

A Characterization of Volatile Carbonyl Compounds Isolated from Meat Fat Subjected to Gamma Radiation^{1, 2}

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IN RECENT YEARS considerable attention and research effort have been given to the problem of "cold," or irradiation sterilization, of foods as a possible commercial processing technique. Major attention, at present, has been concentrated on the use of radioactive materials that are primarily *gamma* ray emitters. Spent fuel rods from atomic reactors and Cobalt-60 have been the principal sources of such radiation.

While there has been little impetus to investigate the irradiation sterilization of simple fat products, such as margarine or shortening, there has been a great interest in complex, fat-containing foods, such as meat and condensed milk. It was early recognized that sterilizing dosages of *gamma* radiation created a definite problem of off-odors and flavor in many meat products. Two degradation products, methylmercaptan and hydrogen sulfide, occur in minute quantities in irradiated meat (2, 11). Carbonyl compounds are present in much larger quantities than are the sulfur compounds. Of these carbonyl compounds, only a small percentage are of lipide origin (3). Typical concentrations in ground beef irradiated with 2 megarads of *gamma* radiation are: mercaptan 0.03, hydrogen sulfide 0.01, benzene-soluble carbonyls 2.4, and acid-salt soluble carbonyls 64 millimoles/kg. At 10 megarads the concentrations are 0.13, 0.08, 3.0, and 172 millimoles/kg. respectively (3, 19).

It has been suggested that compounds formed by the peroxide-catalyzed condensation of sulfur compounds with *alpha-beta* unsaturated aldehydes (23) are precursors to important components of the odor of irradiated meat. Numerous reports have appeared, stating that 2-enals are formed by the autoxidation of fats and oils (4, 7). An interest in the off-odor and flavor of irradiated meat has therefore led to a need for the characterization and identification of the volatile carbonyl compounds formed by the *gamma* irradiation of meat fat.

Experimental

Sources of radiation used in this work were the Cobalt-60 source at the Argonne Cancer Hospital of the University of Chicago and the spent fuel rod source of the Argonne National Laboratory, Lemont, Ill. The cobalt source had an output of approximately 333,000 rad per hour of *gamma* radiation during this period while the radiation intensity of the spent fuel rod source was dependent on the age of the rods and the position of the samples in the lattice. Variations between 1-6 megarads per hour were normal for this source. While the radiation spectra of these rods had a high peak of low energy *beta* radiation, the energy

reaching the inside of the containers was pure *gamma* radiation.

Hog back-fat extracted with hexane was used throughout these experiments. When amino acids were irradiated with the fat, one ml. of a saturated solution of the amino acid hydrochloride was added to 9 ml. of the melted fat; the sample was vigorously agitated and immediately solidified in a dry ice acetone bath. These samples were loosely stoppered, sealed in a number 2 tin can, and irradiated at 2-10 megarads. Samples for carbonyl isolation were irradiated at 10 megarads. Quantitative analyses were performed according to the procedure of Henick *et al.* (10). Calculations for this procedure were based on the modified equations of Chipault *et al.* (6). Material for qualitative analyses was obtained by vacuum steam distillation of one-pound samples at 180°C. at 100 microns' pressure in the deodorization apparatus of Bailey and Feuge (1). Volatiles were collected in dry ice acetone condensers, and the volatile carbonyl compounds seldom reached the second condenser. Occasionally liquid nitrogen was used as the coolant.

Following distillation, the apparatus was brought to room temperature and the condensate was washed from the condensers with a solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. After 24 hrs. the 2,4-dinitrophenylhydrazones were removed by filtration. The derivatives from several steam distillations were pooled in chloroform, and 25-ml. aliquots containing 246 mg. of solid material (600 μ moles) were placed on 45 x 4.5-cm. chromatographic columns, packed with a 2:1 mixture of silicic acid and celite.

Duplicate chromatographic columns were placed on a fraction collector, and a succession of bands were eluted with hexane, hexane-diethyl ether, benzene, and benzene-methanol. Fifteen-ml. fractions were collected continuously until less than 10 μ moles of carbonyl remained on the columns. This required three to four weeks, during which time 18 liters of eluate were collected from each column. An elution diagram was drawn by assaying appropriate tubes, quantitatively, for saturated and unsaturated carbonyls. Absolute molar concentrations of each component were estimated from this elution diagram by use of a differential, compensating, polar planimeter. All concentrations were then converted to mole percentages. These concentrations were subsequently corrected for the dicarbonyl content of the sample.

