

AN INVESTIGATION OF THE CARBONYL
COMPOUNDS IN GAMMA IRRADIATED MILK FAT

by

STAMATIOS EVANGELOS PAPAIOANNOU

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1963

APPROVED:

~~Associate Professor of Food Science and Technology~~

In Charge of Major

~~Head of Department of Food Science and Technology~~

~~Chairman of School Graduate Committee~~

~~Dean of Graduate School~~

Date thesis is presented August 8, 1962.

Typed by Jolene Wuest

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. E. A. Day, Associate Professor of Food Science and Technology for his guidance and suggestions in the laboratory and valuable editing of the manuscript.

The author also thanks Mrs. Jane Wyatt, Dr. L. M. Libbey, and Dr. M. W. Montgomery, as well as all his fellow graduate students for their cooperation in the laboratory. Gratefulness is also extended to the Greek Scholarship Foundation, by whose support this work has been done.

TABLE OF CONTENTS

	Page
AN INVESTIGATION OF THE CARBONYL COMPOUNDS IN GAMMA IRRADIATED MILK FAT	1
INTRODUCTION	1
REVIEW OF LITERATURE	4
Autoxidation of Lipids	4
Factors Affecting the Rate of Autoxidation of Lipids	14
Secondary Autoxidation Products	18
Irradiation-Induced Reaction Mechanisms in Lipids	24
Effect of Radiation on Lipids	30
EXPERIMENTAL	42
Preparation and Irradiation of Milk Fat Samples	42
Analyses of the Milk Fat Samples	43
Isolation and Characterization of the Monocarbonyl Compounds from 4.5 and 0 Mrad Milk Fat	47
Characterization of the Volatile Monocarbonyl Compounds of the 4.5 Mrad Milk Fat : Molecular Distillation	49
Characterization of the Volatile Monocarbonyl Compounds of the 4.5 Mrad Milk Fat: Steam Distillation	53

	Page
RESULTS AND DISCUSSION	54
Results of Chemical Tests on the Control and Irradiated Milk Fat	54
Free Carbonyl Compounds from 4.5 Mrad and Control Milk Fat	57
Identification of Volatile Monocarbonyl Compounds Isolated by Molecular Distillation of 4.5 Mrad Milk Fat	62
Identification of Volatile Monocarbonyl Compounds Isolated by Steam Distillation of 4.5 Mrad Milk Fat	67
SUMMARY AND CONCLUSIONS	75
BIBLIOGRAPHY	79

LIST OF TABLES

<u>Table Number</u>		<u>Page</u>
1	Results of Chemical Tests on Milk Fat Irradiated at Different Dosages	55
2	Free Carbonyls from 4.5 Mrad Irradiated and Control Milk Fats	60
3	Changes in the Free Carbonyl Content of Irradiated Milk Fat as Effected by Molecular Distillation	66
4	Change in the Free Carbonyl Content of Irradiated Milk Fat as Effected by Steam Distillation	67
5	Identification of Carbonyl Compounds Obtained by Steam Distillation of 4.5 Mrad irradiated Milk Fat	71

LIST OF FIGURES

Figure		Page
1	Molecular distillation apparatus.	50
2	Chromatogram of the modified acetonitrile partition column for the free carbonyl derivatives of the 4.5 Mrad irradiated milk fat. Saturated alkanal derivatives.	58
3	Standard curve of the modified acetonitrile partition column for separation of 2, 4-dinitrophenylhydrozones.	59
4	Gas chromatogram of the volatiles obtained by molecular distillation of the 4.5 Mrad milk fat.	64
5	Volatile methyl ketones from steam distillation of 4.5 Mrad milk fat.	69
6	Volatile saturated aldehydes from steam distillation of 4.5 Mrad milk fat.	70

AN INVESTIGATION OF THE CARBONYL COMPOUNDS IN GAMMA IRRADIATED MILK FAT

INTRODUCTION

The application of ionizing radiation for the preservation of food has been very promising in the field of food science and industry, because of certain advantages over conventional methods of food preservation. Ionizing radiations at a sterilizing dose of 2 Mrad can kill the most resistant forms of bacteria. This can be achieved only by overcooking of the foods during conventional processing, with the known harmful consequences. However, irradiation of foods shows some serious disadvantages. The main drawback arises from the fact that the ionizing radiations accelerate lipid autoxidation, even at dosages below the sterilization requirement. As a result serious changes in flavor and color occur during irradiation of foods such as milk, meat, and vegetables.

Much work has been done on the radiation of lipids, to find means for either preventing or reducing the undesirable changes to a level that would not be objectionable. Thus, irradiation effects on lard have been investigated in order to solve the problems arising from the radiation-induced off-flavors during radiation preservation of meat.

Irradiation of milk products has been the subject of intense research, especially from the standpoint of the substitution of pasteurization with irradiation preservation of milk. It is well known that pasteurization, though necessary to kill the pathogenic microorganisms and to inactivate the enzymes of milk possesses disadvantages such as destruction of vitamins and development of cooked or coconut flavors. The use of radiation instead of pasteurization would help solve these problems, but the problems of milk lipid oxidation arise. The main objectionable constituents of lipid oxidation off-flavors have been recognized to be carbonyl compounds and much research has been done to analyze and characterize these compounds. Considerable effort also has been devoted to studies of the mechanisms of carbonyl production during radiation-induced oxidation of lipids. Proctor et al. (101, p. 6) reported that off-flavors developed during irradiation of water-free butterfat, or butterfat in aqueous phase were indistinguishable. Thus, radiation studies to minimize the off-flavors developed from irradiated butterfat, will have direct application on the irradiation-preservation of milk.

Objective characterization of the carbonyl compounds from the radiation-induced oxidation of lipids, is one of the routes followed by many research workers. Chipault (14) very recently

summarized the research findings on carbonyl content of several irradiated fats. No work on the identification of the carbonyls formed during irradiation of milk fat was listed in the summary. Proctor et al. (100, p. 1-101; 101, p. 1-13; 102, p. 1-26; 103, p. 1-20; 104, p. 1-28; 105, p. 1-60; 106, p. 1-34; 107, p. 1-84) investigated the effects of ionizing radiations on milk and milk products, especially from the standpoint of the precursors of oxidation. By fractionating milk fat and irradiating the fractions at 1.8 Mrad, he concluded that the radiation off-flavor was due to carbonyl compounds with six to eight carbon atoms (105, p. 33). A complete chemical characterization of the carbonyls from the radiation-induced off-flavor of milk fat, as well as the identification of the whole spectrum of the carbonyls from irradiated milk fat, is lacking in the literature.

The purpose of this investigation was to analyze and identify the carbonyl compounds formed during the irradiation of milk fat at 4.5 Mrad under vacuum, and attempt to ascertain the carbonyls of greatest significance to the radiation off-flavor of milk fat. A number of chemical tests were used to estimate the changes in the fat at different radiation doses, and to determine which of the tests more closely followed the radiation dose and the off-flavor production.

REVIEW OF LITERATURE

Autoxidation of Lipids

A discussion of the autoxidation of lipids is considered necessary in any attempt to review the effects of the ionizing radiations on lipids. This is because of the similarities, generally accepted today, between the autoxidative and the radiation-induced reaction mechanisms of lipids, and the fact that the literature on the autoxidation is more extensive than that on radiation-induced oxidation. A striking example of the above parallel mechanisms is the work of Slover and Dugan (90, p. 335) who found the same four isomeric hydroperoxides produced in approximately the same proportions by autoxidation or gamma irradiation of methyl oleate.

Berzelius (7, p. 294) was the first to recognize lipid oxidation and to study the phenomenon experimentally in 1827. Since then autoxidation has been studied more extensively than any other food deterioration phenomenon, and as techniques and instrumentation have advanced, especially during the last decade, so has our knowledge of the phenomenon advanced. Even so, the lipid autoxidation picture is not complete today, and controversy still prevails relative to the detailed mechanism of the reactions, the nature of

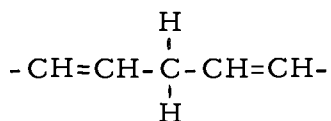
the autoxidation products, and the objective characterization of the resulting off-flavors in lipid containing foods. The lack of full understanding of the autoxidation reaction undoubtedly must be attributed to the complexity of all natural lipid systems, which are mixtures of triglycerides containing many different fatty acids.

To overcome this difficulty the autoxidation reaction mechanisms have been largely studied in model systems of the methyl esters of one fatty acid at a time, and the results of these studies have been applied to the natural lipids.

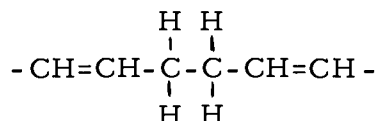
For the purpose of definition, autoxidation is the slow spontaneous catalytic reaction of lipids with atmospheric oxygen with the production of off-flavors. (50, p. 58). The site of oxygen attack was early recognized by Schonbein (85, p. 328-340) as the unsaturated fatty acid portion of the glyceride molecule.

The generally accepted theory of lipid oxidation was developed by Farmer and coworkers (24, p. 228-286; 25, p. 340-348; 28, p. 541-547; 26, p. 121-139; 27, p. 119-122; and 29, p. 10-13), who established the significance of free radical mechanisms, and rejected the earlier theory of additive reaction of polar reagents to the ethylenic bonds.

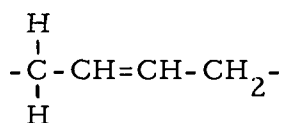
According to Farmer et al. (28, p. 541-547; 30, p. 86-93) the free radical formation from the lipid molecule, depends on the hydrogen lability of the fatty acid portion of the molecule, which in turn depends on the type of unsaturation and the presence or absence of substitution on the carbon atoms of the fatty acid. According to hydrogen lability he distinguished four groups of unsaturation:



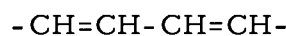
Group I



Group II

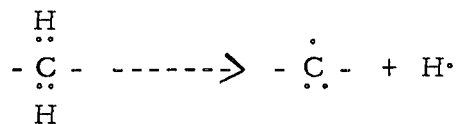


Group III



Group IV

The hydrogen lability decreases from Group I to Group IV, a fact that can be easily explained by the modern theories of organic chemistry. The two double bonds in Group I, by the effect of their unsaturation electrons (π electrons), increase the electron density around the central methylenic carbon atom, facilitating thermodynamically the homolytic cleavage:



and giving the free radical and the hydrogen atom (a free radical itself). The effect of the two double bonds is shared between the two methylenic carbon atoms in Group II, while the effect of one double bond is shared between the two methylenic carbon atoms on both sides of the double bond in Group III. Finally Group IV is stabilized by resonance as a conjugated diene.

It is generally accepted today that autoxidation is propagated through an active α -methylene group and the free radical formed by subtraction of one hydrogen atom from the methylene group sets up a free radical resonance system (68, p. 129).

Farmer et al. (28, p. 597; 27, p. 122) first suggested that autoxidation of unsaturated fatty acid esters starts with free radicals generated by an external energy source such as light, which initiates the chain mechanism. Unsaturated hydroperoxides were primary products of the reaction.

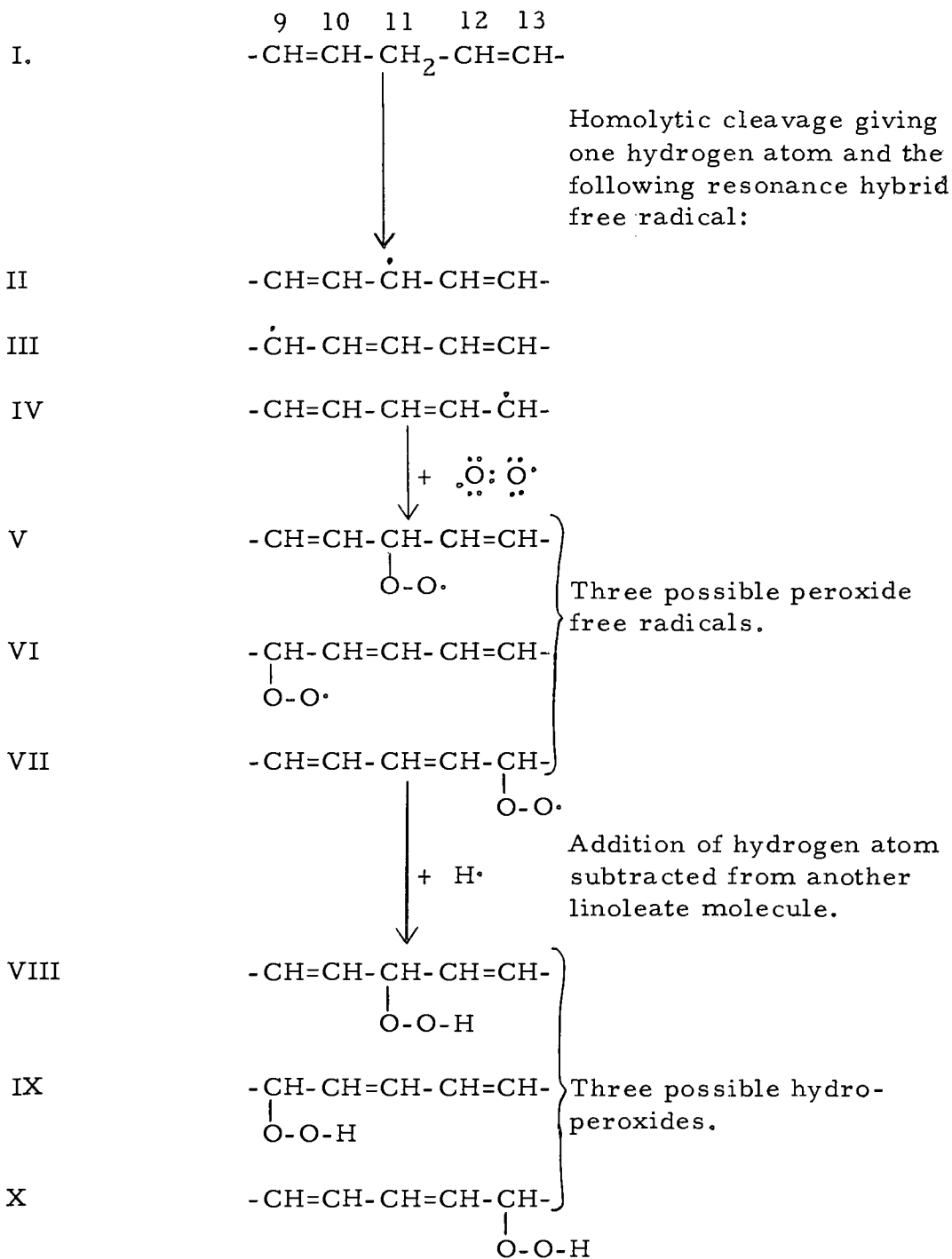
The affinity of the fatty acid ester free radicals towards the oxygen molecule will be best understood if we consider the diatomic oxygen molecule which is strongly paramagnetic as are all free radicals containing an unpaired electron. This

paramagnetism suggests that the oxygen molecule is a diradical having two unpaired electrons (72, p. 567).



Thus the absorption of oxygen by the fatty acid ester free radical is a reaction between two free radicals to form a peroxide free radical, which in turn accepts a hydrogen atom to form a hydroperoxide. Actually the hydrogen atom is more probable to come from another fatty acid ester, which is transformed to a free radical, and so the chain reaction is propagated. Furthermore, the hydroperoxides decompose, especially by light or metal catalysts, to generate more free radicals, which initiate more autoxidation reaction chains.

