AN ABSTRACT OF THE THESIS OF

Lakho Lilaram Khatri for the Ph. D. in Food Technology
(Name) (Degree) (Major)

Date thesis is presented October 25, 1965

Title FLAVOR CHEMISTRY OF IRRADIATED MILK FAT

Abstract approved ____________
(Major Professor)

Increasing interest has been shown in the irradiation sterilization and irradiation pasteurization of foods, but problems of off-flavors and odors are still unsolved, especially in the case of dairy products. From the flavor chemistry point of view, milk lipids are very highly susceptible to irradiation effects. Therefore, this investigation was designed to study some irradiation induced reactions involving flavor changes in the milk fat and to identify the volatile components produced in the milk fat upon irradiation.

Milk fat, prepared from raw sweet cream and washed free of phospholipids, was first irradiated in the presence of air and under vacuum in glass vials at 4.5 Mrad with gamma rays from cobalt-60. The irradiation resulted in increase in TBA number, peroxide value, total monocarbonyls, bleaching of color, slightly rancid and typical candle-like off-flavors. Free fatty acids were also produced upon irradiation. The changes were more drastic in air along with production of a slight oxidized flavor. The monocarbonyls identified by column and paper chromatographic methods in irradiated milk fat

include: C_1 through C_{12}, C_{14}, and C_{16} n-alkanals; C_3 through C_9, C_{11}, C_{13} and C_{15} alk-2-ones with only traces of C_6 and C_8 alk-2-ones; and C_5, C_6, C_9, and C_{12} alk-2-enals.

Irradiation of milk fat that had been dried over calcium hydride also caused free fatty acid production, especially short chain fatty acids. Methyl octanoate treated with calcium hydride and irradiated at 1.5, 3.0, 4.5, and 6.0 Mrad yielded small quantities of free octanoic acid, confirming that irradiation caused fission of the ester linkage even when traces of water were removed. The quantities of octanoic acid formed increased with increasing dose of irradiation.

For identification of volatile components, the milk fat was irradiated in 307x409 'C' enameled cans under vacuum. The headspace analysis showed some air still left in the cans. Irradiation resulted in consumption of oxygen and production of hydrogen, carbon monoxide, carbon dioxide, and methane as identified in the headspace gases. The volatiles were isolated from the irradiated and control milk fats by low temperature, vacuum steam distillation at 40° C and 1-2 mm Hg. The volatile components were then extracted from the aqueous distillate with ethyl ether. The ethyl ether extract exhibited the typical candle-like defect. The ethyl ether concentrate was analyzed by combination of GLC and fast-scan mass spectrometric techniques. Identification of various components was achieved on the basis of mass spectral data and coincidence of gas chromatographic retention times. In the case of the components for which only GLC t_R/t_R evidence was available or the mass spectra obtained were not satisfactory, the identity assigned was only tentative.

The volatile compounds that were positively identified to be present in irradiated milk fat are given below:

n-Alkanes	C_5 through C_{17}
1-Alkenes	C_5, C_7 through C_{17}
Fatty acids	C_4, C_6, C_8 and C_{10}
n-Alkanals	C_5 through C_{11}
Others	γ-decalactone, δ-decalactone, 2-heptanone, benzene, ethyl acetate, chloroform, and dichlorobenzene.

The tentative identification was obtained for the following compounds:

γ-lactones	C_6 and C_8
δ-lactones	C_6, C_8, C_{11}, and C_{12}
1, ?-alkadienes	C_{10}, C_{11}, C_{12}, C_{16} and C_{17}
iso-alkanes	C_{10}, C_{11}, C_{12}, and C_{13}
Others	methyl hexanoate, 2-hexanone, 4-heptanone and n-dodecanal.

The compounds present in unirradiated control milk fat included: short chain fatty acids (C_4, C_6, C_8, and C_{10}), C_8, C_{10}, and C_{12} δ-lactones, 2-heptanone, chloroform, dichlorobenzene, benzene, toluene, and ethyl-benzene. Only tentative identity was established for most of these components in control milk fat. Possible reaction mechanisms are presented for the formation of the compounds in irradiated milk fat.

FLAVOR CHEMISTRY OF IRRADIATED MILK FAT

by

LAKHO LILARAM KHATRI

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1966

APPROVED:

Professor of Food Science and Technology
In Charge of Major

Head of Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented October 25, 1965

Typed by Marcia Ten Eyck

ACKNOWLEDGEMENT

I wish to express my gratitude to Dr. E. A. Day for his inspiration, guidance and always willing assistance throughout this investigation. I also would like to thank the faculty and fellow graduate students of this department for their assistance.

My special thanks to Dr. L. M. Libbey for his help in obtaining and interpreting the mass spectra.

I also wish to express my gratitude to my parents; without their help this work would not have been possible.

A special expression of thanks to my wife, Ratna, and son, Sunil, for their patience and continuous encouragement.

TABLE OF CONTENTS

Page

INTRODUCTION 1

REVIEW OF LITERATURE 3

- Irradiation Induced Changes in Lipids 3
  - Organoleptic Changes 3
  - Changes in Appearance 6
  - Chemical Changes 7
    - Free Radicals 9
    - Irradiation-Oxidation 11
    - Hydroperoxides 12
    - Carbonyl Compounds 16
    - Free Fatty Acids 22
    - Hydrocarbons 24
    - Gaseous Products 26
    - Polymerization 28
    - Cis-Trans Isomerization 29
    - Double Bond Displacement 29
    - Miscellaneous Changes 30
- Factors Affecting Irradiation-Induced Changes in Lipids 32
  - Types of Ionizing Radiations 32
  - Dose Rate 33
  - Physical State of Fat 34
  - Temperature 34
  - Storage 36
  - Oxygen or Air 37
  - Presence of Water 39
  - Antioxidants 40
- Mechanisms Involved in Irradiation-Induced Reactions 42
  - Oxidation 46
  - Chain Scission 48
  - Polymerization 48
  - Decarboxylation 49
  - Hydrolysis 49
  - Dehydrogenation 51
  - Hydrogenation 52
  - Cis-Trans Isomerization 52

TABLE OF CONTENTS Continued

Page

EXPERIMENTAL 54

Preparation and Irradiation of the Milk Fat 54
Analysis of the Irradiated Milk Fat 55
  TBA Number 56
  Peroxide Value 57
  Free Fatty Acids 57
  Monocarbonyls 57
    Preparation of Solvents 57
    Formation and Separation of Monocarbonyl DNP-hydrazones 58
    Partition Chromatography of Monocarbonyl Mixture 59
    Thin-Layer Chromatography of Separated Monocarbonyl Bands 60
    Paper Chromatography of the Separated Monocarbonyl Bands 60
Effect of Traces of Water Present on the Free Fatty Acid Content of Irradiated Milk Fat 60
Irradiation-Hydrolysis of Methyl Octanoate 61
  Purity of Methyl Octanoate 61
  Preparation of Anhydrous Methyl Octanoate 62
  Irradiation of Methyl Octanoate 63
  Analysis of Irradiated Methyl Octanoate 63
Identification and Characterization of Steam Volatile Components of Gamma Irradiated Milk Fat 66
  Preparation and Irradiation of the Milk Fat 66
  Analysis of the Head-Space Gases 67
  Vacuum Steam Distillation of the Irradiated Milk Fat 68
  Purification of Ethyl Ether 69
  Extraction and Concentration of Volatiles 70
  Analysis of Monocarbonyls in the Ether Extract 71
  Gas-Liquid Chromatography (GLC) 72
  GLC Combined with Fast-Scan Mass Spectrometry 75

RESULTS AND DISCUSSION 83

Effects of Irradiation under Air and Vacuum 83
  Changes in Physical and Chemical Properties 83
  Monocarbonyl Compounds 86
  Free Fatty Acids 89
Effect of Traces of Water on the Free Fatty Acid Content of Irradiated Milk Fat 93

TABLE OF CONTENTS Continued

Page

Hydrolysis of Methyl Octanoate upon Irradiation 95
Analysis of Head-Space Gases of Irradiated Milk Fat 97
Identification of Volatile Components 100
    GLC Analysis 100
    GLC in Conjunction with Mass Spectrometry 103
    Analysis of the Mass Spectra 123
Possible Mechanisms for the Production of Some of the Compounds Identified 128

SUMMARY AND CONCLUSIONS 133

BIBLIOGRAPHY 137

LIST OF FIGURES

Figure		Page
1	DEGS column bleed obtained with the temperature programming.	77
2	Gas chromatogram of free fatty acid methyl esters from 4. 5 Mrad milk fat (irradiated in air).	90
3	Head-space gases from 4. 5 Mrad milk fat (A) and unirradiated control.	98
4	Gas chromatogram of steam volatiles of 4. 5 Mrad milk fat using a DEGS column at 125° C.	101
5	Gas chromatogram of 4. 5 Mrad milk fat steam volatiles obtained on 24 ft. DEGS column using temperature programming.	105
6	Gas chromatogram of the steam volatiles of 4. 5 Mrad milk fat using a 10 ft. DEGS column at 150° C.	107
7	Gas chromatogram of the steam volatiles of 4. 5 Mrad milk fat using a 10 ft. DEGS column at 175° C.	110
8	Gas chromatogram of 4. 5 Mrad milk fat steam volatiles using a 2 ft. base pre-column + a 12 ft. Apiezon M column with temperature programming.	114
9	Gas chromatogram of high boiling components of 4. 5 Mrad milk fat steam volatiles using a 6 ft. Apiezon M column at 220° C.	118
10	Mass spectral charts for GLC fractions shown in Figure 8.	124
11	Mass spectral charts for GLC fractions shown in Figure 8.	127

LIST OF TABLES

Table		Page
1	Products expected from irradiation of fats.	9
2	Composition of the monocarbonyl fraction isolated from 4.5 Mrad fat.	21
3	Gross changes in gamma irradiated milk fat.	84
4	Monocarbonyl compounds produced in milk fat upon gamma irradiation in air and under vacuum.	87
5	Major free fatty acids in 4. 5 Mrad milk fat.	91
6	Effect of removing traces of water before irradiation on the free fatty acid content of milk fat.	94
7	Mg free octanoic acid produced per 100 g irradiated methyl octanoate.	95
8	Tentative identification and odor characteristics of some GLC peaks shown in Figure 4.	102
9	GLC and mass spectral identification of the steam volatile components of 4. 5 Mrad milk fat.	106
10	GLC and mass spectral identification of some high boiling components of 4. 5 Mrad milk fat volatiles using 10 ft. DEGS column at 150° C.	108
11	GLC and mass spectral identification of some high boiling components of 4. 5 Mrad milk fat volatiles using a 10 ft. DEGS column at 175° C.	111
12	GLC and mass spectral identification of the steam volatile components of gamma irradiated milk fat using a 2 ft. base pre-column plus 12 ft. Apiezon M column with temperature programming.	115
13	GLC and mass spectral identification of high boiling components of 4. 5 Mrad milk fat steam volatiles using a 6 ft. Apiezon M column.	119

FLAVOR CHEMISTRY OF IRRADIATED MILK FAT

INTRODUCTION

Ever since the bactericidal and germicidal properties of electromagnetic radiations were discovered, interest in radiation sterilization or "cold sterilization" of foods has grown. Heat sterilization is known to change the flavor, color and texture properties of foods. This is especially true in the case of dairy products, where bacterial spoilage is a great problem and heat sterilization brings about many undesirable changes.

Ionizing radiations have been successfully employed in the preservation of some foods. In the case of milk and milk products, however, the use of ionization radiations has created some acute problems. When milk is irradiated even with one-hundredth of the dose required for complete sterilization, the undesirable off-flavors are detected (La Fuente, Goldblith and Proctor, 1959; Goldblith and Proctor, 1956).

The off-flavors of irradiated milk arise from skim milk as well as fat portions (Wertheim et al., 1957). Off-flavor components originating from skim milk include acetaldehyde and some sulfur compounds (Day, Forss and Patton, 1957, 1957a). However, the irradiation-induced off-flavor compounds of milk fat are more significant (Hoff, Wertheim and Proctor, 1959) and include many volatile and non-volatile carbonyls and other compounds which have not yet been characterized. The irradiated-odor of milk fat has been described as tallowy, chalky, metallic, and candle-like by various investigators (Wertheim *et al.*, 1957; Hoff, Wertheim and Proctor, 1959; Day and Papaioannou, 1963). The formation of lipoidal off-flavors occurs independently of any interaction with the aqueous phase of the milk because they are obtained in equal strength and character when anhydrous butter fat is irradiated (Wertheim *et al.*, 1957).

Day and Papaioannou (1963) analyzed the monocarbonyl fractions of irradiated milk fat and also observed that the candle-like component in the irradiation-induced off-flavor was predominant. They suggested that apparently this flavor is comparable to the chalky fraction of Wertheim *et al.* (1957), who attributed it to hydroperoxides. Day and Papaioannou (1963) presented evidence suggesting that the long-chain saturated aldehydes are responsible for this defect. However, they did not try to identify these components or other irradiation-induced off-flavor components of milk fat.

The purpose of this investigation was to study various changes in milk fat brought about by high doses of gamma radiation and to attempt to characterize the odor components formed in the milk fat as a result of gamma irradiation.

REVIEW OF LITERATURE

Irradiation Induced Changes in Lipids

In general, the term irradiation applies to the process of irradiating with ionizing radiations. For the purpose of food sterilization, however, only gamma rays (X-rays) and beta rays (cathode rays or high-energy electrons) have been found to be advantageous due to their high energy and penetration power (Proctor and Goldblith, 1951; Danald, 1957). Irradiation is known to effect a number of organoleptic, color and chemical changes in lipids.

Organoleptic Changes

The irradiation-induced off-flavors in lipids are distinct and differ from other off-flavors (Merritt and Angelini, 1965). However, it is also generally accepted that the irradiation-induced off-flavors of fats are similar, but not identical, to the reversion and rancid organoleptic changes associated with autoxidation (Chipault, 1962; Coleby, 1959).

Astrack *et al.* (1952) reported formation of fishy odor in linseed oil and slightly sweetish odor in cottonseed and castor oil as a result of irradiation with high intensity electron bursts. They also suggested that oxygen as well as air must play a role in the formation of irradiation-induced off-flavors since treatment in vacuum or under inert gases successfully inhibits the occurrence of organoleptic changes. This was also supported by Huber, Brasch and Waly (1953) while studying the effect of high intensity electrons on organoleptic changes of various food-stuffs. However, irradiation-induced organoleptic changes also develop during irradiation under vacuum (Chipault et al., 1957; Coleby, 1959).

The specific compounds responsible for the irradiation-flavors of fats have not been identified, but according to many investigators, carbonyl compounds produced during and after irradiation may be responsible for the irradiation-flavors (Chipault et al., 1957; Chipault, 1962; Day and Papaioannou, 1963). Other compounds, however, may not be excluded, since Chipault (1962) isolated a fraction from methyl oleate which consisted mostly of the methyl ester of an epoxy acid and had a strong irradiation odor. Chipault (1957) also suggested that other conceivable flavor and odor components in irradiated fats are low molecular weight hydrocarbons and aliphatic acids. Recently, Merritt et al. (1965) and Merritt and Angelini (1965) reported saturated and unsaturated hydrocarbons in irradiated methyl oleate and fats and stressed the contribution of short chain hydrocarbons to irradiation-induced off-flavors.

Wertheim et al. (1957) fractionated anhydrous milk fat on the basis of solubility of various fractions in acetone at different temperatures and upon irradiation of these fractions found that the fraction responsible for the chalky off-flavor in irradiated milk fat contained saturated fatty acids. They could not find the chemical nature of the chalky off-flavors, but they suggested that peroxides or hydroperoxides of the saturated fatty acids of 16 carbon chain or longer may be responsible, since no evidence of fatty acid chain breakage during their formation was obtained.

In order to study further the precursors of irradiation-induced oxidation flavor of milk fat which seemed to be arising from unsaturated fatty acids (Wertheim _et_ _al_., 1957), Hoff, Wertheim and Proctor (1959) fractionated the fatty acids of milk fat into saturated and unsaturated fractions. The unsaturated fatty acids were further fractionated by urea adduct formation and finally the fraction containing the greatest amount of diene and triene unsaturation was further separated into six fractions by countercurrent distribution. These fractions were then irradiated. Fraction VI which was identified to be linoleic acid gave fishy flavor and fraction II, which could not be identified except that it contained the vinyl group and chain-branching, gave a candle-like odor. These two fractions when combined gave typical irradiation induced oxidation flavor.

Recently, Day and Papaioannou (1963) observed that when milk fat was irradiated under reduced pressure it gave rise to a flavor typical of that encountered through autoxidation and that the candle-like flavor component was predominant in it. They observed that irradiation of milk fat resulted in three types of flavor fractions: hydrolytic rancidity, oxidative rancidity, and candle-like flavor (similar to C_{12}-C_{14} n-alkanals). Rancid-like odor of irradiated fat has also been described by Chipault and coworkers (1957, 1962); whereas, Wertheim et al. (1957), Hannan and Shepherd (1954), Coleby (1959), Lea (1959), and Cook (1963) have reported the irradiation-induced oxidative rancidity in lipids.

Changes in Appearance

Many investigators have observed the changes in color and appearance of fats and oils as a result of irradiation. Long and Moore (1927) were probably first to study the effect of ionizing radiation (cathode rays) on linseed and other drying oils. They reported the bleaching of the color of oil after a very short exposure. Changes in physical and chemical properties of irradiated olive oil were also reported by Kovalev (1931). Dunn et al. (1948) observed that the orange yellow color of butter was progessively destroyed with increased radiation. Bleaching of color and destruction of pigments of milk fat and other oils have been described by many investigators (Hannan and coworkers, 1952, 1954, 1956; Huber, Brasch and Waly, 1953; Lea, 1959; Zhuravlev, 1961; Chipault, 1962; Day and Papaioannou, 1963). Bleaching of butter fat appears to be directly related both to the dose (Hannan and Boag, 1952; Huber, Brasch and Waly, 1953) and to the water content of the system (Hannan and Boag, 1952).

Astrack et al. (1952) and Lück and Kohn (1961) obtained an increase in the viscosity of irradiated fat at sterilizing doses. Long and Moore (1927) noted the increase in molecular weight and thickening of the irradiated oils. Lück and Kohn (1961) also found that at doses greater than 10^7 rads a rise in density, depression in melting point and increase in refractive index of the fat occurred.

Chemical Changes

Various reviews and books have been published in this field which describe the effects of ionizing radiations on biological and organic systems (Hannan, 1956; Coleby, 1959; Desrosier and Rosenstock, 1960; Chipault, 1962).

Due to its complex nature, the chemical investigation of fats and lipids has been notoriously difficult. The study of the effects of irradiation on fats is still more complicated because of the complexity of the radiochemical reactions, coupled with the very small concentrations of products formed (Hannan, 1956; Coleby, 1959).

The chemical effects of irradiation in organic and biological systems are described as direct and indirect. The indirect effect takes place, generally, in aqueous systems where most of the energy is absorbed by the water present. This results in $H^{\cdot}$ and $OH^{\cdot}$

radicals which react with solute molecules and give rise to organic radicals (Hannan, 1956; Weiss, 1959; Coleby, 1959). The direct effect of ionizing radiation is the homolytic type of bond rupture giving rise to two groups, at least one of these being a free radical and this can take part in a complex series of secondary reactions (Hannan, 1956). Chipault (1962) estimated that the products resulting from the direct effect of radiation on fat should not amount to more than 0. 1 percent. The secondary reactions, through chain mechanism, are often responsible for the greater changes in fats. Table 1 (Chipault, 1962) gives some of the possible reactions and products which may be expected as a result of irradiation on fats.

Table 1. Products expected from irradiation of fats.[a]

Reaction	Products Formed
Oxidation	Carbonyls, hydroxy compounds, peroxides, acids, oxygen-linked polymers, odors and flavors.
Polymerization	Dimers, higher polymers.
Chain scission	Short chain hydrocarbons, short chain acids and longer chain compounds through recombination, odors and flavors.
Decarboxylation	Carbon dioxide, long chain hydrocarbons, long chain carbonyls.
Dehydrogenation	Compounds with new double bonds.
Isomerization	Conjugation and cis-trans isomers.
Hydrogenation	More highly saturated compounds.

[a] Chipault (1962).

Free Radicals. The primary effect of irradiation on fats is free radical formation (Hannan, 1956; Desrosier and Rosenstock, 1960; Chipault and Mizuno, 1964). If oxygen is present during irradiation or if the fat is exposed to oxygen immediately after irradiation, hydroperoxides or peroxide free radicals are formed by the reaction of oxygen with free radicals (Chipault, 1962).