The various mono 2,4-dinitrophenylhydrazone fractions were usually rechromatographed twice on silicic acid-celite columns. During the final elution of monocarbonyls the columns were placed on a fraction collector, and the selection of material for subsequent analyses was based on elution diagrams. The monocarbonyls were tested in the paper chromatographic system of Horner and Kirmse (13), equilibrated according to the suggestions of Sundt and Winter (21). Saturated ketone and aldehyde 2,4-dinitrophenylhydrazones were distinguished by the procedure of

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Jones, Holmes, and Seligman (14). Samples were dissolved in 0.25 *N* alcoholic sodium hydroxide, and the extinction at 520–535 μ was determined after 0 and 90 min.

Crotonal was chromatographed on Whatman No. 1 paper with the methanol-heptane system of Meigh (15) and Heulin (12). A 240- by 5.0-cm. chromatographic chamber was used. Dicarboxyls were examined in the paper chromatographic system of Schmidt, Moriconi, and O'Connor (18). Residual fractions were tested in a variety of keto acid systems (16) and in the semialdehyde system of Nonaka (17).

All visible range spectrophotometric measurements were made in a Beckman model DU spectrophotometer. Infrared spectra were determined on samples in potassium bromide disks in a Perkin-Elmer model 21 infrared spectrophotometer. Melting points were taken on a Fisher-Johns block and are uncorrected.

Results

Seven saturated and three unsaturated aldehydes, comprising 77.5-mole percentage of the total volatile carbonyl compounds isolated from *gamma* irradiated lard, have been characterized (Table I). It is recog-

TABLE I
Monocarboxyl Compounds from *Gamma* Irradiated Lard

Compound	Mole %
Propional.....	0.9
Butanal.....	5.4
Pentanal.....	1.5
Hexanal.....	5.9
Nonanal.....	5.2
Decanal.....	4.7
R _f 0.85 ^a	2.0
Acrolein.....	0.3
Crotonal.....	3.5
2,4-Undecadienal.....	48.1

^a R_f in the system of Horner and Kirmse (13), equilibrated according to Sundt and Winter (21).

nized that because of the extensive distillation employed, some of these volatile compounds may have been formed by decomposition of lipide peroxides. In view of this and the difficulty of stripping all the volatiles from the fat, quantitative results are expressed in terms of the isolated materials. On the 45-cm. silicic acid-celite (2:1) chromatographic columns used for the fractionation of the 2,4-dinitrophenylhydrazones of these aldehydes, the decanal band tended to be rather diffuse but was readily separated from the nonanal band. The R_f values determined by Forss, Dunstone, and Stark (8) for the series of 2-enals and the R_f value determined for the single 2,4-dienal in this study indicate that the most difficult mixtures to resolve are not those composed of adjacent members of the same homologous series. Both nonenal and undecadienal, for instance, have R_f values intermediate between hexanal and heptanal, hence it is apparent that members of two different homologous series may have more nearly similar R_f's than adjacent members of either of the series. Undecadienal and hexanal were only partially separated by one pass through a chromatographic column. The use of elution diagrams greatly reduced the number of times this fraction had to be rechromatographed to attain a reasonable degree of purity.

When the various saturated monocarboxyl fractions, obtained by column chromatography, were dissolved in 0.25 *N* alcoholic sodium hydroxide for

analysis by the procedure of Jones *et al.* (14), all samples gave E_t/E₀ values less than 0.70 after 90 min. None were found to contain ketone derivatives. Inaccurate results were obtained if the samples were added to the alkali in tetrahydrofuran rather than chloroform. The error apparently resulted from the presence of hydroquinone in the tetrahydrofuran. This procedure is not applicable to unsaturated aldehydes and ketones.