The detailed picture of the autoxidation reaction depends on the degree of unsaturation of each fatty acid ester. Bolland and Koch (9, p. 445-447) proposed the following autoxidation mechanism for linoleic acid esters which contain Farmer's Group I.



According to this scheme, the two conjugated resonance formulas III and IV, having more resonance energy than the unconjugated formula II, must contribute more to the hybrid, which means that more than two thirds of the possible hydroperoxides will be conjugated. Since geometrical isomerization reactions during the autoxidation tend to favor the trans configuration, considerable portions of trans-trans conjugated dienes are formed (12, p. 451; 76, p. 65).

Experimental evidence for the formation of conjugated diene hydroperoxides from unconjugated polyunsaturated fatty acid autoxidation, has been given by Farmer and Sutton (27, p. 119-122) by absorption measurement in the ultraviolet region, and by Privett et al. (76, p. 65) by infrared absorption techniques. As it was mentioned before the displacement of double bonds occurring during autoxidation can be accounted by the free radical mechanism.

Bolland and Koch (9, p. 445-447) calculated the autoxidation conjugated products approximately as 70 per cent, but later investigators obtained evidence showing 90 or more per cent conjugation (12, p. 447-451; 46, p. 65). Thus the 9- and 13-conjugated hydroperoxides largely constitute the primary products of linoleate ester autoxidation. Indirect evidence for the formation

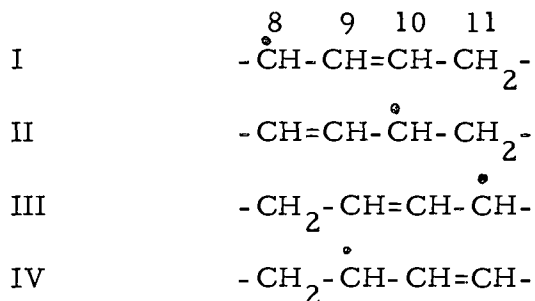
of the 11- unconjugated hydroperoxide in small amounts has been obtained through identification of the carbonyls formed by decomposition of the hydroperoxides (10, p. 238-242; 94, p. 297-300)

Trienoic fatty acids or esters, as linolenates, have not been studied so extensively as dienoic compounds, and some conflicts are found in the literature.

Fugger and coworkers (36, p. 285-289) used countercurrent distribution techniques to fractionate the hydroperoxides of autoxidized linolenate. They found that most of the hydroperoxides produced were dimeric and less than half of the linolenate was converted to conjugated hydroperoxides, while double bonds were destroyed, probably through polymerization.

Privett et al. (77, p. 23-27) studied the autoxidation products of linolenate by infrared spectrometry, and found that all the hydroperoxides were conjugated and 60 per cent were monomeric.

Monoethenoic fatty acids and their esters, such as oleate, give two isomeric hydroperoxides, as shown by Farmer and Sutton (27, p. 119-122). Subsequently Farmer et al. (29, p. 10) proposed the following two resonance hybrid formulas for the free radicals produced by the autoxidation of oleate:



which predict the corresponding four isomeric hydroperoxides. Only the 8 and 11 isomers have been found by Swift et al. (94, p. 297-300), and Farmer and Sutton (27, p. 119-122), a fact that proposes higher contribution of the resonance formulas I and III to the resonance hybrid. However, the detailed nature of the bulk of the hydroperoxides from oleate autoxidation has not been fully elucidated, and Knight et al. (58, p. 498-501) found striking differences in the oleate autoxidation reactions at different temperatures.

The probability of the oleate α -methylene group initiating the autoxidation reaction by itself has been doubted by Hilditch (44, p. 1-16), because of the limited lability of the α -methylenic hydrogens. He proposed that autoxidation of oleate is initiated by poly-unsaturated fatty acid esters occurring as traces in the model system.

Oleate autoxidation differs from linoleate in that significant proportions of peroxides other than hydroperoxides are formed even in the early stages of the reaction. Swern et al. (93, p. 3135-3136) proposed that the non-hydroperoxide portion was cyclic peroxides.

Saturated fatty acids in lipids undergo a slow oxidation if stored at elevated temperatures (48, p. 62). A hydroperoxide is believed to be formed at temperatures above 100° C. by attack of oxygen at the beta carbon atom of the acid.

Factors Affecting the Rate of Autoxidation of Lipids

Many factors influence the rate of autoxidation of lipids. Among them are the degree and type of unsaturation of the fatty acids, the oxygen pressure, the temperature, light, light plus photochemical pigments, ionizing radiations, metals, antioxidants, the physical state of the substrate, and enzymes such as lipoxidase.

Holman and Elmer (47, p. 127-129) showed that the autoxidation rate of lipids is proportional to their unsaturation. Some disagreement, however, exists regarding the effect of conjugated double bonds. Allen et al. (1, p. 395-399) reported that methyl linoleate was oxidized slower when conjugated than unconjugated. Holman and Elmer (47, p. 127-129) did not find any difference in the autoxidation rate of the above linoleates, while Myers et al. (40, p. 107-109) found that conjugated triene esters were oxidized more rapidly than unconjugated triene esters.

At this point it is necessary to point out that the acceleration of the autoxidation can be brought about by increasing the free radical formation, either by a homolytic cleavage of a lipid molecule, or by the decomposition of hydroperoxides.

It has been shown (65) that the decomposition of hydroperoxides is a first order process when they occur at low concentrations, and a second order process when they occur at higher concentrations.

Temperature increase was shown (74, p. 52-57) to increase the rate of lipid autoxidation. However, heating sometimes (61, p. 91) markedly increases the stability of fatty foods against autoxidation. This is due probably to the formation of free sulphhydryl compounds which show antioxidant properties (100, p. 26).

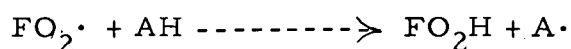
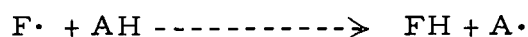
Light, especially that of high quantum energy, as ultraviolet, has been shown (61, p. 91) to have a marked catalytic effect on the autoxidation of fats. The effect is more pronounced when photochemical pigments such as chlorophyll and carotenoids are present.

Ionizing radiations generate free radicals which accelerate autoxidation (See further chapters in this literature review).

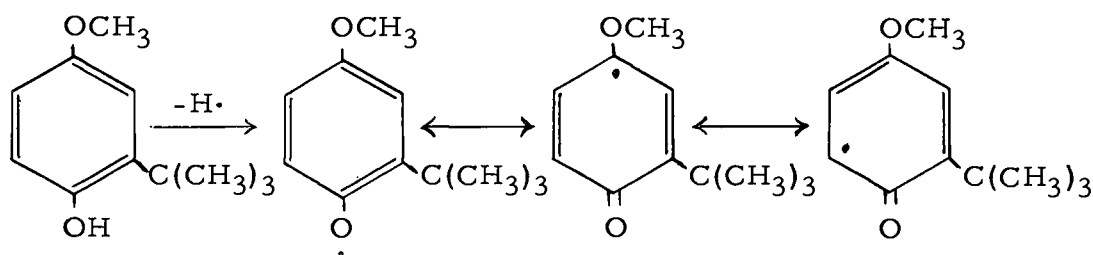
Metals such as copper, iron, cobalt and manganese catalyze, even in trace quantities, the decomposition of peroxides to form free radicals. This has an overall effect of increasing the autoxidation rate (61, p. 91).

In the field of food science, the term antioxidant is used to characterize minor constituents which occur either naturally or

are added to a food product to prevent off-flavor production because of lipid autoxidation. Their common characteristic is that they react with free radicals to form a product which can not propagate the free radical chain reaction. Most of the antioxidants are phenolic, or aromatic amino derivatives, and their reactions with free radicals can be visualized (82, p. 50-63; 98, p. 59-68) as:



where F is the fatty acid molecule, A is the antioxidant molecule, and $A\cdot$ its free radical. The free radical $A\cdot$, is inefficient in propagating the chain reaction since it is a semiquinone stabilized by resonance, as follows:



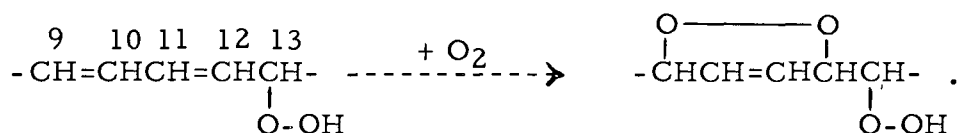
All natural lipid systems contain several natural antioxidants. Milk lipids contain tocopherol, carotene, and cephalin (80, p. 13-16). Among them tocopherol is the most active natural

antioxidant (39, p. 28).

The enzyme lipoxidase was shown to oxidize the lipids giving rise to carbonyls, by the decomposition of hydroperoxides. In the course of the enzymatic oxidation, free radicals are liberated which initiate autoxidation (97, p. 267-281).

Secondary Autoxidation Products

It is well established today that hydroperoxides are the primary reaction products of lipid autoxidation. Cannon et al. (12, p. 450) provided experimental evidence that dihydroperoxides are also formed especially during the later stage of autoxidation of linoleate. The second oxygen molecule forms a cyclic peroxide by a 1, 4 addition (60, p. 1303; 68, p. 127).



The hydroperoxides and peroxides, though harmful from a nutritional and physiological point of view (64, p. 541), have no effect on the flavor of lipids. However, their decomposition products are highly significant in the oxidized flavors of lipids. It has been shown that among the secondary products the carbonyl compounds are the most abundant and important from the flavor point of view (10, p. 238-242; 52, p. 713-718; 51, p. 372-377; 34, p. 211-219; 95, p. 284-291; and 18, p. 585-597).

Coleman and Swern (16, p. 223) found that during oleate autoxidation and after the peak of peroxide formation had passed, 30-35 per cent of the oxidation products were peroxides, 25-30

carbonyl compounds were formed concurrently with the formation of stable peroxides.

Several comprehensive reviews dealing with the type of carbonyl compounds formed in various autoxidized lipids have been published (60, p. 1303-1309; 68, p. 126-132; 80, p. 1-39).

The several oxidized flavors of dairy products have been shown to be due to various combinations of carbonyls. Van der Waarden (108, p. 155) made the first successful attempt to identify the flavor of oxidized milk lipids in storage butter. He reported that the flavor was due to fat soluble compounds that could be easily removed by degassing the fat at low pressure. He postulated that methyl ketones and unsaturated aldehydes were the significant portion of the flavor.

The quantities of the various classes of carbonyls formed from phospholipid autoxidation are different than the carbonyls from milk fat (21, p. 81-89), due to the different fatty acid composition of the two lipid fractions, and the fact that the phospholipids are more unsaturated than the milk fat.

Keeney and Doan (52, p. 713-718; 53, p. 719-727; and 54, p. 728-738) used high-heat vacuum distillation of oxidized milk fat to isolate the volatile carbonyls. They reported that the important odor compounds were ketones excluding methyl ketones,

and that a large portion of them were unsaturated. However, they identified the above ketones through the Girard's reagent, which was later shown to be nonspecific (31, p. 401-402). Furthermore the high-heat treatment was shown to produce methyl ketones (75, p. 49-55) from milk fat.

Tamsma (96, p. 487-496) working with the volatiles of oxidized milk fat reported that unsaturated, unconjugated compounds and conjugated monene compounds were of importance to the flavor. He also reported a dienal being present in the volatile material. These conclusions however, were based on hydrogenation of the flavor distillate under the claim that hydrogenation did not affect the carbonyl groups.

Day and Lillard (18, p. 585-597) contrary to the above findings of Keeney and Doan, and Tamsma, reported that alkanals were 64 per cent of the volatile carbonyl compounds from autoxidized milk fat, and 34 per cent were alk-2-enals, while no evidence of dienals was found. From the aldehyde fraction they identified C_1 to C_{10} n-alkanals, and from the enal fraction C_4 to C_{11} alk-2-enals. They also obtained evidence for the odd numbered C_3 to C_{15} methyl ketones in oxidized milk fat, which however, were not attributed to the autoxidation since they were found in the fresh fat.

Forss et al. (34, p. 211-219) largely supported Day and Lillard's findings, and they also identified the octa-2, 4-dienal as a component of oxidized milk fat.

Subsequent refinement of the techniques used by Day and Lillard (18, p. 585-597) made it possible to obtain evidence for small amounts of hepta-2, 4-dienal (19, p. 1362). Thus the work of Day and Lillard (18, p. 585-597), and Forss et al. (34, p. 211-219) supplemented and supported each other to establish that the volatile monocarbonyl compounds in oxidized milk fat belong mainly to the three classes; alkanals, alk-2-enals, and alk-2, 4-dienals.

Autoxidation products of milk phospholipids were studied by several investigators. Forss et al. studied the copper-induced oxidized flavor of skim milk (33, p. 345-348). They proposed that ethanal, propanal, n-hexanal, 2-butenal, C_4 through C_{11} 2-enals and C_6 through C_{11} 2, 4-dienals, which were identified, originated from the oxidation of the highly unsaturated fatty acids of milk lipids, and though it was not specified, the above carbonyls were largely due to phospholipid oxidation.

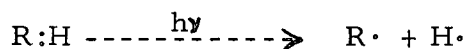
Volatile carbonyl compounds from autoxidized milk phospholipids were isolated and characterized as carbonyls by Riel and Sommer (81, p. 1-73). Three empirical formulas were given for the carbonyls: $C_{15}H_{26}O_3$, $C_{10}H_{18}N_2O$, and $C_{11}H_{22}O$.

Van Duin (21, p. 81-89; 22, p. 90-95) presented evidence of the C_2 to C_{18} alkanals, C_5 to C_{11} alk-2-enals, and C_8 and C_9 alk-2,4-dienals, from autoxidized phospholipids from butterfat. He proposed that carbonyls of longer than twelve carbon atoms in the molecule were not oxidation products but were bound as acetals in the phospholipids. This conclusion was verified by Schogt et al. (84, p. 145) and Parks et al. (73, p. 1941), who reported that the C_{17} and C_{18} normal and branched chain aldehydes were found in the phosphorus-free and phospholipid fraction (plasmalogens) of butter.

Irradiation-Induced Reaction Mechanisms in Lipids

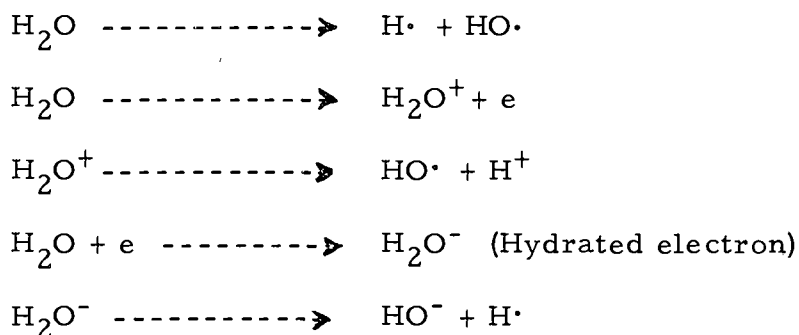
The type and the quantum energy of the radiation and the nature of the matter being irradiated are the main factors upon which irradiation-induced reactions depend. With the exception of neutrons, which induce nuclear reactions, all of the other types of radiation interact with the electrons of matter with an overall result of ionization, excitation and dissociation (35, p. 212-213).