Work of Hannan and Shepherd (1954) suggested that the free radicals formed on irradiation of fats are capable of existence at low temperatures in the solid state. At higher temperatures, however, the system acquires mobility and many reactions could become possible. Truby et al. (Chipault, 1962) studied the effect of temperature before and during irradiation on the type and decay rates of the free radicals produced in tristearin. They observed that the types of free radicals produced and their decay rates were influenced by the temperature. They also found that these free radicals and others resulting from the irradiation of tristearin, stearic acid, or linoleic acid were stable at low temperature under vacuum and reacted with oxygen when exposed to air.

Revina, Aripdzhanov and Bakh (1962) irradiated palmitic acid, tripalmitin and potassium palmitate under vacuum at -196° C and studied the free radicals formed using the electron spin resonance method. They found that palmitic acid gave the highest radical concentration and potassium palmitate, the lowest. Also the radical population declined with the admission of air into the system.

Lück, Deffner and Kohn (1963, 1964) used electron spin resonance spectra to identify the free radicals formed upon irradiation of different fats and related material under varying conditions of temperature, dose and storage in the presence and absence of air. They could identify only two free radicals. The life span of these radicals varied greatly in different fats and could be extended up to weeks at room temperature. But, as soon as the fat melted, no more radicals could be detected. The presence of the peroxide radical could be detected in all samples which were irradiated at -196° C and stored in the presence of air at -80° C. The other was an allyl radical which could be detected in lard and elaidic acid methyl ester when irradiated at -80° C and stored at -80° C for seven or eight days.

The free radicals produced by the direct or indirect effect of irradiation have been found to accelerate the process of autoxidation which is propagated by free radicals. However, since the concentration of free radicals produced by irradiation is high, the number of chains producing free radicals started will be larger than in ordinary autoxidation, and their average length is likely to be shorter (Chipault, 1957; Lea, 1959).

Irradiation-Oxidation. When the fat is irradiated in the presence of air or is exposed to air shortly after irradiation, the changes taking place are very similar to those brought about by autoxidation (Mead, 1952; 1955; Hannan and Shepherd, 1954; Dugan and Landis, 1956; Chipault *et al.*, 1957; Mitchell, 1957; Uri, 1959; Coleby, 1959; Lea, 1959; Chipault, 1962; Chipault and Mizuno, 1964). This has been proven quite satisfactorily by Slover and Dugan (1957). They isolated 8-, 9-, 10-, and 11-hydroperoxidooleates from gamma irradiated methyl oleate at different temperatures. Autoxidation of methyl oleate also gives the same hydroperoxides and roughly in equal amounts (Frankel, 1962) as obtained by Slover and Dugan (1957).

Hydroperoxides. Mead (1952) and Polister and Mead (1954) irradiated linoleic acid solutions and methyl linoleate emulsions with X-rays and observed that chain reactions similar to autoxidation resulting in hydroperoxides formation occurred. Other fatty acids with reactive methylene groups were also found to react in the same manner. Dugan and Landis (1956) observed that oxidation of methyl oleate and oleic acid is induced even at low temperatures by cobalt-60 gamma rays. The peroxide values increased rapidly during irradiation. The formation of peroxides in methyl oleate continued to markedly higher levels than were found in oleic acid before the rate was significantly decreased. The formation of peroxides and hydroperoxides by irradiation-oxidation has also been studied by other workers (Chipault et al., 1957, 1964; Burlakova et al., 1960, 1961). All these workers have found increases in hydroperoxide formation upon irradiation.

Chipault and Mizuno (1964) reported that during irradiation of methyl linoleate in oxygen, approximately one-eighth of the peroxides formed resulted from the direct reaction of irradiation-induced free radicals with oxygen, while the rest were formed through a chain mechanism with an average chain length of seven. Also, peroxides continued to accumulate in irradiated methyl linoleate stored under oxygen at a rate that increased with initial irradiation dose.

Essentially no peroxides were formed (Chipault, et al., 1957) and pre-formed hydroperoxides were destroyed when methyl linoleate was irradiated under vacuum (Chipault and Mizuno, 1964). These results were also confirmed by Milch and Klassen (1965) who could not detect any peroxides using infra-red spectra of linoleic and linolenic acids, irradiated under vacuum at 30° C to a dose of five Mrads.

The saturated fatty acids which normally are resistant to autoxidation also undergo peroxidation upon irradiation (Mead, 1955; Lea, 1959; Täufel and Zimmermann, 1962). Chipault and coworkers (1959, 1962, 1964) reported the peroxide formation in methyl palmitate and methyl stearate irradiated in the presence of oxygen.

In the case of methyl myristate, peroxides were formed in direct proportion to the dose with a G value approaching 3. 3, indicating the formation of one peroxide molecule for each ionization. It appears that the saturated molecule has no site with a bond energy low enough to be activated by a secondary energy transfer. The peroxides found in irradiated methyl myristate apparently represent the primary reaction products of oxygen with the free radicals initially formed as a result of irradiation (Chipault, 1962).

Irradiation of milk fat has been given considerable attention during the past 20 years. Dunn _et al_. (1948) irradiated milk fat and olive oil with roentgen rays and bursts of cathode rays and obtained an increase in peroxide values of the fats. However, the rate of peroxides increase did not appear to be linear with the dose. Various other workers, since then, have also observed the peroxide formation in milk fat upon irradiation (Mukherjee, 1950; Hannan and Boag, 1952; Hannan and Shepherd, 1952; 1954; Becker *et al.*, 1956; Hannan, 1956; Wertheim *et al.*, 1957; Cook, 1963; Day and Papaioannou, 1963).

Mukherjee (1950) demonstrated that peroxides may form during as well as after the irradiation of milk fat. He also observed that milk fat became more susceptible to oxidation upon irradiation. The latter observation was also confirmed by other workers by using different fats and fatty materials (Astrack *et al.*, 1952; Hannan and Shepherd, 1954; Sribney, Lewis and Schweigert, 1955; Hannan, 1956; Burlakova *et al.*, 1960, 1961).

Considerable work has been done on irradiation of other food fats. The effect of irradiation on individual fats and oils is a reflection of their fatty acid makeup and natural antioxidant content. The unsaturated acids are more easily oxidized than the saturated acids. This influences the chemical changes which take place. The nature of fatty acids also determines the physical state of the fat. This has been shown to play a part in irradiation effects (Astrack *et al.*, 1952; Mitchell, 1957).

Astrack *et al.* (1952) studied the effects of high intensity electron bursts upon various vegetable and fish oils. As stated earlier, the magnitude of radiation effect was found to depend largely on the composition of the individual oil. However, all vegetable oils in general showed increases in peroxide values at doses of 1.5×10^6 rep. Increase in viscosity and decrease in iodine number were observed for castor and linseed oils. The changes in general, however, were minor for the vegetable oil. The effects were more pronounced in fish oils. Accelerated peroxide formation was observed in this case.

Increase in the peroxide value of vegetable oils upon irradiation has also been observed by Lück and Kohn (1959); Zhuravlev, Lomova and Benevolenskii (1961); LeClerc _et al._ (1961); and Fomin (1962). However, the increase in the peroxide value in the case of vegetable oils was much less pronounced as compared to meat fats or fish oils.

Meat fats when irradiated under oxygen, show increased peroxide content even at ice water temperatures (Sribney, Lewis and Schweigert, 1955). Egiazarov (1959) reported an increase in the peroxide value of pork and beef fats at doses of 150,000 r but not at 50,000 r or 100,000 r.

Lück and Kühn (1959) observed that spoilage characters (peroxide value, acid number, and free fatty acids) began to rise in lard at 10^7 rads; but could be detected even at a dose of 10^5 rads, while this dose (10^5 rads) caused no marked changes in olive oil and margarine. Similar results were also obtained by LeClerc _et al._ (1961) and Fomin (1962).

Carbonyl Compounds. The most important secondary products of oxidation are carbonyl compounds formed by the degradation of hydroperoxides. Carbonyl compounds are responsible for off-flavors in many products especially high fat containing products. Hence, considerable attention has been given to the determination and identification of these compounds in fats and fatty foods. Irradiated fats have, of course, been no exception to this.

Dugan and Landis (1956), while studying the influence of irradiation on oxidation of oleic acid and methyl oleate, observed that secondary products were formed in appreciable quantities; and that these products may be α, β-unsaturated ketones formed by degradation of hydroperoxides as a result of irradiation-oxidation.

Chipault *et al.* (1957) irradiated various methyl esters and fats under oxygen or air and obtained appreciable amounts of carbonyl compounds, saturated as well as unsaturated. Carbonyl compounds were also formed when methyl oleate, methyl linoleate, lard or methyl palmitate were irradiated under vacuum. Chipault *et al.* (1957) suggested that the oxygen required for carbonyl compound formation under vacuum, was derived from the ester group of the fat compound.

Appreciable amounts of saturated carbonyl compounds were also obtained when methyl palmitate and methyl myristate were irradiated under vacuum (Chipault *et al.*, 1957, 1962).

Täufel and coworkers (1960, 1962) have studied the formation of carbonyl compounds from saturated fatty acids (3:0, 4:0, 6:0, 8:0, 16:0, and 18:0) by gamma irradiation. They report that under oxygen atmosphere the carbonyl formation greatly increased with the dose. Increase in 2-thiobarbituric acid (TBA) number was also reported. Under a nitrogen atmosphere, however, little or no carbonyls were detected.

Recently, Chipault and Mizuno (1964) found that when methyl myristate is irradiated under vacuum, only about one out of seven ionizations result in the production of a carbonyl group. Irradiation under an atmosphere of oxygen, however, results in formation of a carbonyl group for each ionization. This also is true for peroxide formation and the peroxides are not formed through chain mechanism.

Hannan and Shepherd (1952), while studying the effects of irradiation on butter fat, observed that irradiation damage in the butter fat could be followed by determining the concentration of high molecular weight aldehydes which apparently ran parallel to irradiation damage.

Recently, Day and Papaioannou (1963) analyzed milk fat irradiated under vacuum at different doses for free carbonyls by the method of Keith and Day (1963), and found mainly saturated aldehydes present, although traces of alk-2-enals and alk-2, 4-dienals were also found. However, only saturated aldehydes and methyl ketones were found by analysis of the carbonyls for individual members.

Meat fats have been much more intensely investigated than milk fat, as far as carbonyl production by irradiation is concerned. During the irradiation of meat, the main odor contributing fraction is not the fat but protein (Batzer _et al._, 1957). Carbonyl compounds, however, have been determined and identified in irradiated meat fats. Sribney, Lewis and Schweigert (1955) reported the formation of carbonyls in irradiated meat fats when oxygen was present during irradiation. Batzer _et al._ (1957) found that the carbonyl content of both meat and fat increased with increasing doses of irradiation.

The off-odors produced during irradiation of certain meats at sterilization levels do not arise directly from chemical changes in the meat fats, since meats with high fat content do not develop off-odors as do the leaner meats (Sribney, Lewis and Schweigert, 1955; Batzer _et al._, 1957). On the other hand the carbonyls produced may have a role in decreasing the apparent off-flavors by reacting the compounds that do contribute to the off-flavors (Batzer _et al._, 1957).

Monty, Tappel and Groninger (1961) observed that irradiation of meats at 4.6×10^6 rads produced predominantly aldehydes and ketones of chain-length greater than ten carbon atoms. Chromatographic analysis indicated that this group of compounds was largely of chain-length 14 or greater. Some short-chain aldehydes and ketones were also observed but these became predominant at doses of 9.3×10^6 rads. These short-chain carbonyls apparently were produced by hydroxyl radical oxidation of unsaturated fatty acids, while the long-chain carbonyls probably were formed by irradiation-hydrolysis of plasmalogens in meat lipids. The water soluble short-chain carbonyls of irradiated meat were supposed to be coming from proteins of the meat.

Carbonyl formation in irradiated vegetable oils has been reported by many investigators (Lang and Proctor, 1956; Lück and Kohn, 1961, 1962; LeClerc et al., 1961). Lang and Proctor (1956) reported that the yield of the monocarbonyls produced by the action of high-energy cathode rays upon refined vegetable oils depended upon the concentrations and sensitivities of the percursors as well as their products, since some of the products (carbonyls) themselves were sensitive to irradiation.

A considerable amount of work has been done on the identification of various carbonyl compounds in the irradiated meat fats and some fatty acid esters. Chipault (1962) irradiated methyl oleate at $1x10^7$ rads under oxygen. The volatiles vacuum distilled at 50^o C contained C_2, C_3, C_4, C_7, C_9 saturated monocarbonyls, C_{16} or C_{17} unsaturated aldehyde, some alk-2-enals, and dicarbonyls. The non-volatile portion contained C_2, C_3, C_4, C_9, C_{10}, and C_{16} saturated monocarbonyls, C_{11} and C_{12} unsaturated monocarbonyls and an unknown fraction probably containing some other dicarbonyls. Lück and Kohn (1962) reported finding more than 20 different carbonyl compounds in irradiated fats. They reported that C-O-C ester bonds were split and were accompanied by fatty acid chain fracture resulting in essentially C_6-C_{12} carbonyl compounds (aldehydes mostly up to C_9). Irradiation of saturated substances for example, palmitic acid and tripalmitin resulted in a relatively high concentration of unsaturated C_9-ketones and a small amount of ketones with more than 13 C-atoms. None of these higher compounds could be detected in unsaturated oils (olive oil, soya oil and groudnut oil), which contain more C_{11} and C_{12} ketones. The compounds that have been identified in tripalmitin and groundnut oil irradiated at 100 Mrad with electron beam are: pentanal, pentenal, hexanal, hexenone, heptanal, heptenone, octanal, octanone, nonanal, nonenone, decanone, decenone, undecanal, undecanone, undecenone and dodecenone.

Witting and Schweigert (1958) isolated and characterized vacuum steam distallable volatile carbonyl compounds in lard irradiated at $1x10^7$ rads under oxygen. The compounds identified included: propanal, butanal, pentanal, hexanal, nonanal, decanal, acrolein, crotonal, 2, 4-undecadienal, and three different dicarbonyls.

Many types of short and long chain aldehydes and ketones have been reported in irradiated beef (Wick, 1963; Merritt _et al._, 1965; Monty, Tappel and Groninger, 1961), irradiated pork, and irradiated chicken (Monty, Tappel and Groninger, 1961) which will not be individually described here.

Not until very recently was any report available on the identification of various carbonyl compounds formed in milk fat by irradiation. Day and Papaioannou (1963) were the first to report on the identity of various monocarbonyls in gamma-irradiated milk fat at a dose of 4.5 Mrads under reduced atmospheric pressure. They could identify only saturated monocarbonyls, even though they had obtained some evidence of unsaturated carbonyls being present in irradiated milk fat. The results of their analysis of carbonyls isolated by the three different procedures used by them, are given in Table 2.

Table 2. Composition of the monocarbonyl fraction isolated from 4.5 Mrad fat.[a]

Method of Isolation	N-Alkanals[b]	Methyl Ketones
Alumina reaction column (free carbonyls)[c]	C_{16}, C_{15}, C_{14}, C_{12}, C_{10}, C_6, C_8, C_7, C_4, C_9, C_5, C_2, C_1	
Molecular distillation	C_{10}, C_6, C_8, C_7, C_4, isoC_4, C_2, C_1, C_{12}, C_{14}, C_{16}	C_9, C_{11}, C_{15}
Vacuum steam distillation	C_{10}, C_6, C_8, C_7, C_4, isoC_4, C_9, C_5, C_3, C_2, C_1	C_4, C_5, C_7, C_9, C_{11}

[a] Data of Day and Papaioannou (1963).

[b] The alkanals are listed in the order of decreasing concentration in the mixture.

[c] Method of Keith and Day (1963).

Free Fatty Acids. Short chain fatty acids are known to be the secondary degradation products of lipid oxidation (Lea, 1959; Frankel, 1962). That the free fatty acids are produced as a result of irradiation-oxidation was shown by Slover and Dugan (1957). They identified octanoic, nonanoic, octandioic and nonandioic acids among the products of irradiation-oxidation of methyl oleate. However, fatty acids may also be formed in irradiated fats as a result of chain scission (Lück and Kohn, 1961a; Chipault, 1962), or as a result of triglyceride hydrolysis as suggested recently by some Russian workers (Fomin, 1962; Ibragimov and Mukhamedov, 1962; Ibragimov and Arifzhanov, 1962).

Earlier, Sribney, Lewis and Schweigert (1955) had observed that when meat fats packed in cans were irradiated at levels of $2x10^6$ rep and $4x10^6$ rep, a lower peroxide value and a higher free fatty acid content were obtained. It was suggested that at higher dose levels, some cleavage of fatty acid peroxide occurs with the resultant formation of fatty acids. A consistent increase in fatty acids and a decrease in peroxide values was observed when cans were stored.

Lück and Kühn (1959) reported an increase in the acid number and free fatty acid content of the olive oil and other fats at doses of 10^7 rads. The free fatty acid accumulation in irradiated fats and methyl oleate has also been observed by Burlakova et al. (1959, 1960);

Zhuravlev, Lomova and Benvolenskii (1961); and Lück and Kohn (1961a).

While studying the kinetics of the irradiation-oxidation of fish fat and methyl oleate, Burlakova et al. (1959, 1960) found that acids were formed as decomposition products of peroxides and that the accumulation of acids is analogous to peroxide formation.

Lück and Kohn (1961a) have studied the formation of medium chain fatty acids from tripalmitin, palmitic acid, olive oil, and ground nut oil. Palmitic acid and tripalmitin (irradiated at 100 Mrad) gave 8:1, 7:1 and 6:1 acids among unsaturated fatty acids and 15:0, 11:0, 9:0 and 7:0 as saturated fatty acids, indicating that apart from end CH_3-group splitting, the fatty acid chains were also broken down at the middle C-C bonds. Beef fat behaved similarly.

The fatty acids obtained from the irradiated olive and ground nut oils (100 Mrad) were similar and included 8:0, 8:1, 10:1, 10:2, 11:0 and traces of 7:0 and 9:0. Soya oil behaved in the same manner except that higher concentrations of 11:0 and 11:1 acids were produced. The above results thus suggest that chain scission also takes place as a result of irradiation.

It was observed by Ibragimov and Mukhamedov (1962) that the free fatty content of cotton seed oil increases upon irradiation with gamma rays suggesting hydrolysis of the oil. Ibragimov and Arifzhanov (1962) later separated the fatty acids obtained by paper chromatography. They also suggested that hydrolysis of the oil takes place upon irradiation.

Fomin (1962) while studying the influence of gamma irradiation on sunflower seed oil obtained an increase in peroxide number, acidity and free fatty acid content of the oil. He suggested that hydrolysis was taking place. However, since he also observed increase in peroxides and other oxidative changes, the increase in fatty acid content may be due to the irradiation-oxidation of oil rather than hydrolysis.

Hydrocarbons. When a pure fatty acid is irradiated in the absence of oxygen and water (with alpha particles or deuterons), one of the main products formed is hydrocarbon with one carbon atom less than the parent fatty acid. Smaller amounts of other short chain hydrocarbons are also formed among other products of irradiation (Sheppard and Burton, 1946; Burton, 1949; Whitehead, Goodman and Breger, 1951; Johnsen, 1959; Ausloos and Trumbore, 1959; Hummel, 1960).

Sheppard and Burton (1946) irradiated saturated fatty acids (acetic, caprylic, lauric and palmitic acids) with alpha particles in the absence of oxygen. They observed that the main reactions taking place were decarboxylation and dehydrogenation. The main products obtained were hydrocarbons with one carbon atom less than the fatty acid, carbon dioxide and hydrogen. Traces of other short chain hydrocarbons up to butane and carbon monoxide and water were identified in the gaseous phase. Similar reactions were also observed by Burton (1949). She irradiated oleic acid with deuterons and observed that hydrogenation of oleic acid also took place. In addition, she identified a series of saturated and unsaturated hydrocarbons up to seven carbons in chain length among the products. These hydrocarbons were also identified recently by Merritt et al. (1965) in gamma irradiated methyl oleate.

The products of radiolysis of methyl acetate (liquid or gaseous) include methane and ethane indicating that hydrocarbons may be formed by combination of two alkyl radicals (Ansloos and Trumbore, 1959; Hummel, 1960).

Lück and Kohn (1961a) while studying the formation of medium chain fatty acids in irradiated fats, suggested that hydrocarbons were formed via chain fission along with the medium chain fatty acids.