A variety of systems have been reported, which permit the identification of 2,4-dinitrophenylhydrazones by filter paper chromatography. Only those systems using paper impregnated with an organic stationary phase were found to allow resolution of long chain carbonyls. Horner and Kirmse (13) stated the upper limit of their dimethylformamide-decalin system to be octanal. Sundt and Winter (21), without presenting any data, claimed that this limit could be increased by proper equilibration of the solvents in the chromatographic chamber. The original R_f values reported by Horner and Kirmse are shown in column one of Table II. When the chromatographic chamber was lined with Whatman No. 3 filter paper soaked in dimethylformamide saturated with decalin, a beaker of decalin saturated with dimethylformamide placed in the bottom of the chamber, and the chromatogram (Whatman No. 1 filter paper impregnated with dimethylformamide) equilibrated in the chamber for 18 hrs. before addition of the developing solvent, the R_f values in column two were obtained.

From the data in column three it will be seen that the R_f values changed markedly over a period of one week if the same lining sheets were left in the chromatographic chamber. When long-chain derivatives were being tested, it was necessary to reline the chamber daily. Short chain carbonyls however were best resolved in the poorly equilibrated system. A consistent equilibration procedure gave reproducible R_f values from day to day. In practice however a sequence of reference compounds was included on every sheet chromatographed. The apparent upper limit of the system under these conditions was dodecanal.

An unsaturated compound was found having an R_f identical to acrolein in the systems of Horner and Kirmse (13) and of Meigh (15) or Heulin (12). Since a three-carbon unsaturated ketone does not exist, it was presumed that the compound was the aldehyde. A similar assumption could not be made in the case of the fraction corresponding to crotonal. The 2,4-dinitrophenylhydrazones of ketones are stated to have slightly different R_f's from those of the cor-

TABLE II
R_f Values in the Paper Chromatographic System of Horner and Kirmse

System used	1	2	3
Methanal.....	.18	.09	.15
Ethanal.....	.28	.14	.24
Acrolein.....	..	.16	.28
Crotonal.....	.32-.46	.19	.33
Propional.....	.46	.21	.38
Butanal.....	.60	.51	.51
Pentanal.....	..	.40	.60
Hexanal.....	..	.49	.69
Heptanal.....	.83	.56	.74
Octanal.....	.86	.64	.79
Nonanal.....	..	.69	.81
Decanal.....	.92	.74	.86
Undecanal.....	..	.77	.88
Dodecanal.....	.94

1. Original values of Horner and Kirmse (13), Schleicher and Schull No. 2043b filter paper.
2. Chromatographic chamber, equilibrated according to Sundt and Winter (21), Whatman No. 1 filter paper.
3. Equilibrated chamber, used one week without relining, Whatman No. 1 filter paper.

responding aldehydes in the system of Meigh (15). Acetone and propional are reported to have R_f 's of 0.30 and 0.32, respectively. Comparisons were therefore run on paired 205 x 1.9-cm. strips of Whatman No. 1 filter paper formed by splitting a 3.8-cm. strip. Two four-foot lengths of 6-cm. glass tubing were sealed together to form a chromatographic chamber. When methanal, used as the internal standard had been moved 50 cm. from the origin in 40 hrs., crotonal was found to have been moved 80 cm. from the origin. A crotonal standard and the isolated material gave identical R_f values under these conditions. An internal standard was required in this procedure since after the first 20 hrs. the solvent front could not be detected.

The identification of undecadienal was performed according to classical procedures. After three recrystallizations the isolated material melted at 132.5°C. corresponding to the melting point of 133–4°C. reported by Forss *et al.* (9) for the 2,4-dinitrophenylhydrazone of synthetic 2,4-undecadienal. Ultimate analysis substantiated the formula



Calculated: C—58.95%; H—6.38%; N—16.18%.

Found: C—58.62%; H—6.16%; N—16.09%. The infrared spectra of this sample in a potassium bromide disk had the N—H stretching band located at 3.06 microns (3270 cm^{-1}). According to Jones *et al.* (14), this is convincing evidence that the 2,4-dinitrophenylhydrazone was an aldehyde derivative. In the spectra of 2-undecanone this band was found at 3.01 microns (3322 cm^{-1}).