Among the ionizing radiations, only the gamma and beta rays have been used with foods, because of their penetration, efficiency of production, and safety advantages (78, p. 377). With bonded compounds the energy dissipated by the absorbed radiation causes a rupture of covalent bonds of irradiated material. Thermodynamically, the easiest kind of bond rupture is the homolytic, through which two free radicals are formed (72, p. 41). These radicals can then take part in a complex series of secondary reactions. This is the direct effect of the ionizing radiations on organic molecules (103, p. 54)

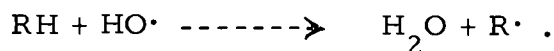


In aqueous systems of organic compounds, however, free organic radicals can be formed indirectly through the reaction of water

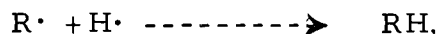
dissociation products. Weiss (109, p. 52-53) studied the effect of radiation on water, and reported the following reactions in the absence of oxygen:



Thus an organic molecule RH can also form free radicals indirectly:



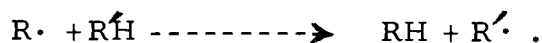
These free radicals, either directly or indirectly formed, can recombine with other free radicals:



can dimerize; $\text{R}\cdot + \text{R}\cdot \text{-----}\rightarrow \text{RR},$

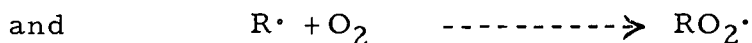
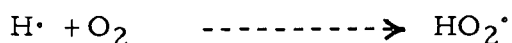
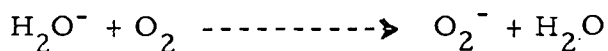
polymerize; $\text{R}\cdot \xrightarrow{+\text{R}} \text{RR}\cdot \xrightarrow{+\text{R}} \text{RRR}\cdot \text{ etc.,}$

or react in a dismutation reaction:



In the presence of oxygen the following reactions were suggested:

(109, p. 53-55)



resulting in hydroperoxide formation. It was further shown that there is no fundamental difference between the direct and the indirect effects of radiation (109, p. 54).

In fats the free radicals produced by the direct or indirect effect of irradiation can accelerate an autoxidation process, which, as we saw before, is initiated and propagated by free radicals. However, because of the high concentration of free radicals produced by radiation, the number of free radical chains started will be greater and their average length shorter, than in the ordinary autoxidation reaction (61, p. 91; 13, p. 1720).

As it was emphasized in the beginning of this review considerable evidence has been produced to establish the general similarity between the oxidative changes that occur during irradiation or ordinary autoxidation of lipids. (66, p. 472; 20, p. 152-154; 90, p. 335). Any discrepancies found, such as in the proportions of the volatile carbonylic products isolated by Gaddis and Ellis (37, p. 745) from rancid pork fatty tissue, and those isolated by Witting and Schweigert (111, p. 413-416) from

γ -irradiated lard, may be due partly to technique differences.

Apart from the general similarities between mechanisms and products of the above two processes, it seems that the effects of irradiation go beyond those of normal autoxidation.

Chipault et al. (13, p. 1715) reported that even saturated fatty acids, which are resistant to autoxidation under ordinary conditions, show appreciable peroxidation when irradiated. He also observed that carbonylic compounds and off-flavors were produced during irradiation of lipids, even with the rigid exclusion of air. To explain this fact, he suggested that the necessary oxygen was derived from the ester group of the glyceride molecules.

Because of the combined complexity of the radiation induced reactions and the natural lipid systems, many workers tried to approach the problem by irradiating simpler systems. Thus Burton (11, p. 4117-4119) used deuterons to bombard oleic acid, and stearic acid, and polymers found by analysis of the irradiation products, suggested hydrogenation and polymerization reactions.

Dugan and Landis (20, p. 152-154) used a cobalt 60 source to radiate oleic acid and methyl oleate with high energy gamma rays. The influence of the radiation on substrate oxidation was apparent even at low temperatures. Except for the increase in peroxide

value, ultraviolet absorption studies showed appreciable quantities of carbonyl compounds which were suspected to be α,β -unsaturated ketones.

The effect of ionizing radiations on methyl esters of palmitic, oleic and linoleic acids were studied with or without oxygen, water and antioxidants at various temperatures and storage conditions, by Chipault et al. (13, p. 1713-1720). Reactions reported were decarboxylation, (de)hydrogenation, polymerization, and oxidation of the esters to the hydroperoxides. Appreciable amounts of peroxides and carbonyl compounds were identified as a result of radiation in the presence of oxygen. Direct ozonization of fatty acid esters produced remarkably similar flavors and odors to the irradiated under oxygen, unozonized samples. The flavor of the direct ozonization sample was more close to the irradiated samples, than to samples rancidified by ordinary autoxidation. Thus, they proposed that ozone and nitrogen oxides may be formed when lipids are irradiated in air, and some of the flavor and odor components may result from the action of these compounds on the fats. Irradiation under vacuum did not produce any peroxides, but off-flavors developed. They proposed that carbonyl compounds are probably formed by deriving their oxygen from the ester group of the substrate. The irradiation of methyl linoleate accumulated high proportions of conjugated diene

hydroperoxides. Maximum peroxide production was observed at -20° C., with subsequent continuation of this production during storage.

Proctor et al. (103, p. 1) tried to evaluate the flavor of the irradiated fatty acids of milk fat, but complications resulted because of the inherent soapy taste of the free acids and specific flavors of the lower fatty acids of milk fat. Thus they preferred to produce the methyl esters of the fatty acids from milk fat, and irradiate these esters, with obvious advantages for flavor evaluation.

Effect of Radiation on Lipids

Long and Moore in 1927 (62, p. 901-903) were the first to study the influence of ionizing radiations on drying oils. They used cathode rays to irradiate linseed and other drying oils, and reported a reduction of drying time, an increase in molecular weight and a decrease in iodine number.

Hannan and Boag (40, p. 152-153) were among the first to study the effects of radiation on food lipids. They reported that the higher the temperature of irradiation, the less was the amount of peroxide produced.

A number of reviews (78, p. 375-380; 71, p. 554-560; 64, p. 541-546; 15, p. 71-75; 110, p. 944-950; and 14) deal with the effects of ionizing radiations on lipids.

Proctor and Goldblith (78, p. 376-380) first discussed the problems involved in food processing by ionizing radiations. The following factors were considered:

- 1) Type of radiation
- 2) Packaging with particular reference to the size of the package
- 3) Dose needed to destroy the microorganisms in question
- 4) Effect of ionizing radiations on enzymes

- 5) Effect of ionizing radiations on flavor, color, texture and nutrients in foods
- 6) Effect of storage life on irradiated products
- 7) Economical considerations.

For sterilization purposes the dose of 4,500,000 rep was sufficient to destroy spore-forming bacteria (sterilization dose), while enzymes such as milk peroxidase are very resistant and are not inactivated, even at a dose of 10,000,000 rep.

Chipault (14) studied the effect of dose rate and the temperature during irradiation or post-irradiation storage on oxidation. For equal doses the lower dose rate resulted in higher oxidative changes, than the higher dose rate. The effect of higher temperatures on the decomposition of the peroxides was also emphasized.

From the standpoint of harmful nutritional and physiological effects, except from the toxicological effects of the hydroperoxides produced, ionizing radiations were reported not to create any harmful effects in irradiated lipids (67, p. 38-39). Becker et al. (6, p. 61-64) carried out a feeding experiment with rats for three generations, in which the butterfat portion of the diet was irradiated. No evidence of carcinogenicity and no significant difference in growth rates were shown between the test and the

control groups of animals. Only the reproductive performance of the test group seemed to be slightly inferior to that of the control group.

Kung et al. (59, p. 142-144) measured the vitamin and enzyme retention in milk and related products irradiated with sterilizing doses of gamma rays. They reported that vitamin A, tocopherols, and ascorbic acid were highly sensitive, carotenoids and riboflavin were moderately sensitive, while the enzyme phosphatase was slightly changed.

Richardson et al. (79, p. 353-360) fed rats with a diet treated with sterilizing doses of ionizing radiations. All the animals (experimental and control) received a supplement of vitamins A and E, which are destroyed by irradiation. Reproductivity and life span tests on the experimental and control rats showed that except for the above two vitamins, the difference between the irradiated and control diets was no greater than would be expected if the diet had been preserved by some other process.

Lea (61, p. 90) emphasized that free radicals destroy pigments, such as haemoglobin, carotenoids, chlorophyll, anthocyanins, flavor constituents, and fat soluble vitamins.

Astrack et al. (4, p. 571-583) studied the effects of sterilizing doses of electrons on some vegetable and fish oils. The radiation

initiated reaction mechanism seemed to be complex, including polymerization, bond breakage and a variety of oxidative changes. Fish oils suffered more pronounced changes than vegetable oils. Antioxidants added before the irradiation prolonged the storage life of the oils.

Antioxidants are, in general, at least partially destroyed during irradiation. Hannan and Shepherd (41, p. 1021-1022) reported that natural antioxidants were destroyed during and after irradiation and Chipault et al. (13, p. 1715) showed that propyl gallate added to lard was completely destroyed during irradiation at ambient temperature with gamma ray doses of 2×10^6 rep.

Irradiation of meat fats were investigated by several workers. (22, p. 958-960; 5, p. 705; 111, p. 413-416). Marked increases in peroxide values were reported when irradiated fats were stored at 5° C. under conditions allowing the oxygen to diffuse in, while exclusion of oxygen showed very little increase in peroxide value.

Carbonyl compounds have been found to increase proportional to radiation dosage. Batzer et al. (5, p. 705) reported that carbonyl compounds formed from meat irradiation were different than those obtained from irradiated meat fat.

Witting and Schweigert (111, p. 413-416) isolated and characterized the volatile carbonyl compounds from irradiated lard. They identified propanal, butanal, pentanal, hexanal, nonanal, decanal, acrolein, crotonal, and 2,4-undecadienal.

Much work has been done on the irradiation of milk fat. Dunn et al. (23, p. 605-616) were among the first to investigate the effects of radiation on butter. They reported an increase in peroxide value, but the rate of increase with dose of radiation was not linear. At the same time the natural color of butter was progressively destroyed with an increase of radiation dosage.

Peroxide formation during, as well as after irradiation was reported by Makherjee (69, p. 589). He also reported that in the presence of oxygen, butterfat was more readily oxidized when irradiated while irradiation under vacuum did not show any appreciable peroxide value. However, Hannan (43, p. 87-89) showed that, if the lipid is of a suitable nature, the radiation-induced free radicals can persist for long periods. If butterfat is irradiated in the absence of oxygen little hydroperoxide is formed, but admission of oxygen after various periods of storage immediately causes peroxide formation.

Hannan and Boag (40, p. 152-153) reported that the temperature during radiation of butterfat had a significant influence on the

peroxide value, which increases with a decrease in temperature. They also confirmed earlier observations that the peroxide value increases with an increase in radiation dosage.

Extensive changes after the radiation of butterfat which were influenced by storage temperatures were reported by Hannan and Shepherd (41, p. 1021-1022; and 42, p. 38-41). Fats irradiated at 0° C. or 70° C. showed a striking increase in peroxide value during the first two days of storage at -20° C. No increase of peroxide value during storage at +20° C. was noticed, while molten fat at 37° C. failed to show any peroxide formation. They explained the above observations on the basis of the higher stability of free radicals around -20° C., which slowly react with oxygen as it diffuses into the fat.

Chipault et al. (13, p. 1715-1720) also studied the effect of radiation of butterfat with or without oxygen, water, antioxidants and at various temperatures and storages. They reported that the maximum peroxide production during irradiation of butterfat was observed at -20° C. with subsequent continuation of this production during storage. Appreciable amounts of peroxides and carbonyl compounds were produced by irradiation of butterfat in the presence of oxygen. Irradiation under vacuum did not produce any peroxides but organoleptic changes still developed.

Proctor and coworkers in a series of reports studied the effect of ionizing radiations on butterfat (99, p. 3; 101, p. 6; 102, p. 10-12; 103, p. 1-4; 100, p. 4-10; 105, p. 14-33; 106, p. 6-9; and 107, p. 9). Comparison between the off-flavors of irradiated whole milk and skim milk suggested that the characteristic chalkiness observed in irradiated whole milk, but not in irradiated skim milk, was apparently a non-volatile component of milk fat. (99, p. 3). Skim milk, skim milk with 4 per cent butterfat, and butter alone were irradiated at 2×10^6 rep. Irradiated butterfat was used to make a 4 per cent emulsion in non-irradiated skim milk, and this sample together with the irradiated skim milk with 4 per cent butterfat was submitted to a taste panel in a triangle arrangement. Statistical evaluation indicated an insignificant difference. Thus they assumed that the off-flavor developed from water free butterfat. Butterfat that was radiated in an aqueous phase was similar, and the water phase had no effect on the development of the radiation off-flavor in butter. Their finding is very important from the point of view that it is possible to produce relatively large quantities of off-flavor condensates with butterfat rather than milk (101, p. 6).

In a subsequent report, Proctor et al. (102, p. 10-12) fractionated anhydrous butterfat by dissolving it in acetone and

fractional crystallizing it at low temperatures. These fractions were irradiated after the removal of the solvent, then they were emulsified in unirradiated skim milk and taste-evaluated. The high melting fractions, composed of the more saturated fatty acid glycerides, showed mainly the taste of chalkiness after irradiation, while the low melting fractions, composed of the more unsaturated fatty acid glycerides, developed an oxidized off-flavor when irradiated. Although there was no significant change in iodine values before and after radiation, a sharp increase in peroxide accompanied the occurrence of chalkiness, whereas a lesser increase, or even decrease was noted in the more unsaturated fractions. Furthermore it was found that the unsaponified components of butter did not develop detectable off-flavors upon radiation.

In an attempt to find the precursors to radiation off-flavor in butterfat, Proctor et al. (103, p. 1-4) prepared the methyl esters of butterfat fatty acids and fractionated them by using urea. Determinations of the conjugated and non-conjugated polyunsaturated fatty acids of the fractions before irradiation, and taste evaluation of the irradiated fractions, showed that the precursors of the radiation-induced off-flavors existed among the non-conjugated trienes and tetraenes. This result was confirmed by

using the Diels-Alder reaction on butterfat, which did not reduce the intensity of the radiation-induced oxidized off-flavor.

The chalky, and the oxidative off-flavors were further identified by Proctor et al. (100, p. 4-10). For the chalky off-flavors it was shown that the precursors were higher fatty acids whose chains were not broken during their formation, that they were not distillable, and they disappeared on heating. Thus it was suggested that chalkiness is due to the higher saturated fatty acid peroxides. Based on the results of Smith and Jack (91, p. 380-389), according to which distillation of milk fat methyl esters at 0.1 mm mercury at temperature below 122° C., only the C₁₆ and lower fatty acid esters distill, Proctor et al. (100, p. 4-10) suggested that oxidized off-flavors come from fatty acids having carbon chains with more than sixteen carbon atoms.

In a different approach to separate the off-flavor compounds of irradiated butterfat, Proctor et al. (104, p. 10) steam distilled irradiated milk fat under vacuum at 50° C. The steam distillate, after appropriate extraction and washings, gave the flavor concentrate which was injected into a vapor fractometer. Their intention was to evaluate the flavor of each peak by trapping it out of the gas chromatograph into milk and tasting. Irradiation under oxygen (105, p. 14-33) gave a 20-fold increase in intensity of the

off-flavor and a certain radiation apparatus proved to give off-flavors that matched the ones of fat irradiated without oxygen. This irradiated butter was steam distilled and the distillate off-flavor injected directly from the traps into the gas chromatograph. Eight compounds were separated. Taste evaluation of the last three peaks suggested that the oxidation flavor should be found in that region of the chromatogram. Infrared spectra of the peaks collected suggested that they were saturated aldehydes. Furthermore the distillate of irradiated milk fat was allowed to react with 2,4-dinitrophenylhydrazine, and the 2,4-dinitrophenylhydrazones were fractionated by column chromatography. One of the fractions was found to be responsible for the oxidation off-flavor. This fraction on further fractionation and identification by paper chromatography techniques, was found to be some carbonyl between six and eight carbon atoms.