Hydrocarbons have also been suggested as secondary products of oxidation (Frankel, 1962). It has been shown that hydrocarbons are formed as oxidation products of Safflower and olive oils (Merritt et al., 1965).

Wick (1963) and Merritt et al. (1965) have reported finding various hydrocarbons in irradiated beef. These, however, are believed to have come from the lipid fraction. That the hydrocarbons are produced in irradiated fats has been demonstrated very recently by Merritt and Angelini (1965).

Gaseous Products. Apart from short chain hydrocarbons, the gaseous products of irradiated fatty acids have been found to contain hydrogen, carbon dioxide, carbon monoxide and water. The hydrocarbon part of the fatty acid chain behaves very similarly to long chain hydrocarbons (Desrosier and Rosenstock, 1960). The main reaction of the hydrocarbon chain is dehydrogenation. The hydrogen gas is produced by both a molecular and free radical mechanism. The carboxyl part of the fatty acids behaves like low molecular weight fatty acids. Here the principle effect of radiation is decarboxylation with the formation of carbon dioxide and carbon monoxide (Desrosier and Rosenstock, 1960).

As stated earlier, Burton and her group (Sheppard and Burton, 1946; Breger and Burton, 1946; Burton, 1949; Whitehead, Goodman and Breger, 1951) have done considerable work on the effects of heavy particle irradiation on pure fatty acids in the absence of oxygen and water. The major reactions that are taking place according to these workers are decarboxylation, dehydrogenation, and some hydrogenation.

The formation of hydrogen and carbon dioxide thus can be easily visualized. However, carbon dioxide may also be formed by some mechanism other than decarboxylation (Sheppard and Burton, 1946).

That decarboxylation does take place on irradiation of fatty acids with gamma rays was shown by Ibragimov and Arifzhanov (1962) and Johnsen (1959).

Whitehead, Goodman and Breger (1951) observed that yields of carbon dioxide and carbon monoxide decreased linearly as the size of the fatty acid molecule increased from C_2 to C_{22}. However, the ratio of yields $\frac{\text{Hydrocarbon}}{CO_2 + CO + H_2O}$ remained constant with increasing chain length of the fatty acids indicating that all these substances must arise from decarboxylation. No mechanism was suggested for the production of carbon monoxide and water. Earlier Sheppard and Burton (1946) had suggested that carbon monoxide and water are produced either directly from the fatty acid molecule or in small part from the action of alpha particles on the carbon dioxide and hydrogen already formed.

The dehydrogenation of fatty acids by alpha particles is a complex function of the size of the molecule. However, the yield of hydrogen increased over that of volatile hydrocarbons with the increase in size of the molecule showing that dehydrogenation may occur in the absence of decarboxylation (Whitehead, Goodman and Breger, 1951).

Johnsen (1959) examined the effects of ionizing radiations on several low molecular weight straight and branched chain fatty acids (C_2 to C_6). He found that C-C scission at the carboxyl group was the predominant reaction in acids of moderate chain length. Also branched chain fatty acids, especially isobutyric acid, gave higher yields of carbon dioxide.

Methyl acetate when irradiated in an air free medium in liquid or gaseous state has been found to give all of the gaseous products (carbon dioxide, carbon monoxide, hydrogen and water) and other volatile compounds (short chain hydrocarbons, carbonyls, alcohols and ethers) (Ausloos and Trumbore, 1959; Hummel, 1960). The production of these gaseous products from methyl esters of long chain fatty acids or fats and oils has not been reported by any investigator so far.

Polymerization. Long and Moore (1927) were the first to observe the thickening of oils and an increase in the molecular weight of the oils when irradiated with cathode rays. The effect was increased with time of exposure. Oleic acid when subjected to deuteron bombardment in absence of air was polymerized to the extent of 52. 5 percent (Burton, 1949).

Polymerization generally takes place as a result of cross-linking between two free radicals formed during irradiation. If the molecule contains unsaturation, the point of cross-linking will depend upon the position of C=C in the chain (Charlesby, 1955).

Hannan and Boag (1952) observed that gelation, presumably due to polymerization occurred in methyl linoleate samples irradiated at $2x10^6$ rep even at a solid carbon dioxide temperature. The increase in viscosity of fat or oil has also been reported by Astrack et al. (1952) and Lück and Kohn (1961).

Cis-Trans Isomerization. Not much has been reported on cis-trans isomerization of unsaturated fats and oils by irradiation. Charlesby (1955) noted that olefins, when irradiated, underwent isomerization from cis to trans form and from trans to cis form.

Lück and Kühn (1959) first studied the cis-trans isomerization in fats using infra red spectra. Later Lück and Kohn (1963) investigated this subject in much detail. They irradiated many fats and oils at a dose of 10 Mrad and concluded that the conversion from cis- to trans- or from trans- to cis-configuration by electron beam irradiation depends upon the amount of cis- and trans- fatty acids present in the fat or oil being irradiated. Thus the energetically more stable form is partially converting to the energetically unstable form.

Double Bond Displacement. Conjugated system of double bonds do not occur in appreciable amounts in natural fats and oils. However, as a result of oxidation of unsaturated fatty acids of fats and oils, a shift in double bonds occurs to give a conjugated system. The formation of a conjugated system can be followed by measuring the ultraviolet absorption spectrum of the system.

Irradiation-oxidation has also been found to result in double bond displacement and conjugation. Mead (1952) observed that conjugated 9, 11-octadecadienoic acid was obtained as one of the products upon irradiation of oleic acid. The concentration of the product increased linearly with dosage.

Chipault *et al*. (1957) irradiated methyl linoleate and observed an increase in diene conjugation in the system. The conjugation of the unsaturated linkages also takes place in natural oils when treated with ionizing radiation (Ibragimov and Mukhamedov, 1962; Lück and Kohn, 1963).

Lang and Proctor (1956) studied the changes in conjugated triene systems of vegetable oils upon irradiation. They found that concurrent with the formation of monocarbonyls, conjugated triene systems were attacked and there was an increase in the conjugated diene system. Irradiation under vacuum gave very similar results.

Increases in diene conjugation and decreases in triene conjugation have also been observed by Lück and Kühn (1959) in fats irradiated at 10^7 and 10^8 rads.

Miscellaneous Changes. Apart from double bond displacement and cis-trans isomerization, irradiation of lipids brings about changes in the number of double bonds present in the system.

The changes in unsaturation are followed by changes in Iodine number. Various workers (Long and Moore, 1927; Astrack *et al*.,

1952) have observed that a decrease in Iodine number and hence decrease in degree of unsaturation takes place when fat is irradiated. However, Lück and Kohn (1961) reported that Iodine number was hardly affected by irradiation at 10^7 rads. No explanation could be given for this observation.

One reason for the decrease in Iodine number may be hydrogenation of unsaturated lipids as observed by Burton (1949) and Coleby (1959). However, Albright, Harrison and Sesonske (1958) observed that hydrogenation of cotton seed oil occurred at slower rates of reaction in a gamma radiation field of cobalt-60 than comparable non-irradiated samples. They explained that gamma radiation apparently degraded the triglycerides to form poisons for the nickel catalyst. Free fatty acids and carbonyls were probably the poisons produced.

In addition to all the compounds and changes listed above, small amounts of hydroxy compounds also have been detected in irradiated fats (Chipault, 1962). Chipault (1962) also reported that in earlier work he and his coworkers had identified a fatty acid methyl ester with approximately 8 to 12 carbon atoms containing a terminal epoxy group.

Factors Affecting Irradiation-Induced Changes in Lipids

Types of Ionizing Radiations

Different types of ionization radiations are not expected to produce qualitatively different chemical effects (Mead, 1955). Heavy particle radiations (alpha-particles, deuterons, etc.) are not suitable for foods because of their low penetration power. They have been used, however, by earlier workers to study chemical changes in fatty acids (Sheppard and Burton, 1946; Burton, 1949).

Neutrons, protons and other heavy particles with high energy are also excluded from use in foods since they bring about nuclear transformations and may result in the formation of radioactive atoms (Hannan, 1956).

Hannan and Shepherd (1954) first noticed that X-rays and high energy electrons of cathode rays did not show any difference in bringing about oxidative changes in fats. Similar results regarding off-flavors produced were also observed by LaFuente, Goldblith and Proctor (1959).

The only detailed study utilizing various types of radiations from different sources was carried out by Burlakova et al. (1959). They reported that the use of various kinds of radiations (gamma rays from cobalt-60, gamma and beta rays from Au-198, beta rays from P-32, and X-rays) did not affect the kinetics and the chemical nature of the oxidation of the fat.

Dose Rate

The dose rate and total dose used are important factors which effect the changes occurring in irradiated lipids. Brasch, Huber and Waly (1952) were first to study the effects of radiation dose rates on ion yields in irradiated foods. According to these workers (Huber, Brasch and Waly, 1953) the effect of dose rate is important in retaining good organoleptic and color and texture qualities of food. High dose rates were considered beneficial in avoiding organoleptic changes. At high dose rates a high concentration of free radical exists and non-oxidative chain termination reactions are more prevalent than when free radicals are formed at a lower concentration over a long period of time at a low dose rate (Chipault _et_ _al_., 1957, 1962). This results in lower concentration of irradiation products at higher dose rates and higher concentrations if lower dose rates are used (Mead, 1952). Also, at lower dose rates, for a given total amount of dose, the time of exposure will be longer resulting in more chemical and organoleptic changes (Dugan and Landis, 1956; Chipault _et_ _al_., 1959, 1962).

For the same dose rate, the total dose applied is also important. The extent of chemical and physical changes occurring is greatly increased with increase in total amount of irradiation dose (Gaden, Henley and Collins, 1951; Batzer *et al.*, 1957; Lück and Kühn, 1959; Egiazarov, 1959).

Physical State of Fat

The physical state of fat during irradiation is regarded as one of the important factors governing chemical changes occurring in the fat upon irradiation. This was demonstrated by Hannan and Shepherd (1954). They fractionated milk fat into solid, semi-solid, and liquid fractions by crystallization from acetone at 0° C and 20° C. They found that upon irradiation, the after effect (peroxide value) was greatest in solid fraction and lowest in liquid fraction. The explanation given was that free radicals formed are more stable in solid fat possibly due to decreased mobility, and that these free radicals react readily with oxygen to form peroxides and hence hydroperoxides. As the fat becomes liquid, the system acquires mobility and many other reactions are possible.

That the free radicals are capable of existence at lower temperatures in solid fats has been clearly demonstrated by Lück, Deffner and Kohn (1963, 1964).

Temperature

Apart from the physical state of the fat, which depends highly upon the temperature, the effect of temperature is very significant.

Hannan and Boag (1952) and Hannan and Shepherd (1952, 1954) have studied the effect of temperature during irradiation and subsequent storage in detail. Their work is fully compiled by Hannan (1956). Hence only their important results will be described below.

The development of oxidized tallowy odors and flavors was at least three times greater for irradiation at 20° C than at -70° C or 37° C (molten). The main influence of temperature, in fact, was observed on the peroxides, which showed a surprising reaction rate in the region of -20° C. Fats irradiated at 0° C or -70° C showed a striking increase in peroxide value during storage at -20° C, compared with much smaller increase at 0° C and barely significant change when stored at +20° C or -70° C. No after effect was observed after irradiation at +20° C; while the molten fat at +37° C failed to show even peroxide formation during irradiation (Hannan and Shepherd, 1952, 1954). Similar results were also obtained when methyl stearate, methyl oleate and methyl linoleate were irradiated earlier by Hannan and Boag (1952).

The temperature of fat during and after irradiation also influences the types of free radicals formed and their stability as described earlier in this review.

The decay rate of free radical is also effected by the temperature (Chipault, 1962). At -78° C the decay rate of free radicals and rates of their reaction with oxygen are very slow so that free radicals persist during storage at this temperature, but peroxides do not accumulate. At 0° C both decay rate and peroxidation rate are more rapid, but the rate of decay is much higher than that of peroxide formation, hence the free radicals disappear before peroxides can accumulate. At intermediate temperatures (-25°C) the rate of peroxide formation is relatively more rapid than the rate of decay of the free radicals and therefore, the peroxides accumulate before the free radicals are completely destroyed.

Storage

It is quite evident from the previous section that the temperatures during and after irradiation have a marked influence on the changes during post-irradiation storage.

In general, when fats are stored in the presence of oxygen or air after irradiation, they show an increase in peroxide formation. Storage under vacuum results in none or a very slight increase in peroxide value (Mukherjee, 1950; Sribney, Lewis and Schweigert, 1955). Recently, Chipault and Mizuno (1964) reported that methyl myristate when stored under oxygen at various temperatures after irradiation did not show any change in peroxide value, reducing compounds or carbonyls after 2, 8 or 20 weeks of storage. However, methyl myristate irradiated and stored under vacuum at various temperatures for 2, 8 or 20 weeks, showed formation of small amounts of peroxides after two weeks. The peroxide contents decreased on longer storage and went back to zero at the end of 20 weeks. These results are quite unusual. The authors suggested that these peroxides were possibly formed during peroxide determination by the reaction of highly reactive free radicals, which may be formed during storage, with oxygen.

Oxygen or Air

The types of physical and chemical changes that take place in irradiated lipids are greatly dependent upon whether the lipid was irradiated and stored in the presence of oxygen, inert gas or vacuum. Even small amounts of oxygen or air dissolved in lipids will have a significant effect. According to Hannan (1956), from the practical point of view the flavor production is very sensitive to the presence of small amounts of oxygen.

In general, irradiation in the presence of oxygen or air results in reactions very similar to oxidation, i. e. , hydroperoxide formation, destruction of carotenoids and oxidized flavor production. The removal of oxygen tends to limit the peroxide formation but oxidation occurs rapidly, if the fat is exposed to air again (Mukherjee, 1950; Hannan, 1956).

Oxygen plays a crucial role in the initiation as well as propagation of irradiation-induced chemical changes (Huber, Brasch and

Waly, 1953). Peroxides and carbonyls are formed even in saturated fatty acids upon irradiation in the presence of oxygen (Täufel, Zimmermann and Huber, 1960; Täufel and Zimmermann, 1962).

According to many workers, irradiation under innert atmosphere or under vacuum prevents both the development of hydroperoxides and off-flavors (Astrack _et al._, 1952; Huber, Brasch and Waly, 1953; Sribney, Lewis and Schweigert, 1955). The reactions are undoubtedly complex, since carbonyl formation and even off-flavor production have been reported even in the absence of oxygen (Lang and Proctor, 1956; Monty, Tappel and Groninger, 1961; Day and Papaioannou, 1963; Chipault and Mizuno, 1964). The oxygen needed in the formation of carbonyls upon irradiation in the absence of oxygen may be derived from the ester groups of the triglycerides (Chipault, _et al._, 1957).

The other effect which the presence of air may have on the irradiation induced changes was reported by Chipault _et al._ (1957). Nitrogen and oxygen of air form ozone and nitrogen oxides upon irradiation. These compounds may react with fat and give rise to flavor and odor compounds. Chipault _et al._ (1957) also observed that direct ozonization of fatty acid esters produced remarkably similar flavors to the unozonized samples irradiated under oxygen. Also the flavor of directly ozonized samples was more close to the irradiated samples, than to the autoxidized samples.

Presence of Water

In aqueous systems, most of the energy from irradiation is absorbed by water with the formation of free radicals. Subsequent changes in a solute can often be explained in terms of the reactions of hydrogen atoms, hydroxyl radicals, hydrogen and hydrogen peroxides which are formed by radiolysis of water (Weiss, 1944, 1959; Proctor et al., 1952).

In lipids, where the amount of water, if present, is very small, the effect may not be very pronounced, but certainly some of the reactions occurring in aqueous systems can be expected. These hydroxyl radicals, hydrogen atoms and hydroperoxides produced from irradiation of water molecules may react with lipid molecules resulting in increased hydroperoxide formation (Nickerson, Proctor and Goldblith, 1953; Proctor et al., 1952).

Hannan and Boag (1952) reported that bleaching of milk fat during irradiation appeared to be directly related to the water content of the system. Moisture content also had a direct effect on the threshold and production of off-flavors (Bierman, Proctor and Goldblith, 1956).

Täufel, Zimmermann and Huber (1960) and Täufel and Zimmermann (1962) reported that the higher the concentration of water present in saturated fatty acids irradiated with gamma rays, the higher were the amounts of hydroperoxides and carbonyls formed.

Wertheim et al. (1957), on the other hand, have reported that the lipoidal off-flavors were produced in milk without any interaction with the water phase and that the off-flavors produced in irradiated anhydrous fat were equal in strength and character to those produced in fat irradiated in the presence of water.

Antioxidants

Antioxidants are added to foods to retard the oxidation of lipids by reacting with free radicals formed and thus preventing their propagation. Some antioxidants occur naturally in minor quantities in fats. These natural antioxidants are responsible for the initial resistance of fats and oils to antioxidation. When the natural antioxidants are destroyed through irradiation, it results in accelerated peroxide formation in fats and oils (Astrack et al., 1952; and Hannan and Shepherd, 1954; and Hannan, 1956). However, it has been observed by several investigators that added antioxidants prevent the irradiation induced changes in fats (Astrack et al., 1952; Hannan and Boag, 1952; Hannan and Shepherd, 1952; Polister and Mead, 1954; Groninger, Tappel and Knapp, 1956). It was also observed by Lang and Proctor (1956) that addition of antioxidants to vegetable oils did not decrease the yield of monocarbonyl compounds and diene conjugation, even though, they were partially effective in reducing the irradiation attack on the unsaturated centers. Fomin (1962) found that antioxidants did not influence the amount of peroxides formed but did decrease their accumulation afterwards.

Some workers have reported that irradiation destroys the antioxidants and decreases their effectiveness, especially of tocopherols (Hannan and Shepherd, 1952; Kung, Gaden and King, 1953; Becker *et al.*, 1956; Chipault *et al.*, 1957). Chipault (1962) reported that irradiation under vacuum caused a partial destruction of antioxidants in methyl myristate which was proportional to the dose. The antioxidants had different sensitivities to irradiation under vacuum, the most easily destroyed being alpha-tocopherol and the most resistant, butylated hydroxyanisole (BHA).

In the presence of oxygen, antioxidants were more easily destroyed and none of the antioxidants had any appreciable effect in preventing or decreasing the formation of peroxides during irradiation of methyl myristate (Chipault, 1962; Chipault and Mizuno, 1964).

It was further observed by Chipault (1962) and Chipault and Mizuno (1964) that antioxidants had some effect in retarding the formation of peroxides during irradiation of methyl linoleate and during post-irradiation storage, but the effect was small compared to their antioxygenic activity toward simple autoxidation. The effect varied with the nature of the antioxidant and with irradiation dose. Propyl gallate was found to be much less effective than BHA. Also the best antioxidant mixture was found to be a combination of BHA and EDTA (Ethylenediaminetetraacetic acid).

Bradshaw and Truby (as quoted by Chipault, 1962) found that alpha-tocopherol used at a concentration of one percent: (1) accelerated the decay rate of irradiation-induced free radicals in milk fat or tristearin, (2) had practically no effect on irradiated stearic acid, and (3) stabilized the free radicals of linoleic acid. These results indicate that alpha-tocopherol acts by some mechanism not associated with direct combination with radiation-induced free radicals. Based on different studies, similar conclusions were also arrived at by Hannan and Shepherd (1954).

Ascorbic acid has no effect on the free radicals formed in anhydrous fats but in aqueous-fat systems, it stabilizes the free radicals formed (Chipault, 1962).

Mechanisms Involved in Irradiation-Induced Reactions

The first effects of ionizing radiations are the production of ions and excited molecules. In air, the production of one ion pair requires 35 ev. Hence, 1 Mev electron (or other type of radiation) can produce from 30, 000 to 40, 000 ionizations. It also produces from 45, 000 to 80, 000 excitations of atoms or molecules (Lück, 1961). As a result of X-ray or gamma ray irradiation, the absorbed electromagnetic energy is largely converted into energy of internally generated high energy electrons. Hence, most of the biological and chemical changes produced by the electromagnetic radiations are basically similar to those produced by cathode or beta rays (Hannan, 1956).