The α dicarbonyl 2,4-dinitrophenylhydrazones and other polar derivatives from irradiated lard have not as yet been adequately characterized. As may be noted in the partial elution diagram (Figure 1), binary mixtures showing no evidence of separation by column chromatography were frequently observed. While certain of these mixtures were readily resolved by paper chromatography, only the *alpha*-dicarbonyl components gave satisfactory R_f values in any of the available systems. The reported values (Table III) were determined in the system of Schmitt, Moriconi, and O'Connor (18). Dicarbonyl B was found as the saturated component of seven successive fractions. Crotonal, acrolein, the three or four unsaturated dicarbonyls with R_f 's between 0.56 and 0.63 grouped as dicarbonyl C, and the unsaturated dicarbonyl A appeared as the second component of these mixtures.

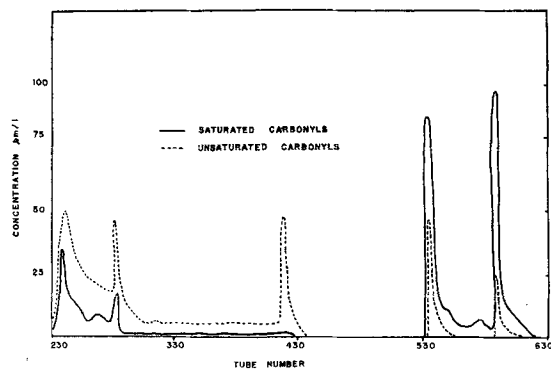


Fig. 1. Partial elution diagram of dicarbonyl 2,4-dinitrophenylhydrazones and other polar materials.

TABLE III
Alpha Dicarbonyls and Other Polar Materials from
Gamma-Irradiated Lard

Compound	R_f^a	Mole %
Dicarbonyl A.....	0.28	2.7
Dicarbonyl B.....	0.43	10.5
Dicarbonyls C.....	0.56–0.63	0.4
Unidentified.....	0.00 or 1.00	8.9
Reference compounds		
Glyoxal.....	0.23
Methanal.....	0.60

^a R_f 's in the system of Schmitt, Moriconi and O'Connor (18).

A number of fractions, both saturated and unsaturated, eluted during the final stages of the development of the chromatographic columns were not identified. These compounds were neither α keto acid derivatives nor *bis* dinitrophenylhydrazones of α dicarbonyls. Negative results were also obtained when these compounds were tested in the semi-aldehyde system of Nonaka (17). The unidentified residue amounted to 8.9-mole percentage of the total volatile carbonyl compounds isolated from irradiated lard.

The 2,4-dinitrophenylpyrazoles of malonidialdehyde, acetylacetone, and glutaridialdehyde were prepared. In the procedure of Henick *et al.* (10) these derivatives displayed the spectral characteristics of saturated hydrazones. Since E_{max} were not determined, it cannot be said to what extent these compounds would interfere with the normal analysis. These derivatives however could be chromatographed in the system of Schmitt *et al.* (18).

When lard was irradiated in the presence of amino acids, the observed percentage of carbonyls was found to be dependent on the nature of the amino acid and on the irradiation dose (Table IV).

TABLE IV
Carbonyl Compounds in Lard Irradiated in the
Presence of Amino Acids

Treatment.....	0 ^a Megarads		2 Megarads		10 Megarads	
	Total ^b carbonyls	% U ^c	Total carbonyls	% U	Total carbonyls	% U
None	3.9	33	6.0	30	10.2	27
Glutamic acid	4.1	32	3.0	23	9.1	21
Tryptophane	3.6	33	7.1	21	11.3	27
Arginine	3.7	32	2.9	24	9.4	25
Cystine	5.0	24	4.6	16	10.9	24
Cysteine	9.6	36	5.4	32	10.6	23
Tyrosine	4.8	27	9.0	22	15.5	22

^a Samples were held three days at 0°C. prior to analysis to duplicate the time interval required to transport irradiated materials to and from the source of radiation.

^b Expressed as millimoles per kilogram.

^c Percentage of total carbonyls found to be unsaturated by the procedure of Henick *et al.* (10), using the equations of Chipault *et al.* (6).