In a later report by Proctor et al. (106, p. 6-9) further work was carried out in order to isolate the precursors to the specific "oxidized" radiation off-flavor of milk fat. The previously (100, p. 4-10) isolated fraction of butterfat fatty acids proved to contain most of the precursors of the "oxidative" off-flavor. Thus it was further fractionated by counter-current liquid-liquid extraction. Seven peaks were reported, each peak was characterized,

and the most likely precursor of the "oxidized" off-flavor was the fifth peak containing highly unsaturated fatty acids. The typical irradiation off-flavor, however, could not be found in any of the seven fractions when irradiated separately, while all the fractions mixed together matched the radiation-induced off-flavor. A mixture of fractions II and VI, however, in the proportions 1 to 3 gave an off-flavor that could not be distinguished from the flavor of irradiated butterfat emulsified in milk. The cis, cis, cis - 9, 12, 15 linolenic acid was referred as one of the precursors of oxidation flavor (107, p. 9).

Analogous procedures were used by Hoff and coworkers (45, p. 468-475) to identify the precursors of the radiation-induced oxidation flavor of milk fat. They isolated the fatty acids of milk fat and fractionated them. The significant fraction from the oxidation flavor point of view, was further fractionated by counter-current distribution. Six peaks were obtained. By the process of elimination it was found that only the mixture of two of them gave the characteristic flavor when irradiated. One of the two peaks was identified by infrared spectrometry to be linolenic acid. The other significant peak was not identified but it is believed to contain vinyl groups and branched chains. Linolenic acid produced a fishy

flavor on irradiation, while the other unidentified peak gave a candle-like flavor.

Recently Chipault (14) studied the effect of high energy radiation on several lipids and fatty acid esters. He used the "G value" (defined as the number of molecules of a compound formed or destroyed for each 100 electron volts of energy absorbed), as an indication of a chain reaction, and he calculated that when $G = 3.3$ every ionization results in a change of one molecule, and when $G = 10$ or more a chain reaction is functioning. Thus the G value of methyl lineoleate, linolenate, and arachidonate were found to be 16, 26, and 80 respectively for a dose of 3 Mrad., while methyl oleostearate was 1.3.

He further listed the recent papers on carbonyls produced by irradiation of several lipids under various conditions. The G values for carbonyl formation were calculated to be below 10, indicating that a chain reaction was not required for carbonyl production.

EXPERIMENTAL

Preparation and Irradiation of Milk Fat Samples

Raw sweet cream was warmed in a water bath to 45° F. and then churned for about half an hour, until the emulsion broke. The buttermilk was drained, and the butter was washed five times with distilled water. The washed butter was melted at 45° C., giving a fat and aqueous layer. The fat layer was washed in a separatory funnel, with distilled water at 45° C., until the washings were clear. Finally the milk fat was filtered at 45° C. and canned in (307 x 409) "C" enameled cans. This procedure of preparing the milk fat was adequate for removal of the phospholipids (18, p. 586). A small headspace was left in the cans and the cans were sealed under vacuum. The canned milk fat was stored at 0° F. before and after irradiation.

Irradiation of the milk fat was carried out at ambient temperature in the high flux chamber of a Cobalt-60 irradiator, under a dose rate of 0.25 Mrad per hour. Dosages of 0, 1.5, 3.0, and 4.5 Mrad were given to different cans of milk fat.

Analyses of the Milk Fat Samples

The time between irradiation and analysis was less than 10 days. Each can containing the irradiated or control milk fat was removed from the 0° F. storage room and melted in a water bath at 45° C. Complete melting was followed by opening the can, tasting the sample, and immediately analyzing for peroxide value, TBA number, iodine number, total carbonyl, and free carbonyl content.

When analysis for carbonyls was conducted the solvents; hexane, benzene and chlorform were treated according to Schwartz and Parks (87, p. 1396-1398) to reduce their carbonyl content to zero. The peroxide values of the control and the fat samples treated at the above indicated dosage levels were determined by the A. O. C. S. method (2, Cd. 8-53). According to this method the peroxides and similar compounds are expressed as milliequivalents of peroxide per kilogram of fat.

The TBA procedure described by Yu and Sinnhuber (112, p. 104-108) was used, with certain modification, since pure fat samples were analyzed instead of complex food systems. The 5 ml of pyridine hydrochloride solution plus 10 ml trichloroacetic acid solution used before refluxing (112, p. 105) was replaced with 15 ml

of 0.6N HCl and again the pyridine hydrochloride-trichloroacetic acid solution was omitted after the main refluxing step. A sample size of 5 to 10 grams of fat was used (5 grams for the higher dose irradiated milk fat, and 10 grams for the control and the low dose samples). The results were expressed in milligrams of malonaldehyde per kilogram of fat.

The iodine absorption numbers were determined according to the method of Hanus (3, p. 494-495) and the results were expressed in grams of iodine absorbed per 100 grams of fat.

The total carbonyl value was determined by the procedure of Berry and McKerrigan (8, p. 693-701). This method can separately determine the saturated, and unsaturated carbonyls because of the different maxima wavelengths of the saturated and unsaturated carbonyl 2, 4-dinitrophenylhydrazones when reacted with alcoholic KOH.

The amount of free monocarbonyl compounds as, alkanals, alk-2-enals, and alk-2, 4-dienals were separately determined by the method of Keith and Day (56). According to this method, a chromatographic tube 12 mm I. D. x 40 cm, plugged at the constricted end with glass wool, was clamped at the outlet and filled up to a level of 5 cm with treated benzene. Fifteen per cent hydrated alumina was added to a depth of 3 cm, and the benzene was

allowed to drain to the alumina level. Ten milliliters of 0.05 grams 2, 4-dinitrophenylhydrazine in 100 ml benzene, was added carefully, and more 15% hydrated alumina to make a column 8 to 9 cm long. When all excess benzene was drained from above the column, an additional 5 ml of benzene were used to wash the column. Three to five ml of a 50% solution of fat in benzene were added onto the column, and washed with small aliquots of benzene. A total of 100 ml benzene was allowed to pass through the column, and it was collected in an erlenmeyer flask. The solvent was evaporated at reduced pressure and 5 ml benzene, 35 ml absolute ethyl alcohol, and 10 ml 4% alcoholic KOH solution were added to the flask in the sequence listed. Ten minutes after the addition of the KOH solution the absorbance was read at 430, 460, and 480 $m\mu$, with distilled water as the blank. A blank of the lipid solution that had been passed through the same type of column without 2, 4-dinitrophenylhydrazine reagent, and a benzene blank were run and read in the same way. The two blank readings are subtracted from the reacted sample readings, and the quantity of each of the monocarbonyl classes were calculated in micromoles per 50 ml solution by means of the following equations:

$$\text{Alkanals} = 7.163 A_{430} - 11.157 A_{460} + 6.506 A_{480}$$

$$\text{Alk-2-enals} = -5.484 A_{430} + 15.392 A_{460} - 11.102 A_{480}$$

$$\text{Alk-2,4-dienals} = 1.516 A_{430} - 6.641 A_{460} + 6.428 A_{480}$$

Isolation and Characterization of the Monocarbonyl Compounds
from 4.5, and 0 Mrad Milk Fat

Twenty grams of 4.5 Mrad milk fat were dissolved in benzene to make a solution of 50 per cent. This solution was passed through a free carbonyl column different from the one previously described in the I. D. and the amount of the reagent used. The column was 24 mm I. D., and 30 ml of 2, 4-dinitrophenylhydrazine reagent were used to make sure that there was an excess of reagent for the larger quantity of carbonyls. A total of 150 ml benzene was passed through the column into an erlenmeyer flask, and the solvent was evaporated.

The method of Schwartz et al. (86, p. 15A) was used to separate the bulk of the fat from the 2, 4-dinitrophenylhydrazones. The last traces of fat were removed from the hydrazones by the ion exchange method of Schwartz et al. (89, p. 39)

The isolated hydrazones were separated into classes by means of the class separation method of Schwartz, Parks, and Keeney (88, p. 669-671). To obtain better resolution of the higher carbonyl derivatives a 20 mm I. D. column was used, and the 0.5 ml of water used during the column preparation according to the original method, were not added. No alkanone band could be detected. A strong alkanal

band, and a second weak band, well separated from the alkanal band, were eluted and collected separately. A third band could not be eluted from the magnesia column (dicarbonyl derivative).

The acetonitrile partition column (17, p. 322-329) was used to resolve each of the two DNP-hydrazone classes. Each individual peak was checked to confirm its class, by finding its wavelength of maximum absorption in chloroform solution and by the paper chromatography method of Gaddis and Ellis (38, p. 870-875). Furthermore, the chain length of each peak from the acetonitrile partition column was determined by the paper chromatographic method of Huelin (49, p. 328-334) for the C_1 to C_6 , and Klein and DeJong (57, p. 1285-1288) for C_7 and longer carbonyls.

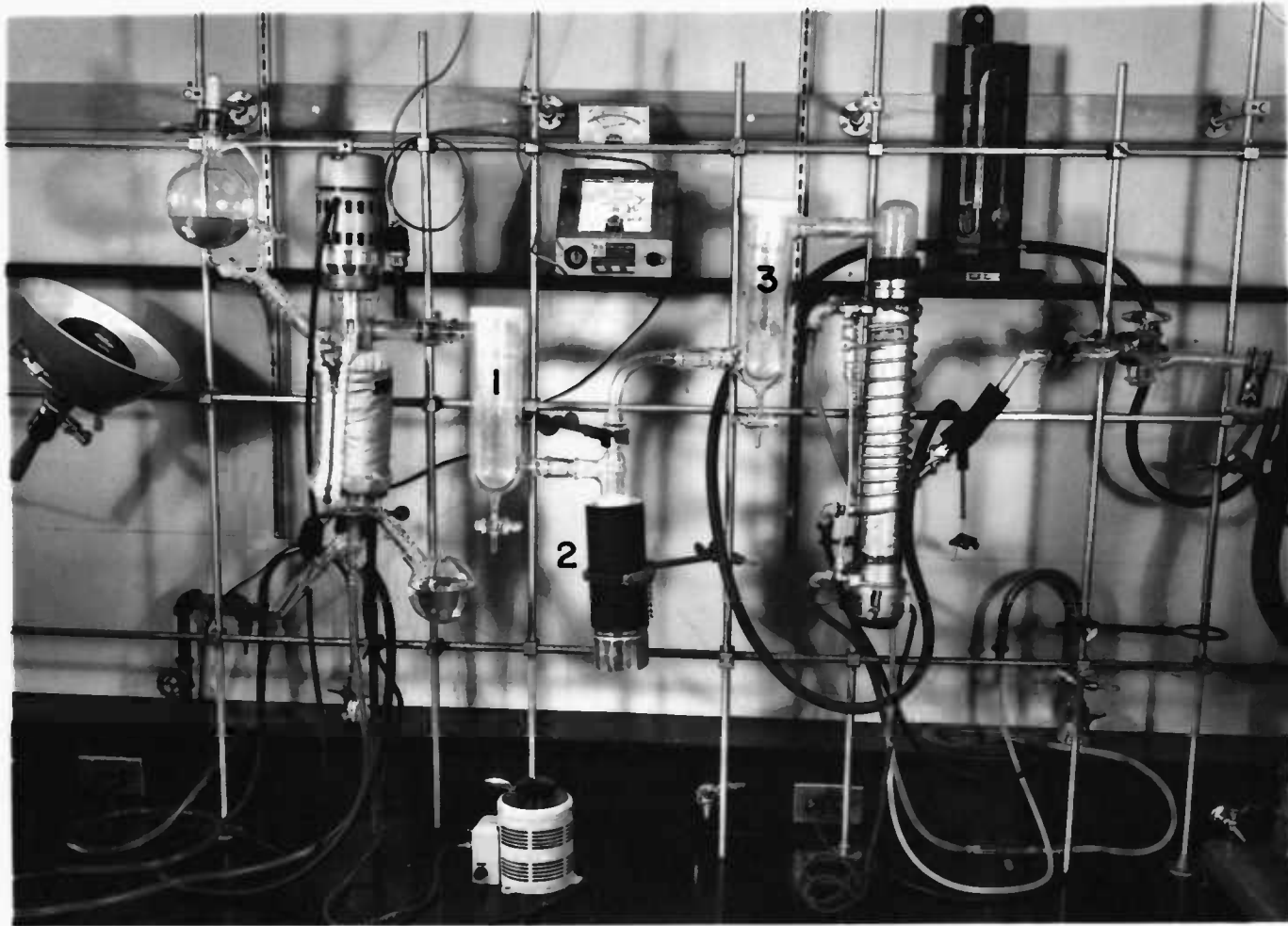
Exactly the same series of procedures as the one used with 4.5 Mrad milk fat, was also used with the control milk fat, in order to estimate the effect of the irradiation dose of 4.5 Mrad on the fresh milk fat.

Characterization of the Volatile Monocarbonyl Compounds of the
4.5 Mrad Milk Fat:Molecular Distillation

The molecular distillation apparatus shown in Figure 1 was used to isolate the volatile material from the 4.5 Mrad irradiated milk fat. Eight hundred grams of irradiated milk fat were distilled at temperatures not exceeding 48° C. and a vacuum of 3 μ Hg for about 8 hours (flow rate of 160 drops per minute). When the distillation was completed, the glass bead trap, identified as No. 2 on Figure 1, was disconnected and part of its distillate was transferred by manipulation of temperature and pressure into a coil injector, which was used to inject the volatile sample into a Barber-Colman Model 20 gas chromatograph. Some of the peaks from the resulting chromatogram were tentatively identified as carbonyl compounds by their retention time.

The main part of the distillate in the glass bead trap (No. 2, Figure 1) was dissolved in ethyl alcohol and allowed to react with a saturated 2,4-dinitrophenylhydrazine solution in 5 N HCl. The hydrazones formed were extracted with chloroform and separated from the excess of the reagent by the cation exchange column of Schwartz et al. (89, p. 38-39). The purified hydrazones were

Figure 1. Molecular distillation apparatus.



separated into classes (88, p. 669-671) on Seasorb. Only one aldehyde band was eluted from the column. Other bands evident on the column were very strongly adsorbed at the top of the column and did not elute by the solvents used in the method for the mono-carbonyls. The eluted band was resolved further by means of the acetonitrile column (17, p. 322-329). A double peak indicating incomplete separation of two components was obtained and it was further identified by spectrophotometric and paper chromatographic techniques (38, p. 870-875; 49, p. 328-334; and 57, p. 1285-1288).

The distillate from trap 1 of the molecular still (Figure 1) was extracted with ethyl ether. After the removal of ether, a part of the distillate was injected into the Aerograph A-100. A broad peak corresponding to the retention time of ethyl ether was the only peak obtained. However, the No. 1 trap distillate did react with 2, 4-dinitrophenylhydrazine reagent to give visible hydrazone precipitate. These hydrazones were further analyzed by using the already described column and paper chromatographic techniques (88, p. 669-671; 38, p. 870-875; 49, p. 328-334; and 57, p. 1285-1288), and several alkanals and 2-alkanones were identified.