The molecules which have been ionized and activated by the radiations have considerable chemical reactivity. However, the emphasis is generally placed on the reactivity of ionized molecules because, it is difficult to determine the number of excitations and to distinguish between their effects and those of the ions. In spite of the high energies of the ionizing radiations, the total amount of chemical change produced in foods is relatively small (Lück, 1961; Hannan, 1956). The possible reactions which may occur as a result of ionizing radiations are as follows (Lück, 1961):

I. Formation of Ions.
  (1) Ionization $A \rightsquigarrow A^{+} + e$
  (2) Ion dissociation $A^{+} \longrightarrow U^{+} + V + \text{------}$
  (3) Ion-molecule reaction $A^{+} + BC \longrightarrow (ABC^{+}) \rightarrow AB^{+} + C$
  (4) Capture of an electron $AX + e \longrightarrow A^{\cdot} + X^{-}$

II. Formation of Activated Molecules.
  (5) Neutralization of ion $A^{+} + e \longrightarrow A^{*}$
  (6) Transfer of activation energy $A^{*} + B \longrightarrow A + B^{*}$ + kinetic energy

III. Formation of Radicals.
  (7) Dissociation of an activated molecule $AB^{*} \longrightarrow A^{\cdot} + B^{\cdot}$
  (8) Dissociative capture of an electron $AB^{+} + e \longrightarrow A^{\cdot} + B^{\cdot}$
  (9) Radical combination $A^{\cdot} + B^{\cdot} \longrightarrow AB$
  (10) Radical-radical reaction $AB^{\cdot} + CD^{\cdot} \longrightarrow AC + BD$
  (11) Radical-molecule reaction $AB^{\cdot} + CD \longrightarrow ABC + D^{\cdot}$

Reactions of type (2), (3), and (4) are of secondary importance in foods. Reactions (1), (5) or (6) do not bring about any basic changes in the chemical properties of the substances. However, formation of radicals (equations 7 through 11) is responsible for most of the changes observed in irradiated foods.

The effects of ionization by radiations, in general, are distinguished as direct effects and indirect effects. The direct effects of ionization depend upon the nature of the molecule ionized. The molecule may be stabilized by resonance or bond breakage may occur. However, in most cases, one of the products of bond cleavage is a free radical which takes part in secondary reactions (Hannan, 1956).

Primary reaction.

$$AX^{+} \longrightarrow A^{\cdot} + X^{+}$$

Secondary reactions.

(1) $A^{\cdot} + BH \longrightarrow AH + B^{\cdot}$ (forms new free radical)
(2) $A^{\cdot} + C^{\cdot} \longrightarrow AC$ (free radical combination)
(3) $A^{\cdot} + A \longrightarrow AA^{\cdot} \xrightarrow{A} AAA^{\cdot}$ --- (polymerization)
(4) $A^{\cdot} + O_2 \longrightarrow AOO^{\cdot} \xrightarrow{H\cdot donor} AOOH$ (peroxide formation)

Direct effects are the main cause of irradiation damage in lipids and dried foods.

Indirect effects are important in aqueous systems, where free organic radicals can be formed indirectly through the reaction of water dissociation products. When water is present in the system, much of the energy is absorbed by the water, forming free radicals, hydrogen atoms, hydrogen, hydroxyl radicals, and hydroperoxides as products of dissociation of water. Weiss (1944, 1959) and Proctor et al. (1952) studied the mechanisms of radiolysis of water and aqueous solutions. They reported various reactions that take place. In the absence of oxygen, the reactions are:

$$
\begin{array}{lcl}
H_2O & \longrightarrow & H^{\cdot} + OH^{\cdot} \\
H_2O^{*} & \longrightarrow & H^{\cdot} + OH^{\cdot} \\
H_2O & \longrightarrow & H_2O^{+} + e \\
H_2O + e & \longrightarrow & H_2O^{-} \\
H_2O^{+} & \longrightarrow & H^{+} + OH^{\cdot} \\
H_2O^{-} & \longrightarrow & H + OH^{-} \\
2H_2O^{+} & \longrightarrow & H_2O_2 + 2H^{+} \\
2H_2O^{-} & \longrightarrow & H_2 + 2OH^{-} \\
2H_2O^{*} & \longrightarrow & H_2 + H_2O_2 \\
2OH^{\cdot} & \longrightarrow & H_2O + O^{\cdot} \\
2O^{\cdot} & \longrightarrow & O_2
\end{array}
$$

In presence of oxygen, the additional reactions are:

$$
\begin{array}{lcl}
H^{\cdot} + O_2 & \longrightarrow & HO_2^{\cdot} \\
O_2 + H_2O^{-} & \longrightarrow & H_2O + O_2^{-} \\
2HO_2^{\cdot} & \longrightarrow & H_2O_2 + O_2 \\
H_2O_2 + H^{\cdot} & \longrightarrow & H_2O + OH^{\cdot} \\
H_2O_2 + OH^{\cdot} & \longrightarrow & HO_2^{\cdot} + H_2O \\
H_2O_2 + O_2^{-} & \longrightarrow & O_2 + OH^{\cdot} + OH^{-}
\end{array}
$$

These free radicals react with solute molecules to give solute free radicals.

$$RH + {}^{\cdot}OH \longrightarrow H_2O + R^{\cdot}$$

The solute free radicals can further react as already shown in secondary reactions of direct effects. Thus, there is no fundamental difference between direct and indirect effects of ionization.

As stated earlier, the total effect is not only a direct effect, but due to the plasticity of lipid systems, indirect effects are important too.

Many types of reactions are expected to take place in irradiated fats, as shown in Table 1. Some of the mechanisms studied and suggested for these reactions will be considered here.

Oxidation

It has already been pointed out in the earlier part of this review that the irradiation-oxidation process is very similar to the autoxidation process. The hydroperoxides formed in gamma irradiated methyl oleate are the same and in almost the ratio predicted by autoxidation (Slover and Dugan, 1947).

The mechanisms of autoxidation have been reviewed in detail in "Symposium on foods: Lipids and Their Autoxidation" (Schultz, Day and Sinnhuber, 1962) and in "Autoxidation and Antioxidants" (Lundberg, 1961). The mechanism of free radical formation, hydroperoxide formation and hydroperoxide deomposition in irradiated fats are similar to autoxidation (Coleby, 1959). These mechanisms will not be discussed here as they are discussed in the books referred to above.

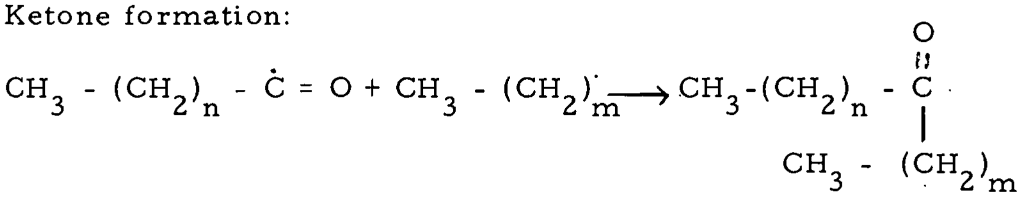
It has been observed, however, that carbonyls are formed when fats are irradiated under vacuum (Lang and Proctor, 1956; Day and Papaioannou, 1963). Various saturated and unsaturated aldehydes and ketones were reported formed in irradiated fats and oils by Lück and Kohn (1962) and Kohn (1964). They suggested the following mechanism for carbonyl formation:

Aldehyde formation:

$$CH_3 - (CH_2)_n - COOR \longrightarrow CH_3 - (CH_2)_n - \dot{C} = O + \dot{O}R$$

$$CH_3 - (CH_2)_n - \dot{C} = O + H \longrightarrow CH_3 - (CH_2)_n - CHO$$

Ketone formation:



$$CH_3 - (CH_2)_n - \dot{C} = O + CH_3 - (CH_2)_m^{\cdot} \longrightarrow CH_3\text{-}(CH_2)_n - \overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle CH_3 - (CH_2)_m}{|}}{C}}$$

The shorter chain aldehydes and ketones may be formed by splitting the fatty acid chain during irradiation, which will also give rise to hydrocarbon free radicals needed for ketone formation. Day and Papaioannou (1963) have also suggested similar mechanism for long chain aldehyde formation in irradiated milk fat.

Chain Scission

One of the sources of free radical formation in irradiated lipids, is chain scission. This may occur in two ways:

(1) Degradation of ion formed (Hannan, 1956).

$$RH \rightsquigarrow RH^{+} \longrightarrow R_1^{\cdot} + R_2H^{+}$$

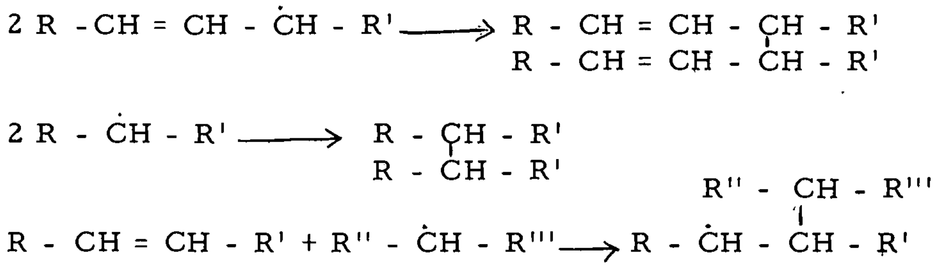
(2) Simple chain scission (Lück, Deffner and Kohn, 1964).

$$\begin{array}{l} R-CH_2-CH_2-CH_2-CH_2-R' \longrightarrow R-CH_2-CH_2^{\cdot} + R'-CH_2-CH_2^{\cdot} \\ R-CH_2-CH_2-CH_3 \longrightarrow CH_3^{\cdot} + R-CH_2-CH_2^{\cdot} \end{array}$$

Polymerization

Polymerization or increase in viscosity of irradiated fats and oils has been observed by many investigators (Long and Moore, 1927; Burton, 1949; Lück and Kohn, 1961; Kohn, Leute and Lück, 1964; Lück, Kohn and Bach, 1964). Charlesby (1955, 1959) has studied the polymer formation in organic systems. He suggests that when olefins and acetylenes are irradiated, they result in cross-linking. When larger molecules are irradiated, chain scission may occur first and then recombination of free radical fragments may occur to form either straight chain, branched chain or interconnected chain polymers.

Lück, Kohn and Bach (1964) have suggested the following mechanism for polymer formation or cross-linking of carbon chains:



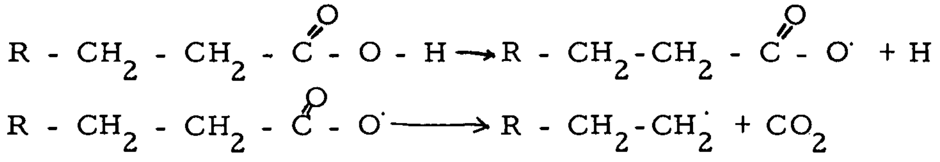
$$2\,R-CH=CH-\dot{C}H-R' \longrightarrow \begin{array}{l} R-CH=CH-\underset{|}{C}H-R' \\ R-CH=CH-CH-R' \end{array}$$

$$2\,R-\dot{C}H-R' \longrightarrow \begin{array}{l} R-\underset{|}{C}H-R' \\ R-CH-R' \end{array}$$

$$R-CH=CH-R' + R''-\dot{C}H-R''' \longrightarrow R-\dot{C}H-\overset{R''-\underset{|}{C}H-R'''}{CH}-R'$$

Decarboxylation

When fatty acids are irradiated, they undergo decarboxylation, polymerization and dehydrogenation (Sheppard and Burton, 1946; Breger and Burton, 1946: Burton, 1949). The main products formed are a hydrocarbon with one carbon atom less in chain than the parent fatty acid, carbon monoxide, carbon dioxide, hydrogen, water and short chain hydrocarbons. The main reaction appears to be decarboxylation.

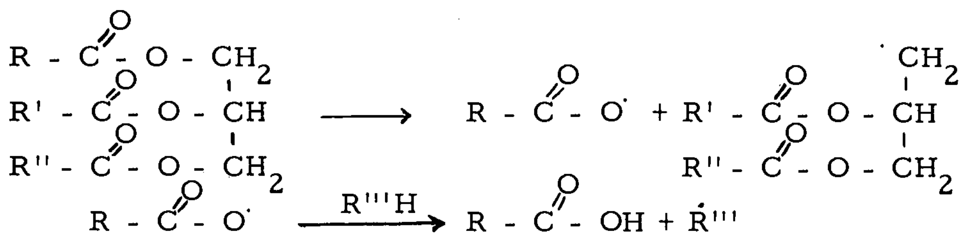


$$R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O-H \rightarrow R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O^{\cdot} + H$$

$$R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O^{\cdot} \longrightarrow R-CH_2-CH_2^{\cdot} + CO_2$$

Hydrolysis

As a result of hydrolysis of lipids, free fatty acids are formed. A reaction given by Lück, Deffner and Kohn (1964) may suggest possible hydrolysis mechanism.



Lück and Kohn (1961) and Kohn (1964) have also suggested mechanism for the formation of medium chain (C_6 to C_{11}) saturated and unsaturated fatty acids in fats.

$$CH_3\text{-}(CH_2)_6\text{-}CH_2\overset{\downarrow}{-}CH_2\text{-}(CH_2)_6\text{-}COOR \begin{array}{l} \xrightarrow{-H} CH_2{=}CH\text{-}(CH_2)_5\text{-}COOR \\ \xrightarrow{+H} CH_3\text{-}(CH_2)_6\text{-}COOR \end{array}$$

In saturated triglycerides, the chain scission is mainly observed at mid point of the fatty acid chain. However, it could also take place at any other place along the chain since other fatty acids were also observed.

In unsaturated fatty esters or triglycerides, the breaking of C-C single bond adjacent to C-C double bond is favored (Lück and Kohn, 1961; Kohn, 1964).

$$CH_3\text{-}(CH_2)_6\text{-}CH_2\ CH{=}CH\overset{\downarrow}{-}CH_2\text{-}CH_2\text{-}(CH_2)_5\text{-}COOR \xrightarrow{\pm H}$$

$$CH_3\text{-}(CH_2)_6\text{-}COOR + CH_2{=}CH\text{-}(CH_2)_5\text{-}COOR + \text{Hydrocarbons}$$

$$CH_3\text{-}(CH_2)_6\text{-}CH_2\overset{\downarrow}{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}(CH_2)_5\text{-}COOR \xrightarrow{\pm H}$$

$$CH_2{=}\ CH\text{-}CH_2\text{-}(CH_2)_6\text{-}COOR + CH_2{=}CH\text{-}CH{=}CH\text{-}(CH_2)_5\text{-}COOR\ +$$

$$\text{Hydrocarbons}$$

Dehydrogenation

Desrosier and Rosenstock (1960) have stated that the hydrocarbon part of a fatty acid chain behaves like long chain hydrocarbons which produce hydrogen and short chain hydrocarbons upon irradiation. The main reaction is dehydrogenation. Dehydrogenation or hydrogen abstraction is a primary process of free radical formation (Hannan, 1956).

$$XH \rightsquigarrow X^{\cdot} + H$$

and $$H + XH \longrightarrow X^{\cdot} + H_2$$ (Manion and Burton, 1952)

Lück, Kohn and Bach (1964) have suggested another mechanism in lipids.

$$R\text{-}CH_2\text{-}CH_2\text{-}R'+H \longrightarrow R\text{-}\dot{C}H\text{-}CH_2\text{-}R'+H_2$$

Toma and Hamill (1964) studied the mechanism of hydrogen formation in gamma irradiated hydrocarbon. They found that in cyclohexane hydrogen may be formed as below:

$$\begin{aligned} &\text{Cyclo - } C_6H_{12}^{+} + e \longrightarrow (\text{Cyclo-}C_6H_{10}^{*} + 2H) \\ &(\text{Cyclo - } C_6H_{10}^{*} + 2H) \longrightarrow \text{Cyclo-}C_6H_{10} + H_2 \\ &\qquad\qquad\qquad\qquad \longrightarrow C_6H_{10} + 2H \end{aligned}$$

Hydrogenation

Hydrogenation also is a free radical process. The hydrogen atoms produced during irradiation could combine at a double bond (Manion and Burton, 1952; Lück, Kohn and Back, 1964).

$$H^{\cdot} + R - CH = CH - R' \longrightarrow R - \dot{C}H - CH_2 - R'$$

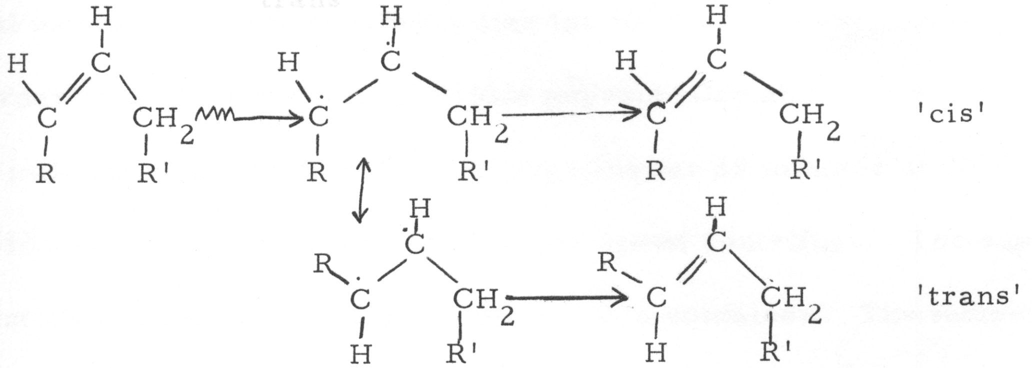
and

$$R - \dot{C}H - CH_2 - R' + H^{\cdot} \longrightarrow R - CH_2 - CH_2 - R'$$

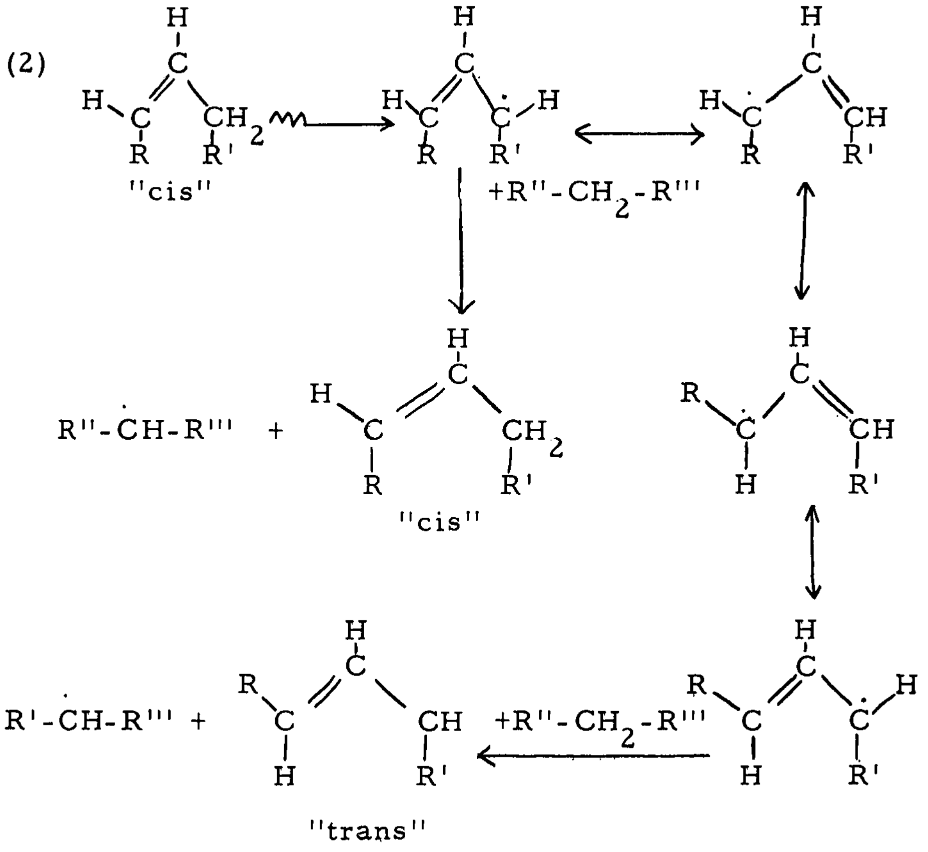
Cis-Trans Isomerization

Lück and Kohn (1963) have proposed two possible mechanisms for cis-trans isomerization.

