Grosh (5) found similar products (primarily propanal, 2-pentenal and 2-hexenal) from both the 9- and 13-linolenic acid hydroperoxides decomposed in the presence of ascorbic acid.

Table 8 also shows the results from the decomposition of the hydroperoxides isolated from the autoxidized methyl linolenate. At 160°C, propanal and 2,4-heptadienal were the major products, with smaller amounts of 2-pentenal and 2-butenal and traces of butanal and 2-hexenal. Frankel *et al.* (10) found all these products in the thermal decomposition of methyl linolenate hydroperoxides produced by autoxidation. At 55°C, only propanal, 2,4-heptadienal and a trace of butanal were found. Only traces of the 2-hexenal expected from the 13-hydroperoxide, and none of the 2,4,7-decatrienal expected from the 9-hydroperoxide was found. Although the formation of 2,4-dienals was favored in the 160°C-decomposition of linoleate hydroperoxides compared with decomposition at 55°C, the amount of 2,4-heptadienal produced from linolenate hydroperoxides is similar at the two temperatures.

The yield of carbonyls from linolenate seems to be somewhat less than that from linoleate. This does not help account for the great flavor-generating ability of linolenyl groups in autoxidized vegetable oils (31).

ACKNOWLEDGMENTS

Journal Paper No. J-13062 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2799.

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[Received May 5, 1988; accepted March 4, 1989]
[J5458]