The residual fat after the molecular distillation of the 4.5 Mrad irradiated fat was tasted and analyzed for free carbonyls (56).

A second pass of the first distillation residue fat was made with the same molecular distillation apparatus (Figure 1) under the same conditions except that the temperature of distillation was increased to 100° C. The distillate was not significant from the irradiation flavor point of view, and it was kept for later research. The material condensed on the cold finger had a characteristic coconut-like odor (55, p. 592-593).

The residual fat after the second run was also tasted and analyzed for free carbonyls (56).

Characterization of the Volatile Monocarbonyl Compounds of the
4.5 Mrad Milk Fat:Steam Distillation

In a second attempt to isolate and characterize the volatile monocarbonyl compounds from the 4.5 Mrad irradiated milk fat, the steam distillation apparatus described by Day and Lillard (18, p. 587-588) was used. Eight hundred grams of irradiated milk fat were steam distilled for 3 hours at 45° C. and a vacuum of 3 mm Hg. The distillate was allowed to react with 2, 4-dinitrophenylhydrazine reagent for 24 hours and the hydrazones formed were extracted with chloroform. After the removal of chloroform under vacuum the hydrazones of the monocarbonyl compounds were dissolved in hexane. The cation exchange column of Schwartz et al. (89, p. 38-39) was used to remove the excess of reagent. The isolated monocarbonyl hydrazones were separated into classes by the method of Schwartz et al. (88, p. 669-671). Three monocarbonyl derivative bands were collected separately and each one was further resolved by the acetonitrile partition column (17, p. 322-329). Each peak from the above column was analyzed to establish the class and the chain length of the monocarbonyl derivative (38, p. 870-985; 49, p. 328-334; and 57, p. 1285-1288).

The residual fat from steam distillation was tasted and analyzed for free carbonyls (56).

RESULTS AND DISCUSSION

The oxidative effect of gamma radiations on milk fat, sealed in cans under vacuum, was evaluated first by a series of chemical tests and sensory methods, and second by analysis for the identification of the radiation-induced carbonyl compounds.

Results of Chemical Tests on the Control and Irradiated Milk Fat

Table 1 shows the results obtained when the control fat and samples irradiated at 1.5, 3.0, and 4.5 Mrad were analyzed for peroxide value, T B A number, iodine number, total carbonyl and free carbonyl content. The data listed for the various chemical tests are the average of duplicate analysis. The results of taste and color evaluation also are listed.

The peroxide value showed a maximum value for the 1.5 Mrad irradiated fat, with a tendency to decrease at higher dosages. Hannan and Shepherd (42, p. 36-41) reported a similar result with irradiated samples of butter. These data are indicative that the effect of radiation above a certain dose on the decomposition of peroxides is greater than the effect on the peroxide formation. It seems that the rate of peroxide formation is proportional to the dose of radiation, but above a certain dose the decomposition rate

TABLE 1

Results of Chemical Tests on Milk Fat Irradiated at Different Dosages.

Samples of Fat	Peroxide Value	TBA No.	Iodine No.	Total Carbonyls	
	(Milliequivalents of peroxide per kilogram fat)	(Milligrams of malon- aldehyde per kilogram fat)	(Grams of iodine per 100 grams fat)	Saturated (Millimoles of carbonyls per kilogram of fat)	Unsaturated (Millimoles of carbonyls per kilogram of fat)
Control Milk Fat	0	0.39	34.55	10.10	0*
1.5 Mrad Milk Fat	0.73	1.33	33.57	7.88	0*
3.0 Mrad Milk Fat	0.68	1.68	30.00	13.85	0*
4.5 Mrad Milk Fat	0.63	1.81	32.36	14.23	0*

Samples of Fat	Free Carbonyls			Sensory Methods
	Saturated aldehydes (Millimoles of carbonyls per kilogram of fat)	Alk-2-enals (Millimoles of carbonyls per kilogram of fat)	Alk-2,4-dienals (Millimoles of carbonyls per kilogram of fat)	
Control Milk Fat	0.039	0.019	0	Typical color and flavor of fresh milk fat.
1.5 Mrad Milk Fat	0.385	0.041	0.016	Partially bleached. Rancid odor, predominantly hydrolytic. Taste like radiated hydrogenated fat (higher carbonyls).
3.0 Mrad Milk Fat	0.761	0.086	0.035	Completely bleached. Strong oxidized flavor. Lard-like taste.
4.5 Mrad Milk Fat	0.997	0.098	0.061	Completely bleached. Strong oxidized flavor. Lard-like taste.

* The results obtained were actually always negative numbers from -0.5 to -2.5.

of peroxides is greater than their formation rate. This sort of transitory nature of peroxides causes a limitation of the value of the peroxide method for measuring oxidative changes of fats irradiated in the absence of oxygen.

The T B A values, and total saturated carbonyl content show an increase with the radiation dose, although the increase was not proportional to the dose.

The formula giving the unsaturated total carbonyls resulted consistently in negative numbers in all the determinations of control and irradiated samples, indicating the questionable value of the method, at least in the case of milk fat.

Iodine numbers showed a slight decrease with irradiation dosage indicating a destruction of double bonds in the unsaturated fatty acids.

The free carbonyl method showed a proportional increase for all three classes of carbonyl compounds with increasing radiation dosage.

The carotene of the milk fat was completely destroyed at doses of 3.0 Mrad and higher. Hydrolytic rancidity developed even at low dosages, suggesting hydrolysis of the ester linkage through irradiation. The lard-like taste indicated the presence of long chain (above C_{10}) carbonyl compounds.

Free Carbonyl Compounds from 4.5 Mrad and Control Milk Fat

The class separation method (88, p. 669-671) of the free carbonyl derivatives from 20 grams of 4.5 Mrad milk fat gave one strong alkanal band, and a second weak band; the only mono-carbonyl bands eluted. These bands were collected separately and resolved by the acetonitrile partition column (17, p. 322-329). Figure 2 shows the chromatogram of the first alkanal derivative band. Because of the modifications made on the original method, a mixture of authentic derivatives in approximately equimolar concentrations of the normal aldehydes and methyl ketones, were separately resolved by the modified method. Figure 3 is a plot of the logarithms of the corrected peak volumes of the known carbonyl derivatives against the number of carbon atoms of the carbonyl moiety in each derivative. The corrected peak volume is the volume of mobile phase corresponding to the peak of each derivative minus the amount of mobile phase held up on the column. The hold up volume was found to be 60 ml. Comparison of the corrected peak volumes of the known and unknown derivatives, as well as spectrophotometric and paper chromatographic techniques (38, p. 870-875; 49, p. 328-334; 57, p. 1285-1288)

Figure 2. Chromatogram of the modified acetone-triethylamine partition column for the free carbonyl derivatives of the 4.5 Mrad irradiated milk fat. Saturated alkanal derivatives.

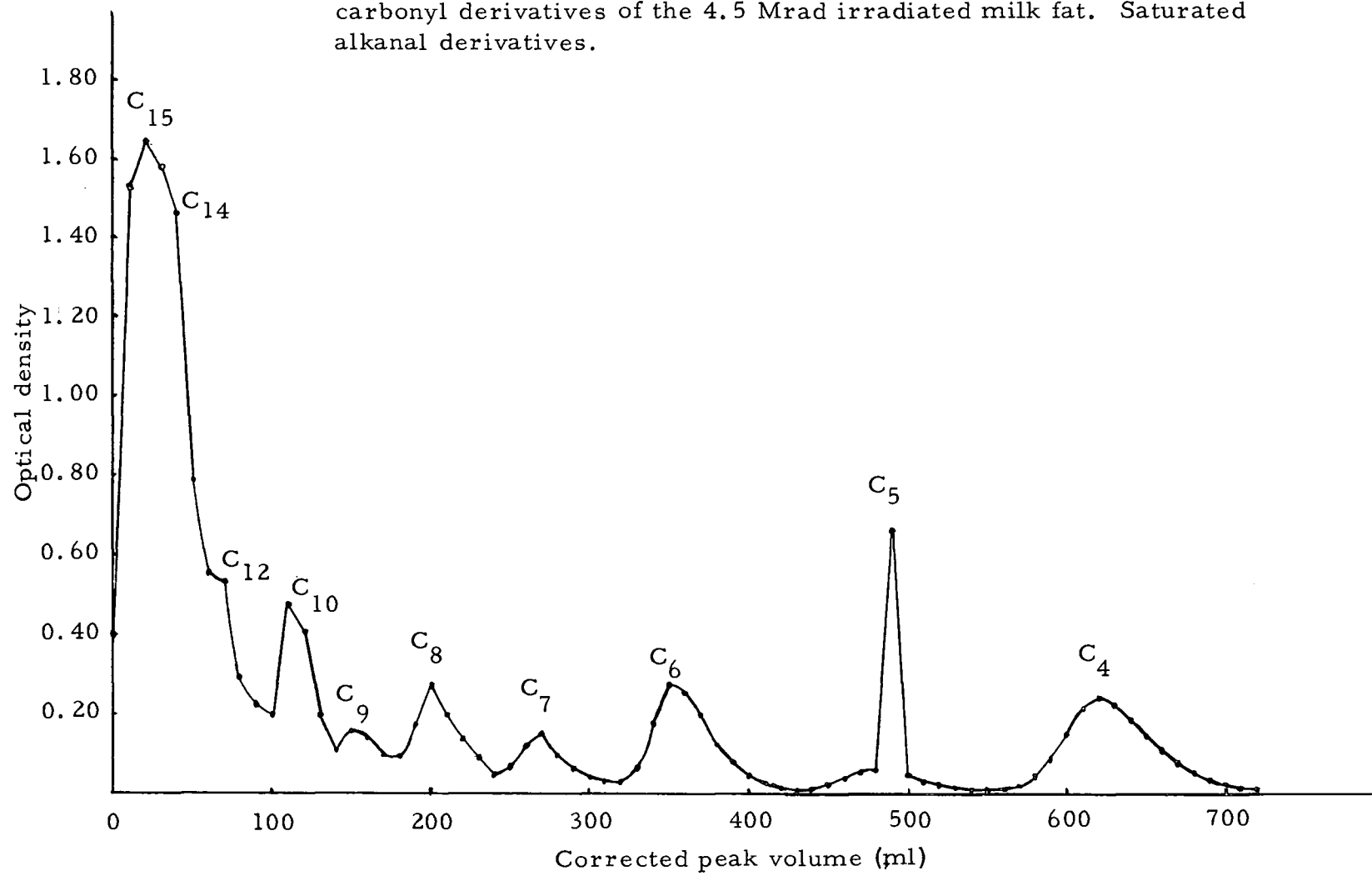
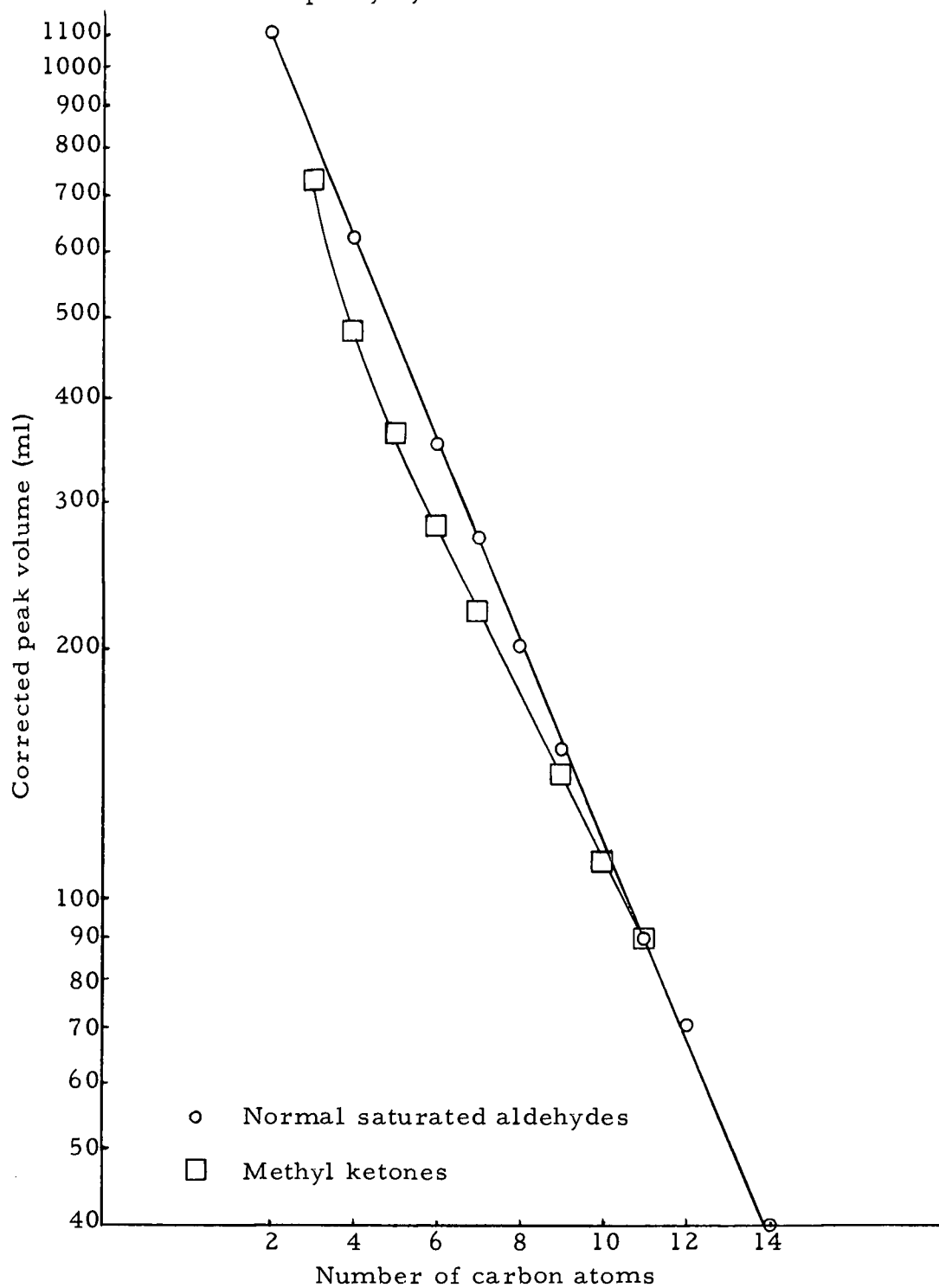


Figure 3. Standard curve of the modified acetonitrile partition column for separation of 2, 4-dinitrophenylhydrazones.



confirmed the identification of each peak of Figure 2.

The second weak band collected from the class separation column (88, p. 669-671) was similarly identified as acetaldehyde.

Exactly the same series of methods were used to identify the free carbonyls from 20 grams of fresh milk fat. However, in this case the amounts of free carbonyls were very small, and the identification was only tentative.

Table 2 summarizes the qualitative results for the carbonyl compounds from the milk fat irradiated at 4.5 Mrad and the control.

TABLE 2

Free Carbonyls from 4.5 Mrad Irradiated and Control Milk Fats.