(1)



(2)



EXPERIMENTAL

Preparation and Irradiation of the Milk Fat

Raw sweet cream (mixed herd) was obtained from the university dairy and cooled to about 10° C in a refrigerator. The cooled cream was churned in a glass churn until the emulsion broke. The buttermilk was drained out and the butter granules were washed repeatedly with cold distilled water (at 10° C) until the wash water was clear. The butter was melted at 40° C in a constant temperature water bath. The melted butter was then transferred into a separatory funnel and the butter serum drained out. The fat was washed five times with equal volumes of warm distilled water (at 40° C). The fat was washed in order to remove the phospholipids present (Day and Lillard, 1960). The milk fat was clarified by centrifugation for 20 minutes at 30, 000 xG (16, 000 rpm) in a Servall SS-3 super speed centrifuge. The supernatant clear milk fat was then poured out in a container. The temperature of the fat at the end of centrifugation was about 40° C. The clear fat was divided into three roughly equal portions. One portion was transferred to glass vials with the help of a long needle (no. 16) hypodermic syringe. The vials were made of pyrex glass (size 1 1/2" in diameter x 3 1/2" long) with a thick-walled glass capillary stem (0. 2 cm i. d. x 1. 0 cm o. d. x about 3 1/2" long). Each vial was about two-thirds full and contained approximately 50 ml of the milk fat. The fat in the vials was degassed for one hour at 20 to 50 microns pressure. The fat was maintained at 40° C by immersing the vials in a constant temperature water bath. After degassing, the fat in the vials was quickly frozen by immersing the vials in liquid nitrogen and the vials were sealed at the stems by means of an oxygen torch. The fat in the vials was warmed up to room temperature The vials were then subjected to gamma irradiation from a cobalt-60 source along with an aliquot of milk fat exposed to air. A third aliquot of the milk fat was used as the unirradiated control and was kept at room temperature.

Irradiation was carried out at room temperature in the high flux chamber of cobalt-60 irradiator. The irradiator had a squirrel cage source configuration with 12 rods and was built by Budd Company. The irradiator chamber was a cylindrical type 5" in diameter and 5" high. The fat was irradiated to a total dose of 4.5 Mrad (megarad) at a dose rate of 4.0×10^5 rads per hour. During irradiation, the temperature of the fat rose to about 45° C.

After irradiation, the fat exposed to air was analyzed immediately, while the sealed vials were stored at 0° F.

Analysis of the Irradiated Milk Fat

Before opening the vials, the fat was melted at 45° C in a constant temperature water bath. All the fat samples were analyzed for organoleptic changes, TBA number, peroxide value, monocarbonyls and free fatty acid content.

TBA Number

TBA number is expressed as mg of malonaldehyde per kg of the sample. The method of Sinnhuber and Yu (1958) with some modification was used in this investigation. About 500 mg of sample weighed accurately was used. To this, 5 ml distilled water, 5 ml 0.6N HCl solution, 10 ml trichloroacetic acid (TCA) solution, and 5 ml TBA reagent were added in a 250 ml ground glass flask. A condenser was very carefully connected to the flask and without shaking the contents, the flask was placed in boiling water bath and refluxed for 30 minutes. After refluxing, 75 ml of 0.6N HCl were added from the top. The flask was shaken several times and refluxed again for an additional 10 min. The flask was then cooled to room temperature in a water bath and the condenser was disconnected. Since the solution was clear, no centrifuging was required. The optical density of the clear solution was read in the Beckman DU Spectrophotometer at 532 mμ. TBA number was determined by the formula:

$$\frac{\text{O. D. at } 532 \text{ m}\mu \times 46}{\text{sample weight (g)}} = \text{TBA number.}$$

Peroxide Value

The peroxide value is defined as milliequivalents of peroxides per kg of fat. The peroxide value was determined by A. O. C. S. Tentative method cd 8-53 (1958).

Free Fatty Acids

The method described by Bills, Khatri and Day (1963) for the quantitative determination of major free fatty acids of milk fat was followed without any modification.

Monocarbonyls

Preparation of Solvents. Hexane and Benzene - High purity grade hexane (Phillips Petroleum Co., Beltsville, Oklahoma) and reagent grade benzene were treated by the method of Hornstein and Crowe (1962) to remove carbonyl impurities.

Chloroform - Reagent grade chloroform was freed of carbonyls by the method of Schwartz and Parks (1961).

Methanol - Reagent grade methanol was saturated with 2, 4-dinitrophenylhydrazine (DNPH) and refluxed over trichloroacetic acid for four hours followed by distillation.

Ethylene chloride - Reagent grade ethylene chloride was distilled over anhydrous potassium carbonate (reagent grade). The purified ethylene chloride was stored over anhydrous potassium carbonate.

Nitromethane - Nitromethane (reagent grade) was redistilled over boric acid.

Formation and Separation of Monocarbonyl DNP-hydrazones. The carbonyls present in fat were directly converted to DNP-hydrazones by reaction column of Schwartz and Parks (1961). The solvent (hexane) in the effluent was removed under vacuum. The residue contained mainly fat along with the DNP-hydrazones of monocarbonyls, semialdehydes, ketoglycerides and other classes of carbonyl compounds whose derivatives are soluble in fat-hexane solution, a small amount of DNPH reagent, and traces of the decomposition products of DNPH (Schwartz, Haller and Keeney, 1963).

The fat and traces of dicarbonyl bis-derivatives soluble in fat-hexane solution were removed by the method of Schwartz, Haller and Keeney (1959, 1963) as modified by Langler and Day (1964). The ketoglycerides were removed by passing the mixture dissolved in hexane through wetted alumina-hexane column (Schwartz and Parks, 1961). A Dowex 50 x 8 cation resin column (Schwartz, Johnson and Parks, 1962) was used to separate the traces of DNPH reagent from the monocarbonyl derivatives. The final traces of fat were removed by passing the monocarbonyl DNP-hydrazone mixture through a Dowex 1x4 resin column as described by Schwartz, Johnson and Parks (1962)

Partition Chromatography of Monocarbonyl Mixture. Before attempting liquid-liquid partition chromatography of the monocarbonyl mixture, class separation by the methods of Schwartz, Parks and Keeney (1960, 1962) was tried. The separation of DNP-hydrazones into classes was very poor. The absorption maxima of the separated fractions indicated that each fraction was not a class by itself, but a mixture. Hence, these fractions were combined into one monocarbonyl DNP-hydrazone mixture and subjected to liquid-liquid partition chromatography.

The nitromethane-hexane-Celite column of Day, Bassette and Keeney (1960) was used to separate the monocarbonyl mixture into various multicomponent bands. Each band or fraction would contain C_n alk-2-one, C_{n+1} alkanal, C_{n+2} alk-2-enal, and C_{n+4} alk-2, 4-dienal, if all the four classes of monocarbonyls were present in the band (Day and Lillard, 1960). A 25 g nitromethane-hexane-Celite column was used for the separation. Two to three columns were required to handle the entire monocarbonyl mixture in one sample and the bands were pooled for subsequent analyses. The carbon chain length of each band was estimated by comparing its threshold volume to that of the band obtained with an authentic mixture of monocarbonyl DNP-hydrazones (alk-2-ones and alkanals).

Thin-Layer Chromatography of Separated Monocarbonyl Bands. The carbon chain length of each band separated on the partition column was further confirmed by the reverse phase thin-layer chromatography (TLC) method of Libbey and Day (1964). Each band was spotted on the TLC plate next to the authentic DNP-hydrazone derivatives and developed. The chain length of the unknown spot was determined by comparing its Rf value with that of the authentic. A further confirmation of the chain length was made by enrichment or co-chromatographic techniques.

Paper Chromatography of the Separated Monocarbonyl Bands. The components present in each band were identified by means of class separation paper chromatographic method of Gaddis and Ellis (1959).

Effect of Traces of Water Present on the Free Fatty Acid Content of Irradiated Milk Fat

The milk fat was prepared from raw sweet cream as described earlier. This fat was not completely free of water. Even after degassing at two to five microns pressure for one hour, it was found to contain 0. 27 percent of water (Langler and Day, 1964).

In order to study the effect of this small amount of water on free fatty acid content of irradiated milk fat, the clear fat was divided into three portions. One portion was used as the unirradiated control.

The other portion was irradiated control. It was degassed and sealed in glass vials as described earlier. The third portion was treated with anhydrous calcium hydride (CaH_2) to remove trace amounts of water as outlined by Kliman and Pallansch (1963, 1965). However, instead of chloroform as suggested by Kliman and Pallansch (1963, 1965), isopentane was used as the solvent, since it was easier to remove. It was removed by heating the reaction flask at 40° C. The fat was clarified after the treatment with CaH_2 by centrifugation at 30, 000 x G in Servall Super Speed Centrifuge for 20 min. The clear fat which did not contain any detectable water (Langler and Day, 1964) was finally degassed and sealed in glass vials as described earlier.

The vials containing the treated and untreated fat were irradiated with gamma rays from cobalt-60 (Co-60) source at a dose of 4. 5 Mrad. At the end of the irradiation process the samples were analyzed for the free fatty acid content by the method of Bills, Khatri and Day (1963).

Irradiation-Hydrolysis of Methyl Octanoate

Purity of Methyl Octanoate

Methyl octanoate (white label) was obtained from Eastman Kodak Company. An Aerograph Model A-100 gas chromatograph with the thermal conductivity detector was employed for determination of purity of the methyl octanoate. The operating conditions were: 6 ft. x 1/4 in. i. d. aluminum column packed with 20 percent LAC-3R-728 (diethylene glycol succinate-DEGS) on 80 to 100 mesh Celite 545, acid and alkali washed by the method of Farquhar et al. (1958); column temperature 110° C; carrier gas (He) pressure 20 psig; cell current 175 milliamperes; and sensitivity one millivolt. The methyl octanoate was found to be 98. 24 percent pure.

Preparation of Anhydrous Methyl Octanoate

Removal of traces of water dissolved in methyl octanoate was achieved by the method of Kliman and Pallansch (1963, 1965), in the manner described earlier for the milk fat. Forty grams of methyl octanoate were placed into an Erlenmeyer flask along with one gram of anhydrous CaH_2 pellets. No solvent was used since the ester was liquid at room temperature. The flask was covered with an air tight stopper with a glass tube. To the open end of glass tube, a drying tube packed with anhydrous calcium chloride was connected. This way, the reaction flask was open to atmosphere only through the drying tube. The flask and its contents were shaken at room temperature for 18 hours. At the end of the drying period, the cloudy methyl octanoate was clarified by centrifugation for 20 minutes at 30, 000 xG in Servall Super Speed Centrifuge.

Irradiation of Methyl Octanoate

Dried and clarified methyl octanoate was degassed and sealed in glass vials as described earlier. Due to the volatility of the ester, it was cooled in an ice bath during the degassing process. Even at this temperature the vacuum that could be reached was not less than 500 microns. The vials (3/4 in. diameter and 4 in. long) contained about 10 g of methyl octanoate.

The vials were then irradiated with gamma rays at doses of 0, 1.5, 3.5, and 4.5 Mrad. In one case a dose of 6.0 Mrad was also employed. The procedure for irradiation has been outlined earlier.

Analysis of Irradiated Methyl Octanoate

The samples of irradiated methyl octanoate were analyzed for free octanoic acid. The method of Bills, Khatri and Day (1963) was used at first. It was found, however, that the unirradiated control sample gave large amounts of free octanoic acid by this method. There are four possible explanations for the high content of free octanoic acid in unirradiated sample:

(1) The ester methyl octanoate was contaminated with free acid: This was not true since the control sample did not give any acid value on titration. Also GLC analysis on ten percent DEGS

plus two percent H_3PO_4 on Celite 545 column was carried out. Conditions: temperature - 167°; detector - thermal conductivity; instrument - Aerograph Model A-100; sensitivity - one millivolt; and helium gas inlet pressure - 20 psig. No peak corresponding to free octanoic acid was found when the control sample was injected.

(2) The calcium hydroxide formed by reaction of CaH_2 with water, and CaH_2 could have hydrolyzed methyl octanoate: This reasoning was also rejected since the analyses mentioned above were carried out on control sample which had gone through CaH_2 treatment and even degassing process. Also, fresh methyl octanoate, when analyzed by Bills, Khatri and Day (1963) method still gave large amounts of free acid.

(3) Methyl octanoate was adsorbed on ion-exchange resin and was desorbed only after treatment with methanol-HCl.

(4) Ion exchange resin hydrolyzed the ester: This could not be determined for certain. Bills, Khatri and Day (1963) had found no hydrolysis of milk fat by the resin. However, earlier, McCarthy and Duthie (1962) had reported that the resin brings about the hydrolysis of esters and triglycerides.

Since any of the last two explanations could be applicable and the analysis of fresh methyl octanoate still showed large amounts of free acid to be present, the method of Bills, Khatri and Day (1963) was abandoned.

The free octanoic acid content was analyzed by GLC analysis of free acids on the column suggested by Metcalfe (1961). Heptanoic acid was used as an internal standard. The conditions for the GLC analysis were: Aerograph Model A-100 gas chromatograph with thermal conductivity detector; 1/4 in. o. d. x 9 ft. aluminum column packed with ten percent diethylene glycol adipate (DEGA) plus two percent H_3PO_4 on 80 to 100 mesh Chromosorb P, HMDS treated; column temperature, 175 - 177° C; helium inlet pressure, 20 psig; sensitivity, one millivolt; and cell current 170 milliamperes.

About 10 g of methyl octanoate sample to be analyzed was accurately weighed and to this about 10 mg of free heptanoic acid (internal standard) accurately weighed, was added. The sample was thoroughly mixed and an aliquot was analyzed by GLC. The areas under the peaks representing octanoic and heptanoic acids were measured and the weight of octanoic acid present was calculated by the following equation:

$$\begin{array}{l}\text{Wt. of octanoic}\\ \text{acid in sample}\end{array} = \frac{\text{Factor} \times \left[\begin{array}{c}\text{Area under}\\ \text{octanoic acid}\\ \text{peak}\end{array}\right] \times \left[\begin{array}{l}\text{Wt. of heptanoic}\\ \text{acid added to}\\ \text{the sample}\end{array}\right]}{\left[\begin{array}{c}\text{Area under heptanoic}\\ \text{acid peak}\end{array}\right]}$$

The factor represents correction due to recovery and recorder response. It was determined by analyzing a known mixture of heptanoic and octanoic acids by GLC under the experimental conditions. The factor was calculated by the following equation:

$$\text{Factor} = \frac{\left[\begin{array}{c}\text{Wt. of octanoic}\\ \text{acid}\end{array}\right] \times \left[\begin{array}{c}\text{Area under heptanoic acid}\\ \text{peak}\end{array}\right]}{\left[\begin{array}{c}\text{Wt. of heptanoic}\\ \text{acid}\end{array}\right] \times \left[\begin{array}{c}\text{Area under octanoic acid}\\ \text{peak}\end{array}\right]}$$

The factor was found to be unity and deviated very slightly from its value of unity.

The recovery of a known weight of octanoic acid added to a known weight of methyl octanoate was also determined by the above procedure. The recoveries were also found to deviate negligibly from 100 percent.

Identification and Characterization of Steam Volatile Components of Gamma Irradiated Milk Fat

Preparation and Irradiation of the Milk Fat

Milk fat was separated from three gallons of raw sweet cream (mixed herd), obtained from the university dairy, by the method described in the beginning of this chapter.

The clear fat was poured into 307x409 (no. 2) 'C' enameled cans and the cans were sealed under 29 in. of vacuum. A small head-space (about 1/3 in.) was left in the cans. The cans were irradiated at 4.5 Mrad in the Co-60 irradiator. The cans were stored at -18° C before and after irradiation. A total of eight cans were filled with the milk fat. Four of the cans were used as unirradiated control.

Analysis of the Head-Space Gases

The cans, irradiated and unirradiated, were analyzed for head-space gases using the Fisher Gas Partitioner Model 25V and the Zahm head-space sampling device described in Fisher Technical Data Gas chromatographic bulletin 12 (1962). The sampling device was first evacuated with a vacuum pump and checked for leaks. The top of the can, containing irradiated milk fat warmed up to 40° C and placed on the jack, was punctured with the piercing needle by raising the can platform. The head-space gases were allowed to diffuse into the sampling device. The Hg reservoir was raised to apply a slightly positive pressure on the gases and then the gas sample was injected into the Gas Partitioner for analysis. The conditions for analysis were:

Column 1 : 30 in. x 1.4 in. o. d. packed with 30 percent hexamethyl phosphoramide (HMPA) on 30 to 60 mesh fire brick.

Column 2: 6 1/2 ft. x 1/4 in. o. d. packed with 30 to 60 mesh Molecular Sieve 13X.

Column temperature: 23.5° C.

Cell current: 5 milliamperes.

Helium flow rate: 80 ml/min.

Inlet pressure: 19 psig.

This instrument has a thermistor detector. Only CO_2, CO, H_2, O_2, N_2 and CH_4 could be analyzed by the columns used.

Vacuum Steam Distillation of the Irradiated Milk Fat

The volatiles in the irradiated milk fat and unirradiated control were isolated by vacuum steam distillation at 40° C and 1-2 mm Hg. The apparatus described by Day and Lillard (1960) was used with some changes. The steam generator and steam trap flasks were larger in size (one liter), and a one liter reaction flask was replaced by a U-tube with a stop-cock outlet at the bottom. Also, an additional glass bead trap was connected at the end of the trapping chain before the vacuum pump.

The cans were opened after head-space analysis and the melted fat was immediately transferred to the steam striping flask. Contents of one or two cans were used for a single distillation. After about 350 ml of water had distilled over from the steam generator flask, the distillation was stopped. The dry ice from the traps was taken out after they had been disconnected from the rest of the equipment and closed at both ends with glass stoppers. The distillate ice was allowed to melt at room temperature. The distillate was finally drained into a flask and extracted with ethyl ether.

At the end of the distillation, the fat did not have any noticeable odor and was quite bland. In one case the fat still had some odor left; hence the distillation process was repeated for that sample until another 200 ml of distillate was collected.

Purification of Ethyl Ether

The method of Valseth (1953) was used to remove the peroxides present in ethyl ether. Two liters of ethyl ether were stirred with 400 ml of ten percent sulfuric acid containing 40 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) in a large Erlenmeyer flask with a magnetic stirrer for one hour.

The ether was decanted into a distillation flask and subjected to fractional distillation. The distillation column used was a 3 cm x 100 cm air jacketed column packed with glass helices and with a vacuum jacketed fractionation head. The rate of fractionation was controlled electrically in the vacuum jacketed fractionation head. A reflux ratio of 1:3 was used. The distilled ether was passed through a water cooled condenser and collected in a flask cooled with dry ice. After the distillation was over, the ether was quickly filtered through glass wool to remove the ice crystals. The ether was then stored in a brown glass bottle in a cool and dark place.

Extraction and Concentration of Volatiles

The distillate from the low temperature vacuum steam distillation had an odor that was typical of the fat distilled. The distillate was extracted four times with 100 ml portions of peroxide free ethyl ether in a separatory funnel. The distillate then was saturated with reagent grade sodium chloride and again extracted twice with 100 ml portions of ethyl ether. The ether fractions were combined. The aqueous distillate at the end of the extraction smelled only of dissolved ether while the ethyl ether extract had a strong odor of irradiated fat.

The ether extract was dried over reagent grade anhydrous sodium sulfate. The dried extract was concentrated by fractional distillation employing the apparatus described for purification of the ether. A smaller distillation column (1 cm x 60 cm) was used for this purpose and the reflux ratio was changed to 1:4. The ether was removed in this manner until about 30 to 40 ml of extract were left in the distillation flask. The concentrated extract was transferred to a 50 ml pear-shaped ground-glass stoppered flask and stored in the freezer compartment of the refrigerator. The flask was stoppered tightly during storage. Fractions of this concentrate were transferred to glass vials and further concentrated for gas chromatographic analysis by allowing the excess ether to evaporate through loosened stopper in the freezer compartment of the refrigerator.

Analysis of Monocarbonyls in the Ether Extract

About 20 ml of the ether extract concentrate were mixed with 50 ml of 5 N HCl saturated with DNPH reagent in an Erlenmeyer flask. The mouth of the flask was covered with aluminum foil and the mixture in the flask was stirred with a magnetic stirred for 48 hours. The mixture was then allowed to stand in the refrigerator (10° C) for another 48 hours with frequent shaking.

The DNP-hydrazones were extracted five times in a separatory funnel with 50 ml portions of purified ethylene chloride. The extracts were combined and the ethylene chloride was removed at reduced pressure to give a dry residue. The monocarbonyl DNP-hydrazones were separated on the alumina column of Schwartz and Parks (1961); and the excess DNPH reagent was removed by Dowex 50x8 cation resin column of Schwartz, Johnson and Parks (1962) as described earlier in this chapter.

The monocarbonyl derivatives, thus obtained, were analyzed by the liquid-liquid partition chromatography on a nitromethane-hexane-Celite column of Day, Bassette and Keeney (1960) followed by the paper chromatographic method of Gaddis and Ellis (1959). The TLC method of Libbey and Day (1964) was not used. Instead, the carbon chain length of each band separated by partition chromatography was determined by paper chromatographic techniques of Huelin (1952) and

Klein and De Jong (1956). The cochromatography on paper was also used to further confirm the carbon chain length of each band, as described earlier.