It has been suggested that the increase in both total carbonyl and percentage of unsaturated carbonyl noted when cysteine was in contact with fresh, unirradiated samples resulted from interaction of the sulfhydryl group and the peroxide. This cannot be the case since the peroxide value of the sample irradiated in the presence of cysteine was not lower than that of the control.

A similar phenomenon was encountered when irradiated lard was in contact with liquefied methyl mercaptan for 18–20 hrs. The carbonyl value increased from 6.0 millimoles/kg. to 12.3 millimoles/kg. in the simple case and to 20.6 when carbonyl-free benzene was included as a solvent.

The effect of amino acids on the carbonyl content of irradiated lard was more apparent at a dose of 2 megarads than at 10 megarads. In either case however it was apparent that the presence of amino acids during the irradiation affected the quantity and type of carbonyls formed.

Discussion

While lard is quite rich in oleic acid, it is not surprising that nonanal and decanal comprise only 9.9-mole percentage of the volatile carbonyl compounds isolated in this study. During *gamma* irradiation, as during autoxidation, it is the linoleic acid that is principally attacked and degraded. Hexanal, pentanal, and propional have been previously isolated from oxidized linoleate (5). Forss, Pont, and Stark (9) have isolated the hexa- to undecadienals from milk and cite linoleic acid as the common source of this series of compounds.

Swift *et al.* (22) isolated 2,4-decadienal from cottonseed oil while Stapf and Daubert (20) found the same compound in soybean oil. Forss *et al.* (8) found heptadienal and nonadienal to be the most abundant dienals in milk. No simple explanation is available for the observation that the principal degradation product of linoleic acid varies from fat to fat. Our data would seem to suggest that under the influence of *gamma* radiation the principal site of oxidation in lard is the 8 position of linoleic acid.

Starting from the observation that amino acids influence the quantity and type of carbonyl compounds found in irradiated lard, the interaction of sulfur compounds and *alpha-beta* unsaturated aldehydes was investigated (23). These condensation products were suggested as important precursors for the development of the typical odors noted in irradiated meat. It has been long recognized that *alpha-beta* unsaturated aldehydes are important constituents of off-odor and flavor in oxidatively rancid fats and oils. Now, in the case of irradiated meat, it seems possible that

the acrolein and crotonal derived from the meat fat may combine with the degradation products of the protein to contribute significantly to the off-odor and flavor of the meat product.

Summary

The volatile carbonyl compounds from irradiated lard were isolated and characterized qualitatively and quantitatively as the 2,4-dinitrophenylhydrazones. Propional, butanal, pentanal, hexanal, nonanal, decanal, acrolein, crotonal, and 2,4-undecadienal were identified by paper chromatography and spectrophotometric analysis.

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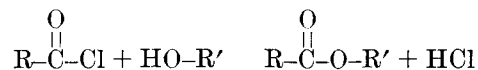
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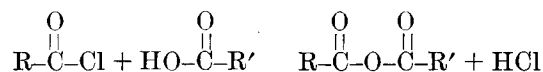
Preparation of Esters and Anhydrides from Long Chain Fatty Acid Chlorides¹

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Fatty acid chlorides combine with alcohols to form esters according to the following general reaction:



Substitution of a free acid for the alcohol results in anhydride formation (1).



These reactions can be made substantially quantitative by removing the HCl formed during the reaction. This has been accomplished in the past almost exclusively by the addition of a tertiary amine, such as

pyridine, quinoline, or trimethylamine, to the reaction mixture. The amine also acts as a solvent for the reactants, either alone or in conjunction with an inert solvent, such as chloroform.

This paper describes the preparation of a number of esters and anhydrides from acid chlorides, in which the reactants are mixed directly and the HCl is stripped from the reaction mixture by operating at reduced pressure. This method is, of course, only applicable to the higher fatty acid chlorides and alcohols of low volatility. Reaction for 1 hr. at 100°C. and 2-mm. pressure gave excellent results. Yields of the order of 90% of the recrystallized product of high purity were obtained for the following compounds, tristearin, pentaerythritol tetrastearate, α -methyl-glucoside tetrastearate, monosaturated triglycerides, stearic anhydride, palmitic anhydride, and a mixed stearic-palmitic anhydride.

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