Corrected Peak Volume (ml)	4.5 Mrad Milk fat	Control Milk Fat		
	Saturated Aldehydes	Saturated Aldehydes	Methyl Ketones	Alk-2-enals
20	n-C ₁₅	n-C ₁₆ to	---	---
30	---	n-C ₁₄	n-C ₁₅	---
40	n-C ₁₄	---	---	---
70	n-C ₁₂	---	---	---
90	---	---	n-C ₁₁	---
110	n-C ₁₀	---	---	n-C ₁₁
150	n-C ₉	---	---	---
200	n-C ₈	---	---	---
270	n-C ₇	n-C ₇	---	---
350	n-C ₆	n-C ₆	---	---
490	n-C ₅	---	---	---
620	n-C ₄	---	---	---
1090	C ₂	C ₂	---	---

Although the list of compounds in Table 2 does not give any quantitative results for the free carbonyls found in the 4.5 Mrad and control milk fat, the area of the peaks (Figure 2) gives a fair estimate of the amount of each carbonyl compound in the free carbonyls of the samples analyzed. Thus with the exception of ethanal whose levels are comparable in the 0 and 4.5 Mrad milk fats, all of the other saturated aldehydes are found in the 4.5 Mrad fat in amounts 3 to 6 times more than in the fresh milk fat.

The effect of the 4.5 Mrad radiation dose was to produce practically the entire spectrum of saturated aldehydes up to a chain length of fifteen carbon atoms. On the other hand the alkanones and enals normally found at low concentrations in fresh milk fat, were not identified in the carbonyls of the irradiated milk fat. Recent observations in this laboratory showed that methyl ketones do not react on the alumina reaction column in the free carbonyl method (56). The negative results for the alkanones were due to this limitation in the above method. On the other hand the small amounts of enals and dienals shown on Table 1, might be of importance to the radiation off-flavor in the 4.5 Mrad milk fat, though no unsaturated carbonyls were detected by the chromatographic methods (88, p. 669-671; 38, p. 870-875; 49, p. 328-334; 57, p. 1285-1288).

Identification of Volatile Monocarbonyl Compounds Isolated by
Molecular Distillation of 4.5 Mrad Milk Fat

This method was used in an attempt to find the individual monocarbonyl compounds of importance to the irradiation off-flavor of milk fat. The original purpose of this approach was to inject the distillate into the gas chromatograph and collect each peak separately in 2,4-dinitrophenylhydrazine reagent for further identification, while in a parallel run each peak would be collected in milk and evaluated by taste. Unfortunately the distillate from the trap No. 1 (Figure 1) of the distillation apparatus, after extraction with ethyl ether and removal of the solvent, did not give but one peak on injection into the Aerograph apparatus. This peak was identified by its retention time to be ethyl ether. A similar observation has been reported by Proctor et al. (106, p. 17) with concentrated ether extracts of the distillates from irradiated milk fat. These distillates left some residue after evaporation of the solvent. The fact that the same distillate which does not give any carbonyl peaks when injected into the Aerograph, gave hydrazones when allowed to react with 2,4-dinitrophenylhydrazine reagent, suggested that the carbonyls condense in an aldol type condensation during the isolation and storage of the distillate.

The distillate, when reacted with 2, 4-dinitrophenylhydrazine reagent, gave hydrazones which were identified by the series of chromatographic techniques used previously. The following compounds were identified:

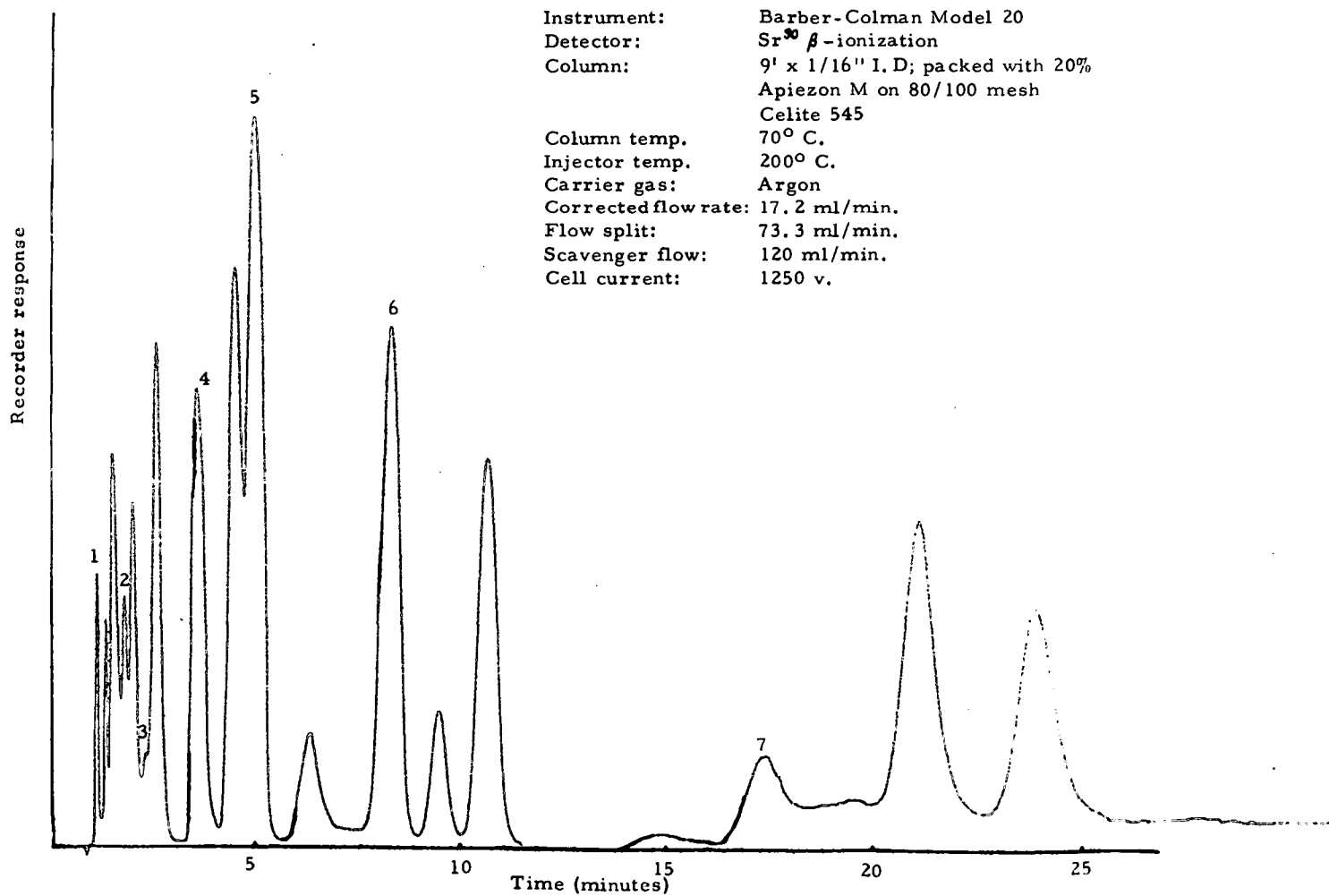
Normal Saturated Alkanals: $C_1, C_2, C_4, C_6, C_8, C_{10},$
 $C_{12}, C_{14},$ and $C_{16}.$

2-Alkanones: C_8, C_{11}, C_{15} or $C_{16}.$

The main difference between the results of the analysis of the free carbonyls and the volatile carbonyls of the 4.5 Mrad milk fat is that in the latter case three ketones were identified. The effect of concentrating the carbonyls of the irradiated milk fat by the molecular distillation is obvious here, while in the former analysis of the free carbonyls these ketones were in very low concentrations and probably were missed during chromatographic separation and analysis.

Additional evidence for the characterization of the carbonyls isolated by molecular distillation was obtained by coil injections of the distillate from the glass bead trap (No. 1, on Figure 1) into the Barber-Colman gas chromatograph. Figure 4 shows one such chromatogram. Some of the peaks shown were tentatively

Figure 4. Gas chromatogram of the volatiles obtained by molecular distillation of the 4.5 Mrad irradiated milk fat.



identified as carbonyls by their retention volumes. Those peaks corresponding to known carbonyls were as follows:

Peak 1 : C₂ al

Peak 2 : C₃ al

Peak 3 : iso-C₄ al

Peak 4 : C₄ al

Peak 5 : iso-C₅ al

Peak 6 : C₅ al

Peak 7 : C₆ al

The results of analysis for free carbonyl content (56) on the fat residues after the first and second pass of the 4.5 Mrad milk fat sample through the molecular still, are given in Table 3. The free carbonyls of the 4.5 Mrad irradiated fat before distillation is also given for the purpose of comparison.

TABLE 3

Changes in the Free Carbonyl Content of Irradiated Milk Fat as Effected by Molecular Distillation.

Sample of Fat	Free Carbonyls (Millimoles of carbonyls per kilogram of fat)		
	Saturated Aldehydes	Alk-2-enals	Alk-2,4-dienals
4.5 Mrad milk fat	0.997	0.098	0.061
Residue of first run	0.848	0.058	0.046
Residue of second run	0.349	0.048	0.014

Parallel taste evaluation of the above two runs showed the presence of free fatty acids in the residue after the first distillation at $3\mu\text{Hg}$ and 48°C . while the residue of the second pass at 100°C . and $3\mu\text{Hg}$ was found not to have any offensive flavor.

It can be seen from the above numerical results that only 20 per cent of the free carbonyls in the irradiated fat was removed by the first molecular distillation. However these carbonyls are the more volatile ones, and more significant from the radiation off-flavor point of view, since the residue fat from the first molecular distillation lacked the characteristic radiation off-flavor.

Identification of Volatile Monocarbonyl Compounds Isolated by
Steam Distillation of 4.5 Mrad Milk Fat

The steam distillation method of Day and Lillard (18, p. 387-388) gave a larger fraction of the volatile carbonyls from the 4.5 Mrad irradiated milk fat than the fraction gained by the molecular distillation procedure. A more complete picture of the irradiation-induced carbonyls was obtained. The results of the free carbonyl determination of the residue from the steam distillation is shown in Table 4, and it is compared with the free carbonyl content of the irradiated fat before the distillation.

TABLE 4

Change in the Free Carbonyl Content of Irradiated Milk Fat as Effected by Steam Distillation.

Sample of Fat	Free Carbonyls		
	(Millimoles of carbonyls per kilogram of fat)		
	Saturated Aldehydes	Alk-2-enals	Alk-2,4-dienals
4.5 Mrad milk fat	0.997	0.098	0.061
Residue after steam distillation	0.789	0.090	0.032

The large decrease of 2, 4-dienals in the residue due to distillation, may involve an important off-flavor 2, 4-dienal component of low flavor threshold and low concentration. No dienals could be detected during chromatographic analysis of the hydrazones from the distillate.

An appreciable amount of the distillate, gained from all the traps of the apparatus, was dicarbonyls because most of the hydrazones formed were not extractable by hexane. Three monocarbonyl bands were separated from the hexane extract by the class separation method (88, p. 669-671). Figures 5 and 6 give the chromatograms of the first (alkanone) and the second (alkanal) band respectively, as resolved by the acetonitrile column (17, p. 322-329). The third band was identified as methanal. Each peak shown in Figures 5 and 6 was identified by a combination of its corrected peak volume according to the standard graph of Figure 3, spectrophotometric absorption studies, and paper chromatographic methods (38, p. 870-875; 49, p. 328-334; 57, p. 1285-1288). The results are given in Table 5. The different area of each peak from Figures 5 and 6 can be used as a good semiquantitative estimate of the amount of each carbonyl in the distillate.

Figure 5. Volatile methyl ketones from steam distillation of 4.5 Mrad milk fat.

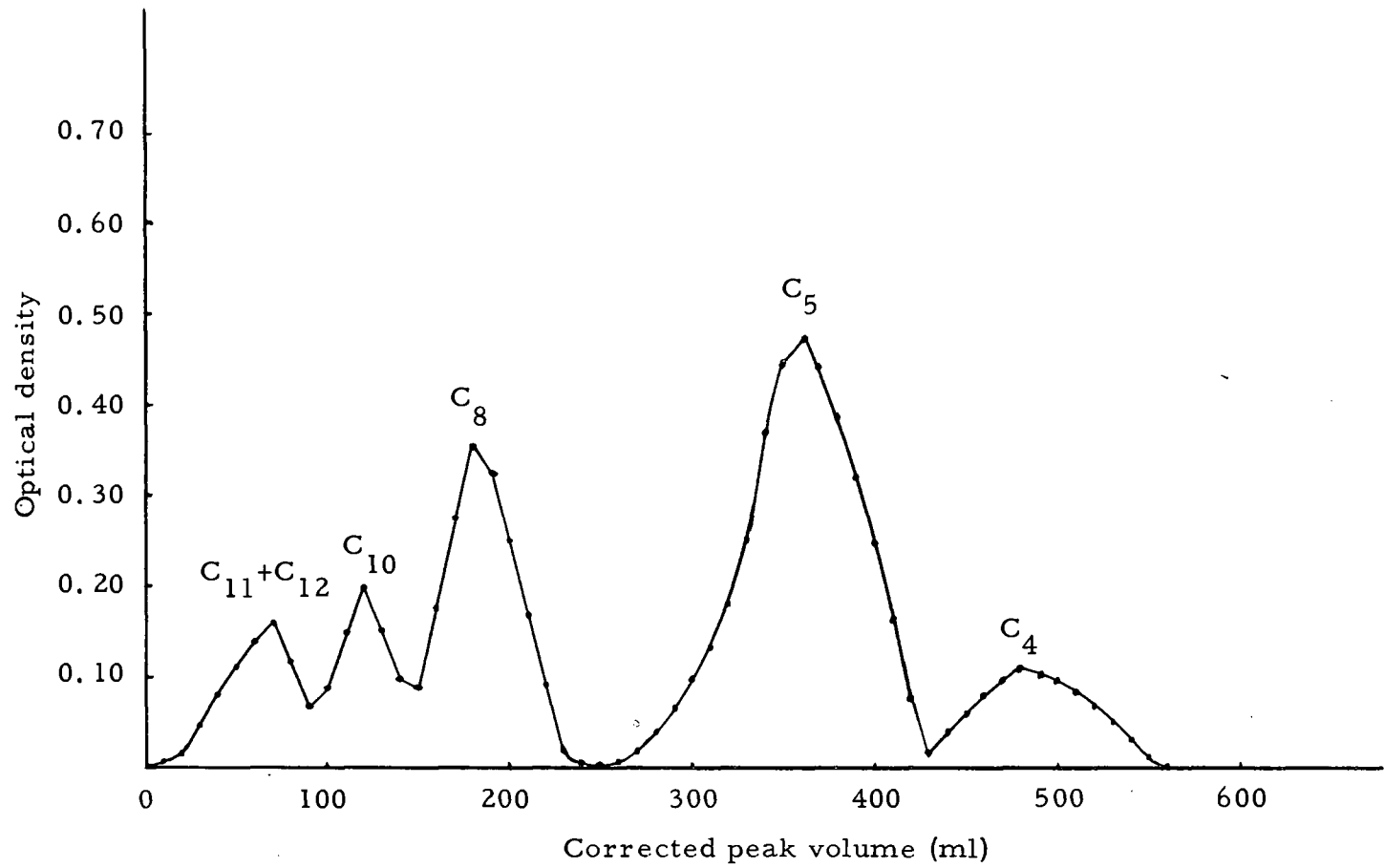


Figure 6. Volatile saturated aldehydes from steam distillation of 4.5 Mrad milk fat.