Gas-Liquid Chromatography (GLC)

The ether extract concentrate was analyzed by gas-liquid chromatography. A Barber-Colman model 20 gas chromatograph equipped with an argon, strontium-90 β-ionization detector was used. The conditions employed were: 10 ft. x 1/8 in. o. d. stainless steel column packed with 20 percent LAC-3R-728 (DEGS) on 80-100 mesh Celite 545; column temperature, 125° C; cell temperature, 220° C, Argon inlet pressure, 20 psig; cell scavenger flow, 120 ml per min.; and cell voltage, 1200 volts. An on-column injection system was used and the effluent gas at the end of the column was split using a "Swagelok" T-fitting. One-half of the effluent gas was introduced into the detector cell and the other half was vented to the atmosphere via a 1/8 in. o. d. stainless steel tubing and a hypodermic needle no. 26. Some of the peaks were tentatively identified by comparing their retention times with those of the authentic compounds and by selectively removing certain classes of compounds by reaction of the ether extract concentrate with appropriate reagents. The characteristic odor of each peak could be determined by sniffing the effluent as each peak came out.

The fatty acids present in the ether extract concentrate were removed by shaking the concentrate with an equal volume of 0.01N sodium bicarbonate. The ether layer was then rechromatographed and the missing peaks noted. The chain length of the acids was identified by comparing the retention times.

Various attempts were also made to remove carbonyls from ether extract concentrate and thus identify the peaks representing them. These attempts were not very successful, since in all the cases the carbonyls were only partially removed from an authentic carbonyl solution. The reagents tried were: 2:4-dinitrophenylhydrazine in 5 N HCl, hydroxylamine-sulfate (method of Bassette, Özeris and Whitnah, 1962), sodium bisulfite reagent (Shriner, Fuson and Curtin, 1960) and 3-methyl-2-benzothiazolone hydrazone-hydrochloride (Sawicki, et al., 1961).

A Barber-Colman Model 5000 gas chromatograph equipped with the flame ionization detector and temperature programming was also used for GLC analysis of the ether extract concentrate. Two different columns were employed for the separation. One was 1/8 in. o. d. x 24 ft. stainless steel column packed with 20 percent DEGS on 80 to 100 mesh Celite 545. The other was 1/8 in. o. d. column with varying lengths packed with 20 percent Apiezon M on 100 to 120 mesh Celite 545. The column lengths, and temperature conditions for GLC had to be varied in order to analyze all the components present in the ether extract concentrate. During some of the GLC analyses temperature programming was used and during other analyses isothermal conditions were used. These conditions will be described later at appropriate places in this thesis.

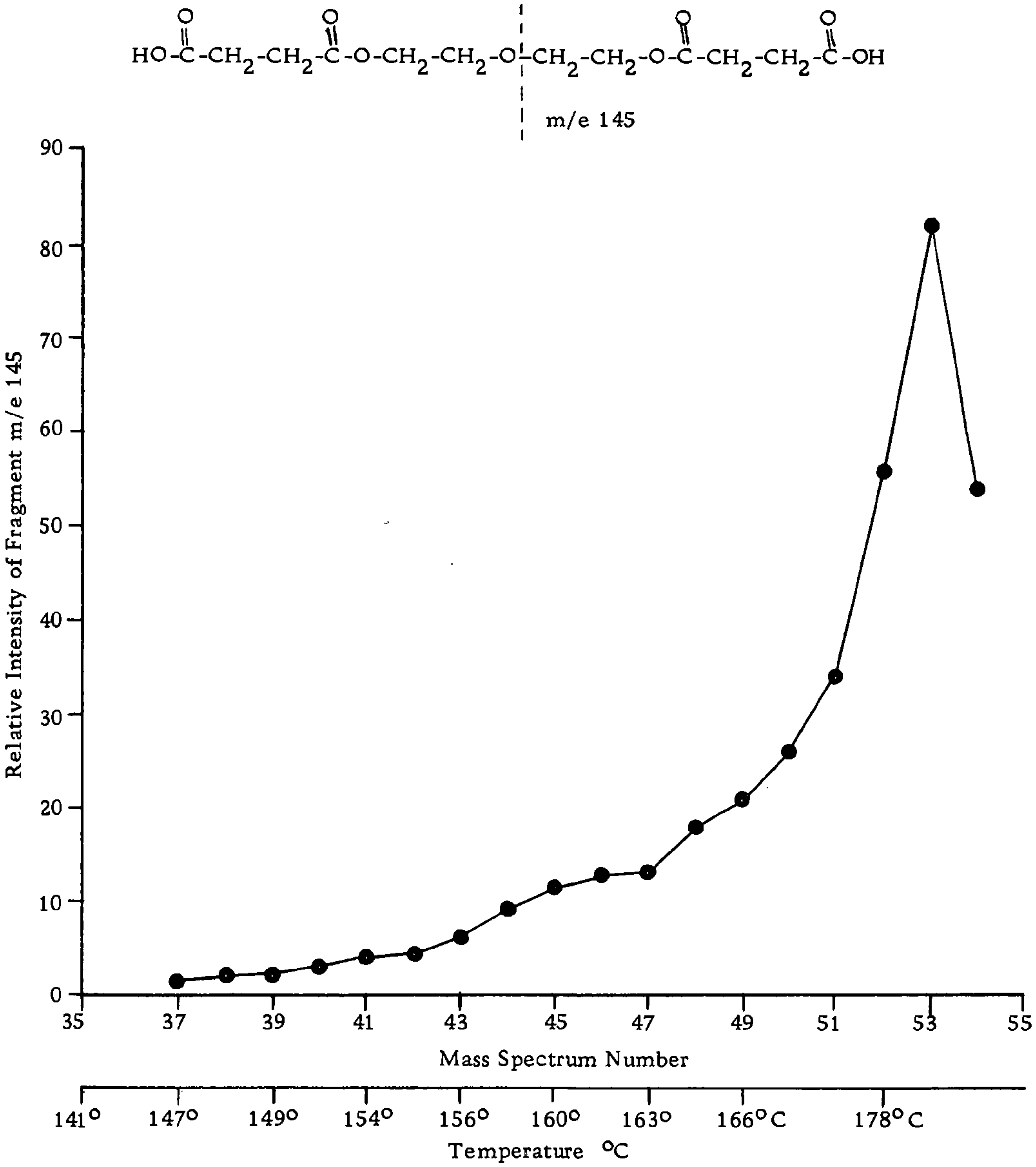
During the course of GLC analysis it became apparent that the large fatty acid peaks, due to their tailing, were hindering the analysis of the neighboring peaks. Consequently a base coated precolumn was devised which effectively removed the acid components from the injected sample. The column was 2 ft. x 1/8 in. o. d. stainless steel, packed with 20 percent Apiezon M on 100-120 mesh Celite 545 coated with five percent by weight of sodium hydroxide. The column packing was prepared as follows: one-half gram of sodium hydroxide pellets were dissolved in about 20 ml reagent grade methanol. The NaOH-methanol solution was mixed with 9. 5 g of 100-120 mesh Celite 545 (acid-alkali washed) and the slurry was heated on a steam bath to remove excess methanol. The slurry was stirred vigorously during evaporation. After all the methanol was evaporated, the Celite-NaOH residue was dried in a vacuum oven at 45^{o} C for six hours. This dried NaOH coated Celite was then recoated with 20 percent Apiezon M in the usual manner. The column was conditioned in the usual manner by heating to 220^{o} C for 24 hours with a small flow of nitrogen or other inert gas flowing through it. The column was good for several analyses.

GLC Combined with Fast-Scan Mass Spectrometry

The final and positive identification of the components separated by GLC was made by fast-scan mass spectral analysis of the effluents from GLC. A Barber-Colman series 5000 unit with temperature programming and flame ionization detector was used in conjunction with the Atlas-MAT CH-4 Nier type mass spectrometer. This is a single-focusing instrument with a nine inch, 60 degree sector magnet. The effluent from the GLC column was split with a "Swagelok" T-fitting at the end of the column, one portion was directed to the detector and the other to the mass spectrometer inlet. Initially, the ratio of the volume of effluent gas entering the GLC detector to that entering the mass spectrometer inlet was 1:10. The amount of sample needed in the mass spectrometer was not enough with a smaller ratio and spectra obtained were very weak. Later, however, the ratio was changed to 1:1 because it became apparent from working with the mass spectrometer that suitable spectra could be obtained even with this ratio. The mass spectrometer inlet system was equipped with the EC-1 gas inlet valve, which could be adjusted to regulate the amount of effluent admitted to the mass spectrometer ion source. The mass spectra were obtained by magnetically scanning the GLC effluent at various points of the gas chromatogram and were recorded with the Honeywell Model 1508 Visicorder. The operating conditions for the mass spectrometer were:

Ionization current	60 μ-amps
Accelerating potential	3000 volts
Electron voltage	35 ev
Multiplier voltage	1. 60 to 1. 85 KV
Analyzer pressure	~2 x 10^{-7} mm Hg
EC-1 opening	0. 2 to 0. 4 μ-amps
Scanning speed	5 seconds for m/e 25 to m/e 250.
Visicorder chart speed	8 inches per sec.

Initial analyses were carried out on DEGS columns. A 24 ft. x 1/8 in. o. d. stainless steel column packed with 20 percent DEGS on 80-100 mesh Celite 545 was used. The GLC operating conditions were: temperature, 63° C for 14 minutes and then programmed at 2° per minute to 170° C; cell, 265° C; carrier gas (helium) flow rate, 30 ml per minute; hydrogen pressure, 15 psig; and air pressure, 65 psig. A split ratio 1:10 was used for all the analyses on DEGS columns. Background spectra were taken in each case before the components came off the column and entered the mass spectrometer. The problem of stationary phase bleed during temperature programming caused excessive background in the mass spectra. As the temperature of the column increased, the spectra of the last few peaks in the chromatogram were indistinguishable from background. Figure 1 shows the increase in the intensity of m/e peak 145 due to DEGS bleed with increase in temperature. The bleed was less significant when the temperature of the GLC column was not varied



$$HO\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH$$

Figure 1. DEGS column bleed obtained with the temperature programming.

during the mass spectral analysis. An isothermal GLC-mass spectral analysis was, therefore, carried out to obtain identity of the peaks which were masked by bleed during temperature programming. The conditions for the GLC analyses were the same as used for temperature programming except that the column temperature was kept constant at 150° C.

During the analyses on the 24 ft. column, the mass spectra of the slow moving components were very weak, probably because the peaks were spread out and not sharp. This resulted in a lower concentration of the components in the mass spectrometer at a given time. In order to analyze these slow moving long chain components, a shorter GLC column was used. The GLC conditions were: 10 ft. x 1/8 in. o. d. stainless steel column packed with 20 percent DEGS on 80 to 100 mesh Celite 545; column temperature, 150° C and 175° C (two separate runs); cell temperature, 260° C, helium flow rate, 30 ml per minute; hydrogen pressure 12 psig; and air pressure, 65 psig.

Even though the volatiles from 4. 5 Mrad irradiated milk fat contained various monocarbonyls (as determined by column chromatography and paper chromatography of DNP-hydrazones), no aldehydes or ketones could be identified by mass spectrometric analysis of the GLC effluents from the DEGS column. The peaks which had odors similar to aldehydes gave mass spectra similar to those of hydrocarbons. This indicated that these peaks did not represent single components and thus represented poor separation. In a search for stationary phases to give better separation of the components, various columns with packings coated with Ucon non-polar; Carbowax 20M, and Apiezon M were tried. The Apiezon M column seemed to give the best separation of all the columns investigated. Hence, the ether extract concentrate was analyzed again on the Apiezon M column. Most of the component peaks were analyzed on long columns. The conditions were: 2 ft. x 1/8 in o. d. stainless steel (SS) base pre-column packed with 20 percent Apiezon M on 100-120 mesh Celite 545 coated with five percent by weight NaOH, (to remove acid components) followed by the 12 ft. x 1/8 in. o. d. SS main column packed with 20 percent Apiezon M on 100-120 mesh Celite 545; temperature, 78° C for five minutes, then programmed at 2° per minute to 215° C and held until the end of analysis; cell temperature, 250° C; helium flow rate, 30 ml per minute, air pressure, 70 psig; and hydrogen pressure, 14. 5 psig. For long chain or slow moving components, a shorter 8 ft. x 1/8 in. o. d. SS main column following the base pre-column was used. The analysis was carried out at 175° C isothermally. The other conditions for GLC were the same as described above.

The only compounds that could be identified were still the hydrocarbons. No evidence of carbonyls was obtained. When a mixture of authentic aldehydes was analyzed, the spectra obtained were completely different from those described in the literature. A clear indication of thermal decomposition was obtained, since the spectra indicated possible aromatic ring compounds. By manipulation, it was observed that if the column temperature, cell temperature, or mass spectrometer inlet temperature was above 220° C, aldehydes were completely decomposed.

Long chain components were analyzed on a 6 ft. column containing the same packing. Another problem which became apparent with the lactones and some very high boiling components, was that they tended to condense in the EC-1 inlet at temperatures of 200° or 205° C. For the analysis of these components a higher column temperature was used. The conditions used were: 6 ft. x 1/8 in. o. d. SS column with 20 percent Apiezon M on 100-120 mesh Celite; column temperature, 220° C (isothermal analysis); cell, 250° C; EC-1, 215° C at pipe leading to EC-1 and 250° C near the connections to GLC; helium pressure, 45 psig; hydrogen, 14. 5 psig; and air, 70 psig. Analysis of the volatiles from unirradiated control milk fat was also carried out by the above method. The conditions used for long chain components were similar to those described above. However, conditions for the remainder of the components were as follows: 14 ft. x 1/8 in. o. d. SS column with 20 percent Apiezon M on 100 to 120 mesh Celite 545; column temperature, 70° C for six minutes then programmed at 15° per minute to 200° C and held until the end of the analysis; cell, 200° C; EC-1, 199° C; helium pressure, 54 psig; hydrogen, 14.5 psig; and air, 70 psig.

Some of the components which were present in very small quantities would not give strong enough mass spectra for a proper interpretation. Hence, a trapping procedure had to be devised to concentrate these components. By using the trapping technique, the components present in larger quantities in the mixture which otherwise would have interfered with the smaller components if whole extract was further concentrated, were eliminated. These components were trapped on a packed column attached to the effluent port of the gas chromatograph. The column was cooled by immersing it in methyl cellosolve (2-methoxyethanol) - dry ice mixture. The trap-column was 2 ft. x 1/8 in. o. d. SS column packed with 20 percent Apiezon M coated on 100-120 mesh Celite 545. The concentration of a fraction was achieved by trapping the same fraction from several injections. After the particular fraction to be analyzed was concentrated in the above manner, the trap-column was connected in front of the main analyzing column in the GLC oven and the GLC and mass spectral analysis was carried out as usual.

It should be mentioned that a 1:1 split ratio of gas flow to detector: EC-1 was used for the later analyses on Apiezon M columns. During these analyses, it was noticed that a particular component did not reach maximum concentration in the GLC detector and mass spectrometer ionization chamber at the same time. There was a lag period before a maximum concentration was obtained in the mass spectrometer. In order to assign the mass spectra to proper peaks of the gas chromatogram, a trial run was made before each analysis with the mixture to be analyzed and the lag period was determined for each component by monitoring the signal for m/e 43 or 57 in the mass spectrometer. During the actual analyses, these lag periods were taken into consideration.

RESULTS AND DISCUSSION

Effects of Irradiation under Air and Vacuum

Changes in Physical and Chemical Properties

The milk fat was irradiated at 4. 5 Mrad in air and in sealed glass vials under vacuum. The control milk fat was allowed to stand at room temperature during the time of irradiation. Apart from the necessary changes, the manipulation of all the samples was similar. Table 3 gives the results of organoleptic tasting and chemical analyses of the unirradiated control, milk fat irradiated at 4. 5 Mrad under vacuum and in air. The monocarbonyls were determined by measuring the absorbancy of the DNP-hydrazone-hexane solution eluted from DNPH reaction column against the hexane blank on the Beckman DU Spectrophotometer (Schwartz, Haller and Keeney, 1963). The reading was converted to micromoles, using E = 22, 500. It was then converted to millimoles per kg. These results indicate clearly that the effect of irradiation is quite pronounced even under vacuum; but it is more so in air. The results for the sample irradiated in vacuum are in good agreement with those of Day and Papaioannou (1963). They reported a total of 1. 156 millimoles of free carbonyls per kg of 4. 5 Mrad fat irradiated under vacuum. They used the method of Keith and Day (1963) for determination of free

Table 3. Gross changes in gamma irradiated milk fat.

Treatment	Peroxide Value[a]	TBA Number[b]	Total Monocarbonyls[c]	Sensory Observations
Control (0.0 Mrad)	0.0	0.75	0.3567	Golden yellow color, clean and sweet odor; sweet taste with no after-taste.
4.5 Mrad in Air	2.51	4.97	1.5678	Pale yellow color (almost completely bleached), slightly rancid, oxidized and candle-like odor; waxy taste with some bitter after-taste.
4.5 Mrad in Vacuum	0.33	0.91	1.2697	Pale yellow color (almost completely bleached), slightly rancid and candle-like odor; waxy taste with some bitter after-taste.

[a] Milli equivalents per kg fat.

[b] mg of malonaldehyde per kg fat.

[c] Milli moles of monocarbonyls per kg fat.

carbonyls. The total monocarbonyls found in this investigation were 1. 2697 millimoles per kg of fat irradiated under vacuum. There is some difference, however, in the TBA no. and peroxide value. The values obtained in this investigation were smaller, probably because of the difference in magnitude of vacuum in the two investigations. The glass vials were sealed under 50 microns of vacuum, after degassing of the sample, while Day and Papaioannou (1963) used commercial vacuum of 28 inches in their cans. This may also explain why they observed a strong oxidized flavor in their samples. The control milk fat in the present investigation showed higher TBA number and total monocarbonyl values. These could be explained on the basis that the control milk fat was kept at room temperature and exposed to atmosphere during the time of irradiation of other samples. The total time involved was close to nine hours (0. 4 Mrad per hour). This may have brought about some autoxidation of the control milk fat.

There is considerable difference in the magnitude of the changes brought about by irradiation in air and under vacuum. As has already been shown (Mukherjee, 1950), the reduction in oxygen pressure results in less hydroperoxide formation. Irradiation in air also results in higher TBA no. and monocarbonyl value.

Irradiation of milk fat in air or under vacuum results in destruction of carotenoid pigments as observed by Hannan and coworkers

(1952, 1954 and 1956) among other investigators. The observation of rancid and candle-like odors has also been reported by Day and Papaioannou (1963), but this is in contradiction to the statement made by Coleby (1959) that removal of oxygen prevented off-flavors on irradiation.

Monocarbonyl Compounds

Table 4 shows the monocarbonyl compounds identified in control milk fat and irradiated milk fat samples. The estimate of the total quantities of free monocarbonyls may be obtained from Table 3. These data show that the amount of monocarbonyls in irradiated fat was four to five times that in the non-irradiated control fat. However, this ratio may be even larger if completely fresh fat was used for the comparison. There does not seem to be much difference between total free monocarbonyls of fat irradiated under vacuum and in air. However, differences are noticeable in individual component content of the irradiated fats.

The milk fat irradiated in glass vials was under almost complete vacuum whereas the fat used for steam distillation was irradiated in cans sealed under 29 inches of vacuum, and still had some air left in the head-space (see analysis of head-space gases). This fact may account for the differences between the aldehyde content of fat irradiated in glass vials (under 20-50 microns pressure) and that of

Table 4. Monocarbonyl compounds produced in milk fat upon gamma irradiation in air and under vacuum.

Milk Fat Treatment	n-Alkanals	Alk-2-ones (Methylketones)	Alk-2-enals
Control (0.0 Mrad in air)	C_9, C_{10}, C_{12}, C_{16}	C_3, C_6(trace), C_7(trace), C_{13}, C_{15}	---
4.5 Mrad in Air	C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{14}, C_{16}	C_3(trace), C_4, C_5, C_6(trace), C_7, C_9, C_{11}, C_{15}	C_9, C_{12}
4.5 Mrad under a Vacuum (in glass vials)	C_4, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{14}, C_{16}	C_4, C_5, C_7, C_8(trace), C_9, C_{11}, C_{13}, C_{15}	C_{12}
Vacuum Steam Distillate Extract (4.5 Mrad under vacuum in cans)	C_1, C_2, C_3, C_4, iso-C_4?, C_5, iso-C_5?, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{14}, C_{16}	C_3, C_6(trace), C_8(trace), C_9, C_{15}	C_5(trace), C_6(trace), C_9, C_{12}

the fat irradiated in cans (29 in. vacuum) (see Table 4). The carbonyls found in the steam distillate agree more closely with those found by Day and Papaioannou (1963). These results also suggest that when air or oxygen is almost completely eliminated, no aldehydes are formed with carbon chain smaller than four carbon atoms. The possible reason will be considered while discussing the mechanisms for aldehyde formation.

The methyl ketones present in fats irradiated in air or under vacuum do not seem to differ much. The smaller differences observed may be due to discrepancy of the method and especially due to smaller amounts of ketones being present. However, during the course of analysis it was observed that the amounts of methyl ketones present in irradiated fats were much larger than those present in the control. The work of Lawrence and Hawke (1963) and Langler and Day (1964) suggests that methyl ketones are formed as a result of lipid hydrolysis. That the irradiation of milk fat brings about the hydrolysis of fat has been suggested by various workers (Fomin, 1962; Ibragimov and Mukamedov, 1962) and also will be shown conclusively later in this discussion. The finding of trace amounts of C_6 and C_8 methyl ketones is hard to explain. The presence of these and many other even- and odd-numbered saturated and unsaturated methyl ketones and their probable mechanism of formation has been reported by Lück and Kohn (1962) and Kohn (1964). This may give the possible explanation as to the formation of some of these trace methyl ketones.

Day and Papaioannou (1963) obtained evidence of alk-2-enals and alk-2, 4-dienals in irradiated milk fat. However, they failed to identify these by chromatographic techniques. In the present work traces of C_5 and C_6, C_9 and C_{12} enals have been identified in irradiated fat samples. These results thus support the finding of Day and Papaioannou (1963) that unsaturated aldehydes are present in irradiated milk fat. However, no dienals could be identified in the irradiated milk fat.

The finding of carbonyls in milk fat irradiated under vacuum supports the results of Lang and Proctor (1956), who also reported that carbonyls were formed even under vacuum.

Free Fatty Acids

The free fatty acids were determined by the method of Bills, Khatri and Day (1963). Figure 2 shows a typical gas chromatogram of the methyl esters of the free fatty acids from an irradiated milk fat sample. The amounts of individual free fatty acids present in irradiated samples and the control are given in Table 5. These results indicate that large amounts of free fatty acids are produced upon irradiation under vacuum and even more so in air.

There are two possible explanations for the large increase in

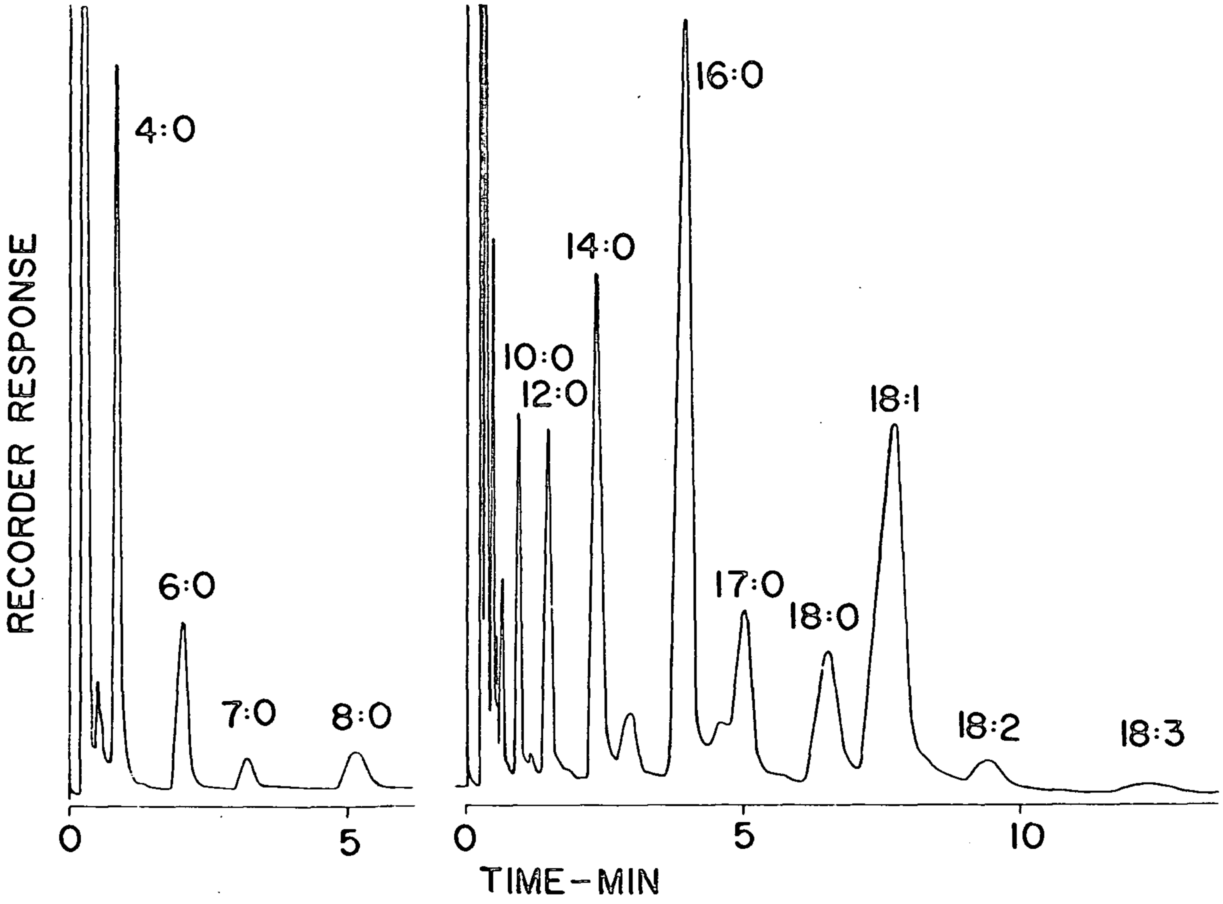


Figure 2. Gas chromatogram of free fatty acid methyl esters from 4.5 Mrad milk fat (irradiated in air).

Table 5. Major free fatty acids in 4.5 Mrad milk fat (mg fatty acid/ kg milk fat).

Free Fatty Acids	Control (0.0 Mrad)	4.5 Mrad in Air	4.5 Mrad in Vacuum (20-50 microns)
4:0	46.6	419.6	138.50
6:0	16.4	143.8	73.81
8:0	13.0	70.8	51.21
10:0	59.0	178.4	119.59
12:0	141.6	282.4	195.10
14:0	301.6	685.8	521.26
16:0	910.6	1601.2	1364.98
18:0	308.6	497.4	461.86
18:1	1073.6	1544.2	1336.88
18:2	133.4	171.4	152.90
18:3	66.2	62.2	84.70
Total	3070.6	5657.2	4500.90

the free fatty acid content upon irradiation. One possible mechanism may be the formation of fatty acids via reactions similar to those involved in autoxidation. This would explain the presence of larger amounts of fatty acids in samples irradiated in air in comparison to those irradiated under vacuum. However, this reasoning cannot explain the large amounts of free fatty acids present in samples irradiated under vacuum, since little or no peroxides are formed upon irradiation under vacuum (Chipault et al., 1957; Chipault and Mizuno, 1964). Oxidation mechanism, on the other hand, can explain the formation of large amounts of short chain fatty acids only. In the present investigation, however, large amounts of all the major fatty acids of the milk fat are formed upon irradiation.

These facts point to the second possibility, namely, the irradiation of milk fat results in hydrolysis of the milk fat. This has also been suggested by some investigators (Zhuravlev, Lomova and Benevolenskii, 1961; Fomin, 1962; Ibragimov and Mukhamedov, 1962; Ibragimov and Arifzhanov, 1962).

The differences in the free fatty acid content of samples irradiated in air and under vacuum may be explained by the assumption that the process of evacuation and degassing of milk fat resulted in some loss of dissolved water necessary for the hydrolysis of triglycerides. A very high content of butyric acid in sample irradiated in air, however, cannot be explained. It may be due to some discrepancy in the method or measurement of GLC peaks, but these values are the results of duplicate analysis.

Effect of Traces of Water on the Free Fatty Acid Content of Irradiated Milk Fat

The milk fat separated from the raw sweet cream by the method described contains about 0.27 percent of water (Langler and Day, 1964). The samples of milk fat containing these traces of water and dried over CaH_2 by the method of Kliman and Pallansch (1963, 1965) were degassed and sealed under vacuum and irradiated at 4.5 Mrad. The free fatty acid content of these samples is given in Table 6. In general, irradiation of the milk fat causes an increase in the free fatty acid content. It is interesting to note that the amount of short chain fatty acids (4:0 to 8:0) is not affected by the presence or absence of water, whereas, long chain fatty acids show a significant increase in samples not treated with CaH_2 over the samples treated with CaH_2. Also, when compared with the control, the samples treated with CaH_2 show an increase in the amounts of shorter chain fatty acids and some decrease in the amounts of long chain fatty acids. From these observations, it may be generalized that the presence of water aids in hydrolysis of triglycerides, but hydrolysis may also be taking place in the absence of water. The lower quantities of longer chain fatty acids in CaH_2 treated samples could be

Table 6. Effect of removing traces of water before irradiation on the free fatty acid content of milk fat (mg fatty acid/ kg fat).

Fatty Acid	Milk Fat Sample No. 1			Milk Fat Sample No. 2		
	0.0 Mrad No CaH_2 treatment	4.5 Mrad No CaH_2 treatment	4.5 Mrad Treated with CaH_2	0.0 Mrad No CaH_2 treatment	4.5 Mrad No CaH_2 treatment	4.5 Mrad Treated with CaH_2
4:0	30.09	93.75	90.24	34.55	87.12	88.18
6:0	13.44	50.09	48.35	6.73	41.02	41.05
8:0	7.07	32.70	30.32	7.14	26.76	24.52
10:0	45.68	81.24	68.25	46.98	81.67	61.75
12:0	134.24	178.56	131.05	126.35	176.50	105.40
14:0	257.03	432.47	325.56	246.96	428.02	275.18
16:0	789.48	1123.97	738.53	719.04	1170.34	687.13
18:0	286.83	382.23	225.91	217.51	370.11	199.79
18:1	958.39	1089.25	913.42	909.29	1145.52	768.04
18:2	142.66	142.90	132.14	116.80	110.17	100.37
18:3	49.72	43.27	26.79	65.05	59.42	39.20
Total	2714.63	3650.43	2730.56	2496.40	3696.65	2390.61

explained on the basis that these acids will form easier targets for the gamma rays and will be further decomposed. Short chain fatty acids may also be formed by chain scission and hydrolysis mechanism of Lück and Kohn (1961a) and Kohn (1964). Sheppard and Burton (1946) and Burton (1949) have also reported finding traces of short chain fatty acids in irradiated long chain fatty acids. Finally, the effects of irradiation in the presence or absence of water appear to be more complex than simple hydrolysis or chain scission of the triglycerides.

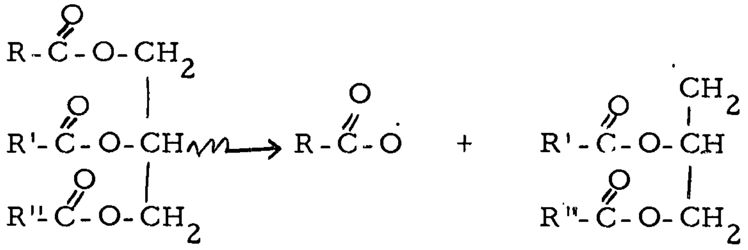
Hydrolysis of Methyl Octanoate upon Irradiation

Table 7 gives the amounts of free octanoic acid formed upon gamma irradiation of methyl octanoate. The methyl octanoate was treated with CaH_2 to remove traces of moisture and then irradiated under vacuum. These results prove conclusively that the irradiation results in the fission of an ester linkage and that water is not necessarily required for the reaction. This suggests a free radical mechanism.

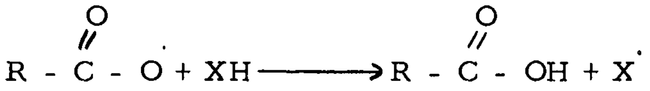
Table 7. Mg free octanoic acid produced per 100 g irradiated methyl octanoate.

Radiation dose	Sample 1	Sample 2	Sample 3
1. 5 Mrad	5. 5413	8. 2649	6. 9863
3. 0 Mrad	35. 8468	16. 8696	18. 5671
4. 5 Mrad	77. 8019	24. 4542	51. 9747
6. 0 Mrad	----	----	68. 4457

Lück, Deffner and Kohn (1964) have proposed that one of the radicals that are formed upon irradiation of triglycerides is a carboxyl radical:



The carboxyl free radical may accept a hydrogen atom from a donor as indicated.



$$R - \overset{\overset{O}{\|}}{C} - \dot{O} + XH \longrightarrow R - \overset{\overset{O}{\|}}{C} - OH + X^{\cdot}$$

It may be noted from Table 7, however, that the extent of hydrolysis is very small and that more free acid is formed with increase in dosage from 1. 5 Mrad to 6. 0 Mrad. The increase in the amount of free octanoic acid with the increase in irradiation dose appears to be somewhat linear. However, non-reproducibility of the results from sample to sample cannot be explained satisfactorily. One reason may be the minute amounts of free acids produced and the other may be the slight unavoidable variations in treatment and preparation of the samples.

Analysis of Head-Space Gases of Irradiated Milk Fat

Figure 3 shows the chromatogram obtained for the head-space gases by the Fisher Gas Partitioner. The columns used were not capable of separating and identifying hydrocarbons larger than methane. It is evident that the cans still contained some air in the head-space at 29 in. of vacuum. However, irradiation of the milk fat in cans affected the head-space gas composition. The O_2 was used up suggesting the occurrence of some oxidation reactions, since irradiation of fats in presence of oxygen is known to accelerate the oxidation (Hannan, 1956). Also, CO_2, CO, H_2 and CH_4 were produced.

The possible mechanisms for the formation of H_2 and CH_4 have already been discussed in the literature review. The formation of CO_2 and CO has been reported in irradiated free fatty acids (Sheppard and Burton, 1946; Burton, 1949). In fats these gases may be formed by free radical mechanism. Lück, Deffner and Kohn (1964) have suggested that a triglyceride may form the following free radicals, among others, upon irradiation:

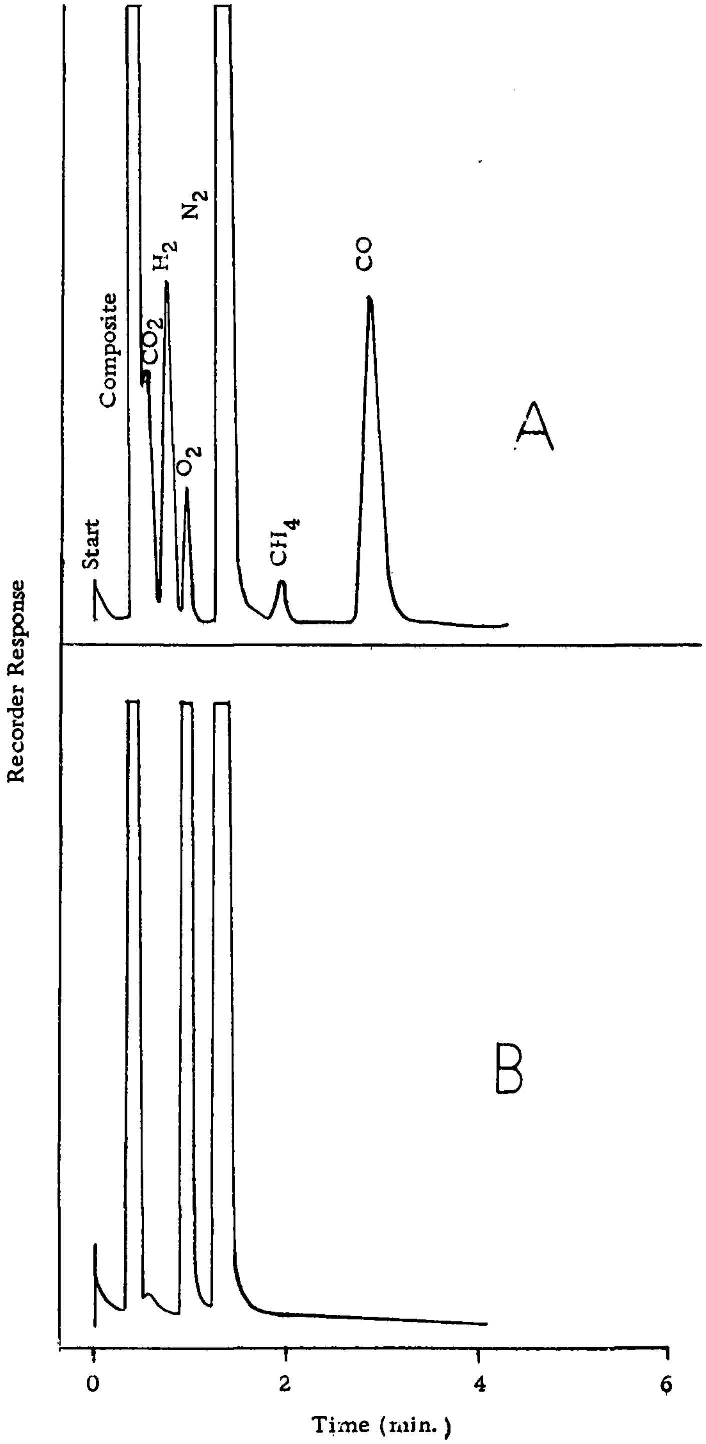
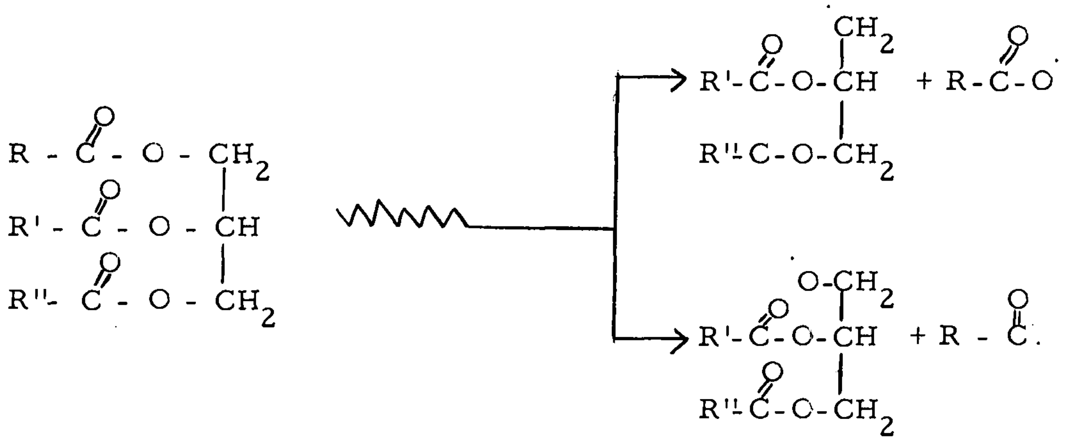
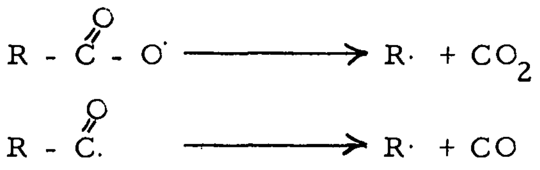


Figure 3. Head-space gases from 4.5 Mrad irradiated milk fat (A) and unirradiated control (B).



These free radicals may further break down and lose CO_2 and CO.



$$R-\overset{O}{\overset{\|}{C}}-O^{\cdot} \longrightarrow R^{\cdot} + CO_2$$

$$R-\overset{O}{\overset{\|}{C}}^{\cdot} \longrightarrow R^{\cdot} + CO$$

However, the head-space of the irradiated milk fat may also contain compounds other than those identified. Irradiation of fatty acids leads to formation of traces of short chain hydrocarbons (Sheppard and Burton, 1946; Burton, 1949). Merritt et al. (1965) reported finding the C_3-C_7 n-alkanes and 1-alkenes in irradiated methyl oleate. Desrosier and Rosenstock (1960) have stated in their book that irradiation of oxygen-free fats produces primarily hydrogen gas, small amounts of methane and still smaller traces of alkanes up to butane. In light of these reports, it can be safely stated that the head-space gases of irradiated milk fat may also contain other short chain hydrocarbons (apart from methane which has been identified in the present studies).