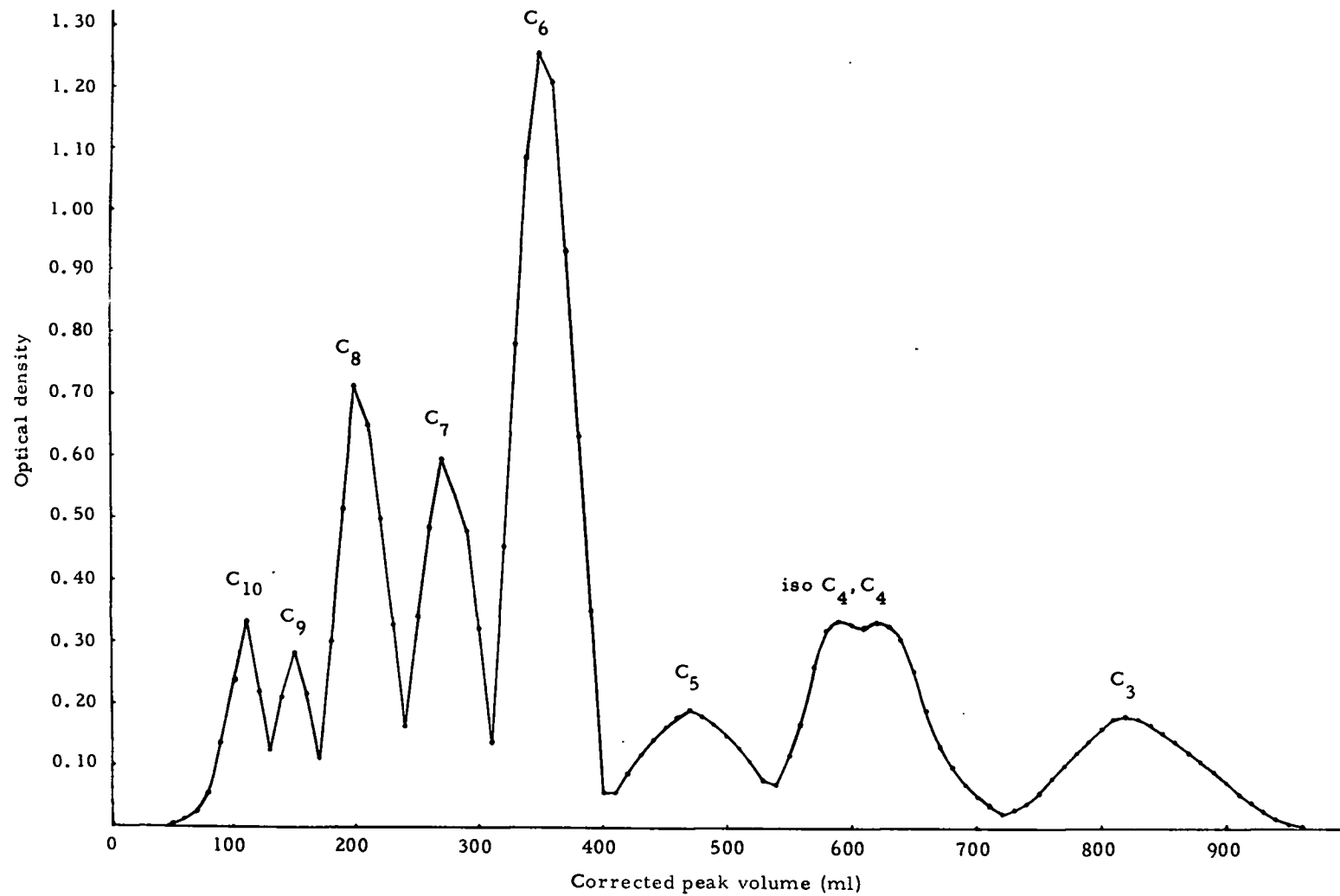


TABLE 5

Identification of Carbonyl Compounds Obtained by Steam Distillation of 4.5 Mrad irradiated Milk Fat.

Corrected peak volume (ml)	Band obtained from class separation column method	Carbonyl compounds
70		C ₁₁ and C ₁₂
120	First band.	C ₁₀
180	Methyl ketones.	C ₈
360	(Figure 5)	C ₅
480		C ₄
110		n - C ₁₀
150		n - C ₉
200		n - C ₈
270	Second band.	n - C ₇
350	Saturated aldehydes.	n - C ₆
470	(Figure 6)	n - C ₅
590		iso - C ₄
620		n - C ₄
820		C ₃
--	Third band	C ₁ aldehyde

Comparisons between Figures 5 and 6 leads immediately to the result that the bulk of the volatile carbonyls of the 4.5 Mrad irradiated milk fat are saturated aldehydes.

The order of decreasing relative concentration of the carbonyls in the distillate for the alkanals was C₆, C₈, C₇, C₄, C₁₀, C₉, C₅ and C₃, while for the alkanones was C₅, C₈, C₁₀, C₁₁ and C₁₂, C₄. It was mentioned earlier that the attempt to separately collect each of the above carbonyls by means of gas chromatography of the distillate, had failed. It was not possible, therefore, to obtain direct evidence as to which of the above carbonyls were more significant from the irradiation off-flavor point of view. However, the fact that the alkanones do not have offensive odors tends to exclude these compounds as the major off-flavor components. Proctor et al. (105, p. 33) reported that the 6 to 8 carbon carbonyl compounds identified from a fraction of 1.8 Mrad milk fat distillate, were most characteristic of "oxidized". This result together with the fact that the C₆ to C₈ saturated aldehydes found previously were the more abundant carbonyls, gives tentative evidence that the C₆, C₈, and C₇ saturated aldehydes are the more significant compounds in the off-flavor of 4.5 Mrad milk fat when irradiated under vacuum.

The several methods for the isolation and characterization of the carbonyl compounds from the irradiated milk fat generally gave comparable results as to the composition of the carbonylic mixture, and even the relative concentration of each carbonyl in the mixture. Thus the peaks of Figures 2 and 6 are very closely comparable. Figure 6 does not contain the higher carbonyls because they are not volatile enough to distill during vacuum steam distillation. Apart from this limitation, the distillation methods had the advantage of isolating a greater amount of carbonyls, so that the alkanones also could be identified.

In this investigation it would appear that each method supports and supplements the results of the other methods and by joining the results of the different methods of isolation of carbonyls it was possible to deduce the whole spectrum of radiation-induced carbonyls from the 4.5 Mrad milk fat. In order of decreasing relative concentration, these carbonyls are as follows:

Saturated aldehydes: $n\text{-C}_{16}$ to $n\text{-C}_{14}$, $n\text{-C}_{12}$, $n\text{-C}_{10}$, $n\text{-C}_6$, $n\text{-C}_8$, $n\text{-C}_7$, $n\text{-C}_4$, iso C_4 , $n\text{-C}_9$, C_3 , $n\text{-C}_5$, C_2 , and C_1

Saturated methyl ketones: C_5 , C_8 , C_{10} , C_{11} to C_{12} , and C_4 .

Among these carbonyl compounds the higher (C_{10} to C_{16}) aldehydes are not volatile enough to contribute to the radiation off-odor, but they apparently cause the chalky or lard-like taste, detectable in the irradiated milk fat.

The saturated aldehydes $n-C_6$, $n-C_7$, and $n-C_8$, on the other hand, are believed to be of more importance to the oxidized off-flavor of irradiated milk fat.

SUMMARY AND CONCLUSIONS

In this investigation an attempt was made to study the effects of gamma radiation on milk fat sealed under vacuum, especially from the point of the flavor-significant radiation-induced carbonyl compounds.

Milk fat, free from phospholipids, was irradiated at different dosages and the oxidative effects of radiation were evaluated by chemical tests that are indicative of lipid oxidation, as well as by tasting the irradiated milk fat.

The radiation-induced carbonyl compounds were isolated from the 4.5 Mrad irradiated milk fat by three general methods: (a) the free carbonyl method, (b) molecular distillation of the fat, and (c) vacuum steam distillation of the fat. The carbonyl compounds obtained by each of the above methods were purified and further fractionated. Gas chromatography was used to fractionate and tentatively identify the more volatile carbonyls isolated by the molecular distillation method. Reaction of the carbonyls with 2, 4-dinitrophenylhydrazine reagent was used in most cases, and the 2, 4-dinitrophenylhydrazones formed were used to further purify, fractionate, isolate and characterize the

carbonyl compounds. A series of spectrophotometric and column and paper chromatographic methods were used to confirm the identity of the derivatives.

The findings of this investigation that are believed to be of importance to the problem of irradiation induced oxidation of milk fat were as follows:

1. Gamma irradiation of milk fat, sealed under vacuum, did not produce large quantities of stable peroxides, but off-flavors and carbonyl compounds were produced in appreciable amounts in proportion to the radiation dosage. This finding is similar to the one reported by Chipault et al. (13, p. 1713-1720). They proposed that in such cases the carbonyls are formed not through peroxide decomposition, but by a parallel mechanism where the carbonyls that are formed derive their oxygen from the ester group of the glycerides.

2. The peroxide value does not correlate with the oxidative changes of milk fat irradiation above a certain dose in the absence of oxygen.

3. The method of measuring the unsaturated total carbonyls (8, p. 693-701), was not accurate for either fresh or irradiated milk fat.

4. The best of the tests used for the estimation of the oxidative changes of milk fat during irradiation was the one for the free carbonyl content (56). All classes of the free carbonyls proportionally increased with the radiation dosage.

5. Although the test for free carbonyl content on the 4.5 irradiated milk fat gave a result of about 0.1 millimole of alk-2-enals per kilogram of fat, no unsaturated carbonyls were identified in the carbonyls isolated from the irradiation procedures. This conclusion is in line with the results of Proctor et al. (105, p. 23) who could not detect any unsaturation from the infrared spectra of the distillate from irradiated milk fat containing the carbonyls.

6. The carbonyl compounds produced by the irradiation of milk fat under vacuum and at a dose of 4.5 Mrad, were the following in the order of decreasing relative concentration of each carbonyl:

Saturated aldehydes: n-C₁₆ to n-C₁₄, n-C₁₂, n-C₁₀, n-C₆, n-C₈, n-C₇, n-C₄, iso-C₄, n-C₉, C₃, n-C₅, C₂, and C₁

Saturated methyl ketones: C₅, C₈, C₁₀, C₁₁ to C₁₂, and C₄.

7. The bulk of the volatile carbonyls from the irradiated milk fat are saturated aldehydes.

8. Indirect indications suggest that the carbonyls significant to the radiation off-flavor of the milk fat are saturated aldehydes, and especially the evil smelling $n\text{-C}_4$ to C_8 , the $n\text{-C}_6$ to $n\text{-C}_8$ being found in higher relative concentrations.

BIBLIOGRAPHY

1. Allen, R. R., A. Jackson and F. A. Kummerow. Factors which affect the stability of highly unsaturated fatty acids. I. Difference in the oxidation of conjugated and nonconjugated linoleic acid. *Journal of the American Oil Chemists' Society* 26:395-399. 1949.
2. American Oil Chemists' Society. Official and tentative methods of analysis. Chicago, 1946 ed. Rev. 1961. Tentative method cd 8-53.
3. Association of Official Agricultural Chemists. Official methods of analysis. 6th ed. Washington, 1945. 932 p.
4. Astrack, A. et al. Effects of high intensity electron bursts upon various vegetable and fish oils. *Food Research* 17:571-583. 1952.
5. Batzer, O. F. et al. Production of carbonyl compounds during irradiation of meat fats. *Journal of Agricultural and Food Chemistry* 5:700-705. 1957.
6. Becker, R. R. et al. Nutritional and biochemical effects of irradiation. *Food Technology* 10:61-64. 1956.
7. Berzelius, J. J. *Lehrbuch der Chemie, IV.* Dresden, Arnold, 1847. 725 p.
8. Berry, N. W. and A. A. McKerrigan. Carbonyl compounds as a criterion of flavor deterioration in edible fats. *Journal of the Science of Food and Agriculture* 9:693-701. 1958.
9. Bolland, J. L. and H. P. Koch. The source of autoxidation reaction in polyisoprenes and allied compounds. Part IX. The primary thermal oxidation products of ethyl linoleate. *The Journal of the Chemical Society* 67:445-447. 1945.
10. Brekke, J. and G. MacKinney. Aldehydes from rancid corn and avocado oil. *Journal of the American Oil Chemists' Society* 27:238-242. 1950.

11. Burton, V. L. The effects of radioactivity on oleic acid. *Journal of the American Chemical Society* 71:4117-4119. 1949.
12. Cannon, J. A. et al. Analysis of fat acid oxidation products by countercurrent distribution method. IV. Methyl linoleate. *Journal of the American Oil Chemists' Society* 29:447-451. 1952.
13. Chipault, J. R. et al. Effects of ionizing radiations on fatty acid esters. *Industrial and Engineering Chemistry* 49:1713-1720. 1957
14. Chipault, J. R. High energy irradiation. In: Harold W. Schultz's Symposium on Foods. Lipids and their oxidation. Westport, Connecticut, AVI, 1962 (In press).
15. Colleby, B. Chemical changes produced in lipids by irradiation. *International Journal of Applied Radiation and Isotopes* 6:71-75. 1959.
16. Coleman, J. E. and Daniel Swern. Reaction of fatty materials with oxygen. XVIII. Catalytic hydrogenation of autoxidized methyl oleate and oleic acid. Preparation of monohydroxystearic acids. *Journal of the American Oil Chemists' Society* 32:221-224. 1955.
17. Corbin, E. A., D. P. Schwartz and Mark Keeney. Liquid-liquid partition chromatography. Separation of the 2, 4-dinitrophenylhydrazones of saturated aldehydes, methyl ketones, 2-enals, and 2, 4-dienals. *Journal of Chromatography* 3:322-329. 1960.
18. Day, E. A. and D. A. Lillard. Autoxidation of milk lipids. I. Identification of volatile monocarbonyl compounds from autoxidized milk fat. *Journal of Dairy Science* 43:585-597. 1960.
19. Day, E. A. Autoxidation of milk lipids. *Journal of Dairy Science* 43:1360-1365. 1960.

20. Dugan, L. R., Jr. and P. W. Landis. Influence of high energy radiation on oxidation of oleic acid and methyl oleate. *Journal of the American Oil Chemists' Society* 33:152-154. 1956.
21. Duin, H. van. Investigation into the carbonyl compounds in butter. II. Preliminary investigation into the autoxidation products of phosphatides from butter. *Netherlands Milk and Dairy Journal* 12:81-89. 1958.
22. Duin, H. van. Investigation into the carbonyl compounds in butter. III. Phosphatide-bound aldehydes. *Netherlands Milk and Dairy Journal* 12:90-95. 1958.
23. Dunn, C. G. et al. Biological and photochemical effects of high energy electrostatically produced roentgen and cathode rays. *Journal of the Applied Physics* 19:605-616. 1948.
24. Farmer, E. Harold. Peroxidation in relation to olefinic structure. *Transaction of the Faraday Society* 42:228-236. 1941.
25. Farmer, E. Harold. α -methylene reactivity in olefinic and polyolefinic systems. *Transactions of the Faraday Society* 38:340-348. 1942.
26. Farmer, E. Harold and Alvappillai Sundralingam. The course of autoxidation reaction in polyisoprenes and allied compounds. Part I. The structure and reactive tendencies of peroxides of simple olefins. *Journal of the Chemical Society* 64:121-139. 1942.
27. Farmer, E. Harold and Donald A. Sutton. The course of autoxidation reaction in polyisoprenes and allied compounds. Part IV. The isolation and constitution of photochemically-formed methyl oleate peroxide. *Journal of the Chemical Society* 65:119-122. 1943.
28. Farmer, E. Harold, H. P. Kock and Donald A. Sutton. The course of autoxidation reaction in polyisoprenes and allied compounds. Part VII. Rearrangement of double bonds during autoxidation. *Journal of the Chemical Society* 65:541-597. 1943.
29. Farmer, E. Harold and Donald A. Sutton. The course of autoxidation reaction in polyisoprenes and allied compounds. Part VI. Double bond movement during autoxidation of a mono olefin. *Journal of the Chemical Society* 68:10-13. 1946.

30. Farmer, E. Harold. Certain fundamental concepts relating to nonpolar mechanisms in olefinic systems. *Journal of the Society of Chemical Industry* 66:86-93. 1947.
31. Forss, D. A. and E. A. Dunstone. The Girard reaction. *Nature* 173:401-402. 1954.
32. Forss, D. A., E. G. Pont and W. Stark. The volatile compounds associated with oxidized flavor in skim milk. *The Journal of Dairy Research* 22:91-102. 1955.
33. Forss, D. A., E. G. Pont and W. Stark. Further observations on the volatile compounds associated with oxidized flavor in skim milk. *The Journal of Dairy Research* 22:345-348. 1955.
34. Forss, D. A., E. A. Dunstone and W. Stark. Fishy flavour in dairy products. II. The volatile compounds associated with fishy flavour in butterfat. *Journal of Dairy Research* 27:211-219. 1960.
35. Friedlander, G. and J. W. Kennedy. *Nuclear and radio-chemistry*. New York, Wiley, 1960. 468 p.
36. Fugger, J. et al. Analysis of fat acid oxidation products by counter-current distribution method. III Methyl linolenate. *Journal of the American Oil Chemists' Society* 28:285-289. 1951.
37. Gaddis, A. M. and Rex Ellis. Volatile saturated aliphatic aldehydes in rancid fat. *Science* 126:745. 1957.
38. Gaddis, A. M. and Rex Ellis. Paper chromatography of 2, 4-dinitrophenylhydrazones. Resolution of 2-alkanone, n-alkanol, alk-2-enal, and alk-2, 4-dienal derivatives. *Analytical Chemistry* 31:870-875. 1959.
39. Godel, A. Vitamins as a method of controlling the development of bitter taste in butter. *Molochnaya Promyshlennost* 10:28. 1949. (Abstracted in *Chemical Abstracts* 43:9282. 1949)
40. Hannan, R. S. and J. W. Boag. Effects of electronic irradiation on fats. *Nature* 169:152-153. 1952.

41. Hannan, R. S. and H. J. Shepherd. An aftereffect in butterfat irradiated with high-energy electrons. *Nature* 170:1021-1022. 1952.
42. Hannan, R. S. and H. J. Shepherd. Some after effects in the fats irradiated with high energy electrons and x-rays. *British Journal of Radiology* 27:36-41. 1954.
43. Hannan, R. S. Department of Scientific and Industrial Research. Food Investigation. H. M. Stationery office. London 1955. 192 p. (Special Report No. 61)
44. Hilditch, T. P. Mechanism of oxidation and reduction of the unsaturated groups in drying oils. *Journal of the Oil and Color Chemists' Association* 30:1-16. 1947.
45. Hoff, J. E., J. H. Wertheim and B. E. Proctor. Radiation preservation of milk and milk products. V. Precursors to the radiation-induced oxidation flavor of milk fat. *Journal of Dairy Science* 42:468-475. 1959.
46. Holm, V., G. Wode and K. E. Thome. Oily flavors in butter and α,β -unsaturated carbonyl compounds. Malmo, Sweden, 1952. 12 p. (Meddelande Fren Statens Mejeriforaok No. 37).
47. Holman, Ralph T. and Otto C. Elmer. The rate of oxidation of unsaturated fatty acids and esters. *Journal of the American Oil Chemists' Society* 24:127-129. 1947.
48. Holman, Ralph T. Autoxidation of fats and related substances. *Progress in the Chemistry of Fats and Other Lipids* 2:51-98. 1959.
49. Huelin, F. E. Volatile products of apples. III. Identification of aldehydes and ketones. *Australian Journal of Scientific Research* 5B:328-334. 1952.
50. Jenness, R. and S. Patton. Principles of dairy chemistry. New York, Wiley, 1959. 446 p.
51. Kawahara, F. K. and H. J. Dutton. Volatile cleavage products of autoxidized soybean oil. *Journal of the American Oil Chemists' Society* 29:372-377. 1952.
52. Keeney, Mark and F. J. Doan. Studies on oxidized milk fat. I. Observations on the chemical properties of the volatile flavor material from oxidized milk fat. *Journal of Dairy Science* 34:713-718. 1951.

53. Keeney, Mark and F. J. Doan. Studies on oxidized milk fat. II. Preparation of 2, 4-dinitrophenylhydrazones from the volatile material from oxidized milk fat. *Journal of Dairy Science* 34:719-727. 1951.
54. Keeney, Mark and F. J. Doan. Studies on oxidized milk fat. III. Chemical and organoleptic properties of volatile material obtained by fractionation with various solvents and Girard's reagent. *Journal of Dairy Science* 34:728-734. 1951.
55. Keeney, P. G. and S. Patton. Isolation and identification of δ -decalactone as the compound responsible for the coconut like flavor of butteroil. *Journal of Dairy Science* 38:592-593. 1955.
56. Keith, R. W. and E. A. Day. Determination of the classes of free monocarbonyl compounds in oxidizing fats and oils. Corvallis, Oregon. Oregon State University, Department of Food Science and Technology. (In press).
57. Klein, F. and DeJong, K. Paper chromatography of 2, 4-dinitrophenylhydrazones of aliphatic carbonyl compounds. *Recueil des Travaux Chimiques des Pays-Bas* 75:1285-1288. 1956.
58. Knight, H. B., Joseph E. Coleman and Daniel Swern. Reaction of fatty material with oxygen. IV. Analytical study of the autoxidation of methyl oleate. *Journal of the American Oil Chemists' Society* 28:498-501. 1937.
59. Kung, H.-C., E. L. Gaden, Jr. and C. G. King. Vitamins and enzymes in milk. Effect of gamma-radiation on activity. *Journal of Agricultural and Food Chemistry* 1:142-144. 1953.
60. Lea, C. H. Recent developments in the study of oxidative deterioration of lipids. *Chemistry and Industry* 41:1303-1309. 1953.
61. Lea, C. H. Chemical changes in stored foods when microbial spoilage is not limiting. *International Journal of Applied Radiation and Isotopes* 6:86-94. 1959.

62. Long, J. S. and C. N. Moore. Action of cathode rays on drying oils. *Industrial and Engineering Chemistry* 19:901-903. 1927.
63. Lundberg, W. O. and J. R. Chipault. The oxidation of methyl lineoleate at various temperatures. *Journal of the American Chemical Society* 69:833-836. 1947.
64. Lundberg, W. O. and J. R. Chipault. Recent advances concerning the stability of foods. *Food Technology* 8:541-546. 1954.
65. Lundberg, W. O. Mechanisms and products of lipid oxidation. In: Harold W. Schultz's Symposium on Foods. Lipids and their oxidation. Westport, Connecticut, AVI, 1962. (In press)
66. Mead, J. F. The irradiation-induced antoxidation of linoleic acid. *Science* 115:470-472. 1952.
67. Meinke, W. W. Does irradiation induce radioactivity in foods? *Nucleonics* 12(10):37-40. 1954.
68. Morris, Steward. Fat rancidity. Recent studies on the mechanism of the fat oxidation in relation to rancidity. *Journal of Agricultural and Food Chemistry* 2:126-132. 1954.
69. Mukherjee, S. Studies on rancidity of butterfat. *Journal, Indian Chemical Society* 27:589. 1950.
70. Myers, J. E., J. P. Kass and C. O. Burr. Comparative rates of oxidation of isomeric linoleic acids and their esters. *Oil and soap* 18:107-109. 1941.
71. Nickerson, J. T. R., B. E. Proctor and S. A. Goldblith. Public health aspects of electronic food sterilization. *American Journal of Public Health* 43:554-560. 1953.
72. Noller, R. Carl. *Chemistry of organic compounds*. 2d ed. London, W. B. Saunders, 1958. 978 p.

73. Parks, Owen W., Mark Kenney and Daniel P. Schwartz. Bound aldehydes in butteroil. *Journal of Dairy Science* 44:1950-1943. 1961.
74. Paschke, R. F. and D. H. Wheeler. Formation and decomposition of peroxides of unsaturated fat esters. *Oil and Soap* 21:52-57. 1944.
75. Patton, S. and B. W. Tharp. Formation of methyl ketones from milk fat during steam distillation or saponification. *Journal of Dairy Science* 42:49-55. 1959.
76. Privett, O. S. et al. Structure of hydroperoxides obtained from autoxidized methyl linoleate. *Journal of the American Oil Chemists' Society* 30:61-66. 1953.
77. Privett, O. S. et al. Evidence for hydroperoxide formation in the autoxidation of methyl linolenate. *Journal of the American Oil Chemists' Society* 31:23-27. 1954.
78. Proctor, B. E. and S. A. Goldblith. Food Processing with ionizing radiations. *Food Technology* 5:376-380. 1951.
79. Richardson, L. R. and Ruth, Brock. The nutritional value of a synthetic diet sterilized by gamma rays, as measured by reproduction and life span of rats. *Journal of Nutrition* 65:353-360. 1958.
80. Riel, R. R. and H. H. Sommer. Oxidized flavor in milk. I. Review of literature. La Trappe, P. Q., Canada, 1954. 39 p. (Contribution de l'Institut d' Oka. Université de Montreal, No. 10)
81. Riel, R. R. and H. H. Sommer. Oxidized flavor in milk. III. Characterization of some oxidation products associated with oxidized flavors. La Trappe, P. Q., Canada. 1955. 73 p. (Contribution de l'Institut d' Oka. Université de Montreal, No. 11)
82. Riemenschneider, R. W. Oxidative rancidity and the use of antioxidants. *Transactions, American Association of Cereal Chemists* 5:50-63. 1947.

83. Saunders, D. H., C. Ricciuti and D. Swern. Reactions of fatty materials with oxygen. XVI. Relation of hydroperoxide and chemical peroxide content to total oxygen absorbed in antoxidation of methyl oleate. *Journal of the American Oil Chemists' Society* 32:79-83. 1955.
84. Schogt, J. C. M., P. H. Begemann and J. H. Recourt. Composition of aldehydes derived from some bovine lipids. *Journal of Lipid Research* 2:142-147. 1961.
85. Schonbein, C. F. Uber das verhalten des Bitterman deloles zum Sauerstoffe. *Journal fur Pracktische Chemie* 74:328-340. 1858. (Cited in Klave S. Markley's *Fatty Acids*. New York, Interscience, 1947. p. 454)
86. Schwartz, D. P. et al. Separation of fat from hydrazones. *American Chemical Society Abstracts of Papers* 136:15A. Sept. 1959.
87. Schwartz, D. P. and O. W. Parks. Preparation of carbonyl-free solvents. *Analytical Chemistry* 33:1396-1398. 1961.
88. Schwartz, D. P., O. W. Parks and Mark Keeney. Separation of 2, 4-dinitrophenylhydrazone derivatives of aliphatic-monocarbonyls into classes on magnesia. *Analytical Chemistry* 34:669-671. 1962.
89. Schwartz, D. P., A. R. Johnson and O. W. Parks. Use of ion-exchange resins in the microanalysis of 2, 4-dinitrophenylhydrazones. *Microchemical Journal* 6:37-44. 1962.
90. Slover, H. T. and L. R. Dugan. Influence of high energy radiation on the oxidation of oleic acid and methyl oleate. II. Sites of oxygen attack. *Journal of the American Oil Chemists' Society* 34:333-335. 1957.
91. Smith, L. M. and E. L. Jack. The unsaturated fatty acids of milk fat. I. Methyl ester fractionation and isolation of monoethenoid constituents. *Journal of Dairy Science* 37:380-389. 1954.

92. Sribney, M., V. J. Lewis and B. S. Schweigert. Effects of irradiation of meat fats. *Journal of Agriculture and Food Chemistry* 3:958-960. 1955.
93. Swern, D. et al. Reactions of fatty materials with oxygen. XIV. Polarographic and infrared spectrophotometric investigation of peroxides from autoxidized methyl oleate. *Journal of the American Chemical Society* 75:3135-3137. 1953.
94. Swift, S. E. et al. The aldehydes produced during autoxidation of cottonseed oil. *Journal of the American Oil Chemists' Society* 26:297-300. 1949.
95. Tamsma, A. F. A study of the volatile fraction isolated from oxidized milk fat. I. Isolation, preliminary characterization, and chromatographic separation of volatile fraction. *Journal of Dairy Science* 38:284-291. 1955.
96. Tamsma, A. F. A study of the volatile fraction isolated from oxidized milk fat. II. Further characterization of compounds responsible for the oxidized flavor. *Journal of Dairy Science* 38:487-498. 1955.
97. Tappel, A. L., P. D. Boyer and W. O. Lundberg. The reaction mechanism of soybean lipoxidase. *Journal of Biological Chemistry* 199:267-281. 1952.
98. Uri, N. Some aspects of radiation-induced free radical reactions and the significance of free radical acceptors. *International Journal of Applied Radiation and Isotopes* 6:59-68. 1959.
99. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1955 [11] p. (B. E. Proctor, M.I.T. Contract No. DA 19-129-QM-522. S-501 - Rpt. #2)
100. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1955 [101] p. (B. E. Proctor, M.I.T. Contract No. D.A. 19-129-QM-522. S-501 - Rpt. #8)

101. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1956. [13] p.
(B. E. Proctor, M.I.T. Contract No. DA-19-129-QM-522. S-501 - Rpt. #5)
102. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1956. [26] p.
(B. E. Proctor, M.I.T. Contract No. DA-19-129-QM-522. S-501 - Rpt. #6)
103. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1956. [20] p.
(B. E. Proctor, M.I.T. Contract No. DA 19-129-QM-522. S-501 - Rpt. #7)
104. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1956 [28] p.
(B. E. Proctor, M.I.T. Contract No. DA 19-129-QM-522. S-501 - Rpt. #9)
105. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1956. [60] p.
(B. E. Proctor, M.I.T. Contract No. DA 19-129-QM-905. S-501 - Rpt. #2)
106. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1957. [34] p.
(B. E. Proctor, M.I.T. Contract No. DA 19-129-QM-522. S-501 - Rpt. #10)

107. U. S. Army. Quartermaster Corps. Studies of the fundamental physical and biological changes that occur as a result of radiation treatment of foods resulting in modifications of flavor, color and texture. Chicago, Ill., 1957. [84] p. (B. E. Proctor, M.I.T. Contract No. DA 19-129-QM-522. S-501-Rpt. #11)
108. Waarden, van der, M. Chemical and physical investigations of dairy products. New York, Elsevier, 1947. 215 p.
109. Weiss, J. Effects of radiations on aqueous systems under aerobic and anaerobic conditions. International Journal of Applied Radiation and Isotopes 6:52-58. 1959.
110. Wertheim, J. H. et al. Irradiation preservation of milk and milk products. Journal of Agricultural and Food Chemistry 5:944-950. 1957.
111. Witting, L. A. and B. S. Schweigert. A characterization of volatile carbonyl compounds isolated from meat fat subjected to gamma radiation. The Journal of the American Oil Chemists' Society 35:413-416. 1958.
112. Yu, T. C. and R. O. Sinnhuber. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technology 11:104-108. 1957.