Identification of Volatile Components

GLC Analysis

The concentrate of volatiles from irradiated milk fat was first analyzed on a Barber-Colman Model 20 gas chromatograph with Sr-90 β-ionization detector. Figure 4 shows the gas liquid chromatogram obtained. A 10 ft. x 1/8 in. o. d. SS column packed with 20 percent DEGS on 80-100 mesh Celite 545 was used for the separation. The conditions of GLC were: column temperature, 125° C (isothermal); cell temperature, 220° C; Argon (carrier gas) inlet pressure, 20 psig; scavenger flow, 120 ml/min. ; and voltage, 1200 volts. An "on-column" injection system was used for the sample injection. The tentative identification of some of the components shown in Table 8 was obtained by comparing their relative retention times (t_R/t_R) with those of authentic compounds. Most of the components had aldehyde-like odors and their t_R/t_R agreed quite well with those of the authentic aldehydes. The presence of the acids was confirmed by injecting the ether extract concentrate after shaking with 0. 1 N sodium bicarbonate. The peaks due to acids were missing. Aldehydes and methyl ketones have already been identified in the irradiated milk fat and in its steam distillate.

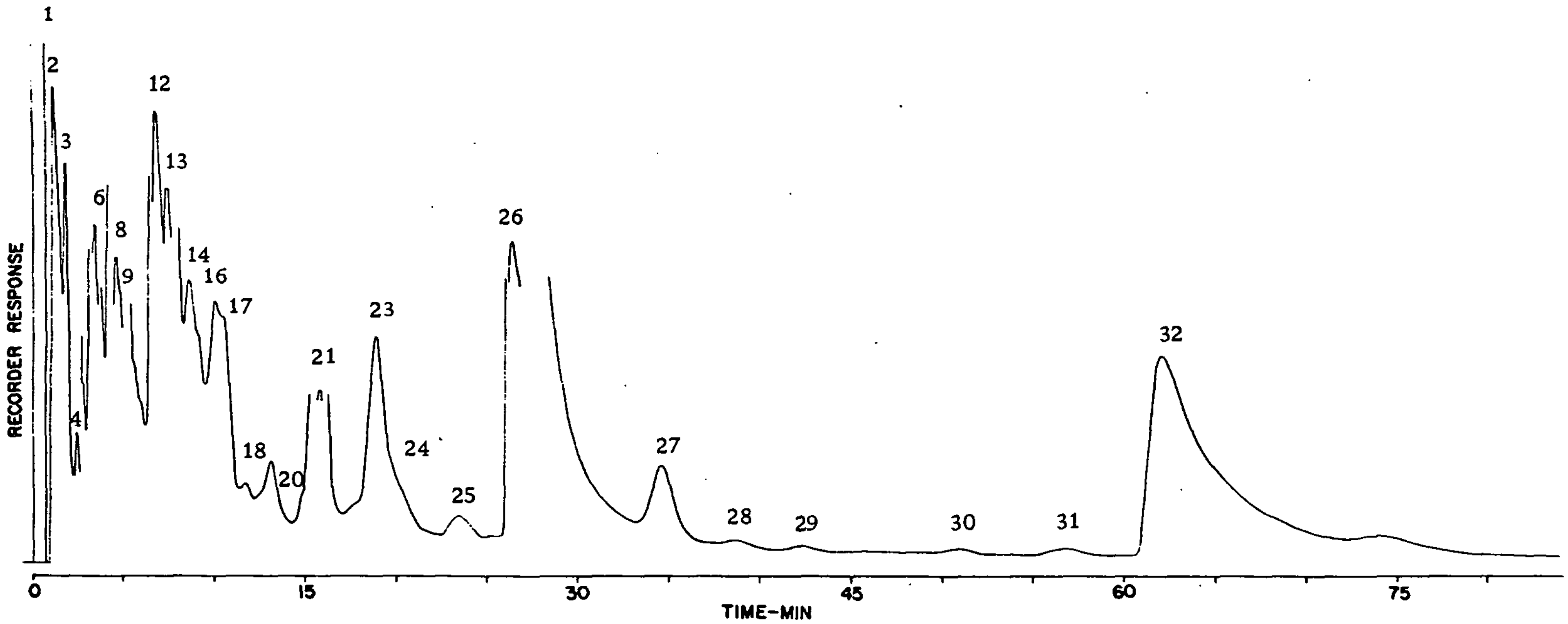


Figure 4. Gas chromatogram of the steam volatiles of 4.5 Mrad milk fat using 10 ft. DEGS column at 125°C. (See Table 8 for peak designation.)

Table 8. Tentative identification and odor characteristics of some GLC peaks shown in Figure 4.

Peak No.	Compound	t_R/t_R [a, b] Irradiated Milk Fat	t_R/t_R [a, b] Authentic	Odor Characteristics
1	Ethyl ether	0.112	0.112	The authentic ether also gave two peaks.
2	Ethyl ether	0.184	0.184	Odor characteristic of ether.
3	Butanal	0.254	0.267	Odor of aldehyde.
4	Pentanal	0.334	0.357	----
5	---	0.387	---	----
6	Hexanal	0.484	0.491	Odor very similar to hexanal.
8	---	0.638	---	"Meaty" odor--also like cooked potato.
9	Heptanal	0.678	0.695	Heptanal-like odor.
13	Octanal	0.998	1.000	Octanal-like odor.
16	---	1.359	---	Aldehyde-like odor.
17	Nonanal	1.461	1.463	Has candle-like characteristics.
20	---	2.020	---	Typical candle-like odor.
21	Decanal	2.120	2.152	Aldehyde-like odor.
24	---	2.654	---	Strong candle-like odor.
25	Undecanal	3.172	3.152	Odor similar to undecanal odor.
26	Butyric Acid	3.701	3.681	Odor same as of C_4 acid.
27	Dodecanal	4.621	4.843	Aldehyde-like odor.
30	Tridecanal	6.945	6.942	Aldehyde-like odor.
32	Hexanoic Acid	8.730	8.731	Odor similar to C_6 acid.

[a] t_R/t_R = Relative retention times with t_R of Octanal = 1.000.

[b] Packed column, 10 ft. x 1/8 in. o. d., 20 percent DEGS on 80-100 mesh Celite 545; column temperature: 125°C; Barber-Colman Model 20.

However, no component coming off the GLC column during the analysis had a ketone odor. Many peaks did have t_R/t_R close to those of methyl ketones, but the odor characteristics were not those of ketones. Apart from the components shown in Figure 4, several other components were observed to be present by GLC analysis at higher temperature. A column temperature of 175° C was used with other conditions the same as those described for the chromatogram in Figure 8. A total of six more peaks were observed, two of which were identified by t_R/t_R, odor, and reaction with sodium bicarbonate to be octanoic and decanoic acids.

It should be noted that the identification of aldehydes was tentative and the flavor concentrate (ether extract of 4.5 Mrad milk fat steam distillate) contained many other components.

To determine the presence of other classes of compounds in the flavor concentrate, spot tests for esters and primary and secondary alcohols were carried out on the flavor concentrate (Feigl, 1960). The results of these tests indicated that no alcohols were present and that some esters may be present.

GLC in Conjunction with Mass Spectrometry

Earlier work on identification of the components present in the flavor concentrate using GLC combined with fast scan mass spectrometry was carried out with DEGS columns. The conditions for the mass spectrometer have already been described along with the type of mass spectrometer and gas chromatograph used.

The gas chromatogram in Figure 5 was obtained on a 24 ft. x 1/8 in. o. d. SS column packed with 20 percent DEGS on 80-100 mesh Celite 545. The conditions for the GLC were: temperature, 63° C for 14 minutes then programmed at 2° per minute to 170° C and held there until the end of the analysis; cell temperature, 260° C; helium flow, 30 ml per minute (inlet pressure, 50 psig); hydrogen, 15 psig; air, 65 psig.

The results of the mass spectral analysis of components shown in Figure 5 are given in Table 9. The programmer in the gas chromatograph was not functioning properly, and the programming could not be duplicated exactly. Hence, the confirmation of t_R/t_R was obtained by co-chromatographic or enrichment techniques.

Compounds with long retention times were analyzed on shorter column using a 10 ft. x 1/8 in. o. d. 20 percent DEGS column at isothermal temperatures of 150° C and 175° C. The other GLC conditions were the same as used for 24 ft. column analysis. The gas chromatograms obtained are depicted in Figures 6 and 7 and the mass spectral identification of the components shown in these figures is given in Tables 10 and 11, respectively.

It may be pointed out that the compounds identified by mass spectrometry and GLC on DEGS columns include saturated and

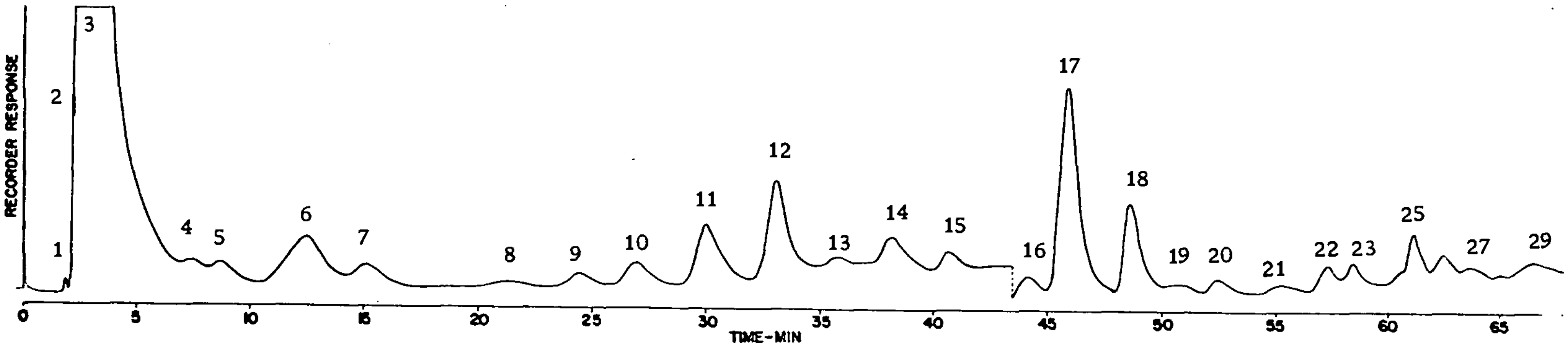


Figure 5. Gas chromatogram of 4. 5 Mrad milk fat steam volatiles obtained on 24 ft. DEGS column using temperature programming. (See Table 9 for peak designation.)

Table 9. GLC and mass spectral identification of the steam volatile components of 4.5 Mrad milk fat (gas chromatogram shown in Figure 5).

Peak No.	Identity	t_R/t_R [a] Confirmed by Co-chromatography	Mass Spectral Identification [b]
1	n-Pentane	No	Positive
2	1-Pentene	No	Positive
2	n-Hexane	Yes	Positive
3	Ethyl ether	Yes	---
4	1-Heptene	---	Tentative
4	1-Octene	---	Tentative
4	n-Nonane	Yes	Positive
5	1-Nonene	---	Tentative
5	n-Octane	---	Tentative
6	n-Decane	Yes	Positive
7	1-Decene	Yes	Positive
7	Ethyl acetate	No	Tentative
7	Benzene	Yes	Positive
8	n-Undecane	Yes	Positive
9	1-Undecene	Yes	Tentative
10	n-Dodecane	Yes	Positive
11	1-Dodecene	Yes	Positive
13	n-Tridecane	Yes	Positive
14	1-Tridecene	Yes	Positive
18	1-Tetradecene	Yes	Positive
19	n-Pentadecane	Yes	Positive
22	1-Pentadecene	---	Tentative
26	1-Hexadecene	---	Tentative
28	1, ?-Hexadecadiene	---	Tentative
28	n-Heptadecane	Yes	Positive
29	1-Heptadecene	Yes	Positive

[a] 24 ft. x 1/8 in. o. d. column packed with 20 percent DEGS on Celite 545; column temperature: 63°C for 14 min., then programmed at 2° per min. to 170°C and held until the end of the analysis; Barber-Colman Series-5000.

[b] Reference for mass spectrometry: API (1948 to date), ASTM (1963).

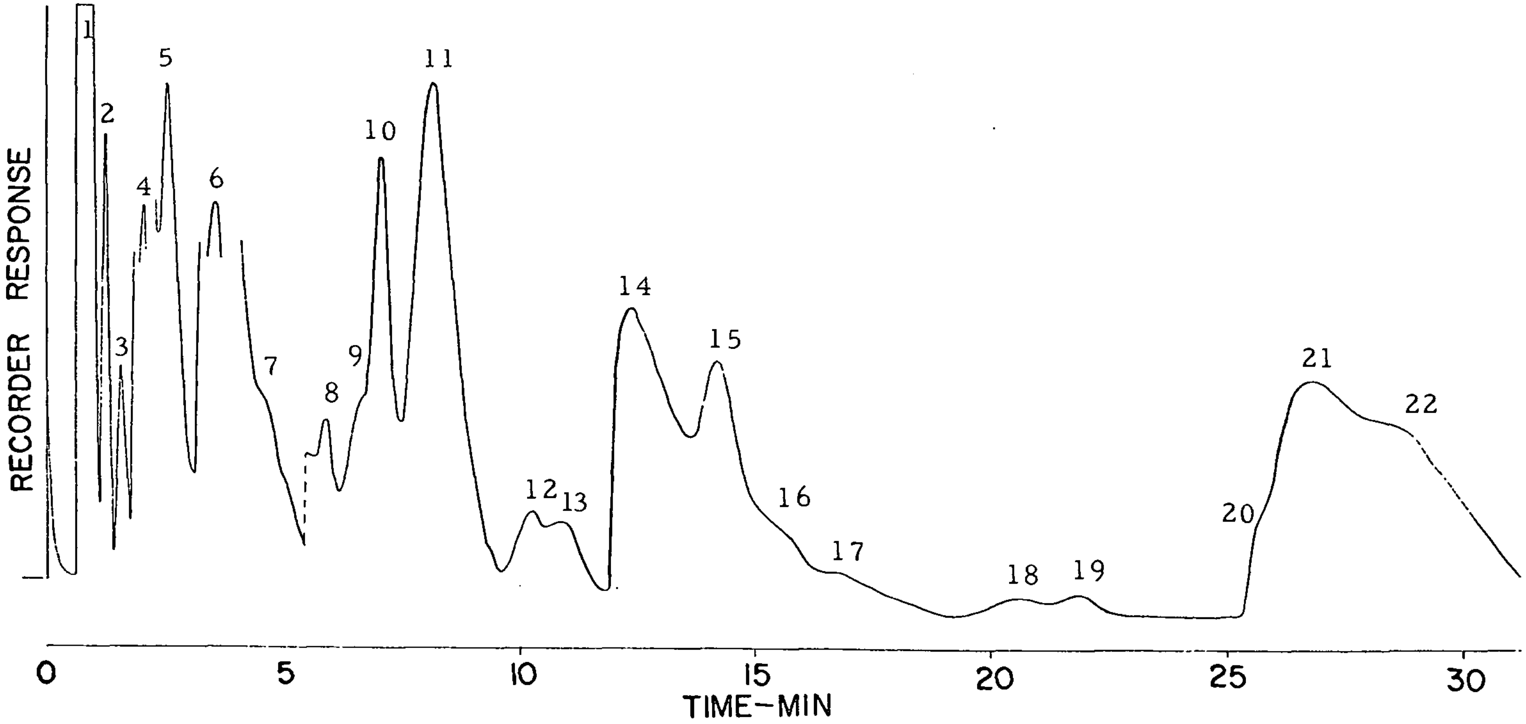


Figure 6. Gas chromatogram of the steam volatiles of 4.5 Mrad milk fat using a 10 ft. DEGS column at 150° C. (See Table 10 for peak designation.)

Table 10. GLC and mass spectral identification of some high boiling components of 4.5 Mrad milk fat volatiles using 10 ft. DEGS column at 150^{o}C (gas chromatogram shown in Figure 6).

Peak No.	Identity	t_R/t_R [a] Confirmed by Co-chromatography	Mass Spectral Identification [b]
6	n-Pentadecane	No	Tentative
9	1-Hexadecene	Yes	Positive
11	1, ?-Hexadecadiene	No	Tentative
11	n-Heptadecane	Yes	Positive
12	1-Heptadecene	Yes	Positive
13	1, ?-Heptadecadiene	No	Tentative
14	Butyric Acid	Yes	Positive
21	Hexanoic Acid	Yes	Positive

[a] 10 ft. x 1/8 in. o. d. column packed with 20 percent DEGS on Celite 545; column temperature: 150°C; Barber-Colman Series-5000.

[b] Reference for mass spectrometry: API (1948 to date), ASTM (1963).

unsaturated hydrocarbons, fatty acids and lactones. However, no carbonyl compounds could be identified. Even the components which had aldehyde-like odor and retention times were identified as hydrocarbons. This suggested that the peaks representing these components were probably due to more than one component with t_R/t_R either the same or very close to each other; the major components in each peak were identified by mass spectrometry. This was further confirmed by the fact that a very poor separation of an authentic mixture of aldehydes and hydrocarbons was obtained by GLC under similar conditions. About that time, Merritt et al. (1965) reported the finding of hydrocarbons in irradiated beef and methyl oleate. This supported the results obtained in the present work that hydrocarbons are also present in the irradiated milk fat. The identification of hexadecadiene and heptadecadiene is positive. However, the position of the double bonds could not be ascertained by analysis of fast scan mass spectra.

The free fatty acids are known to be present in milk fat. Increases in their concentration upon irradiation has already been demonstrated in the present work. However, the finding that δ- and γ-lactones are present in irradiated milk fat is quite interesting. The presence of γ- and δ-lactones in heated milk fat has been reported by several workers (Keeney and Patton, 1956; Tharp and Patton, 1960; Boldingh and Taylor, 1962; Jurriens and Oele, 1965;

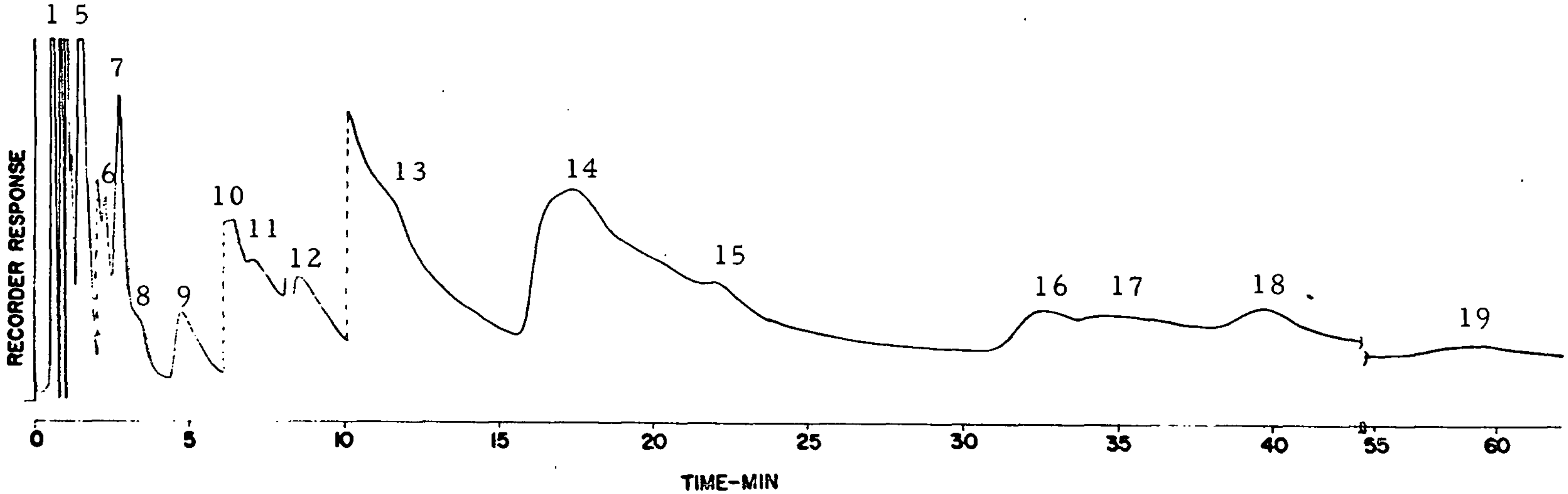


Figure 7. Gas chromatogram of the steam volatiles of 4.5 Mrad milk fat using a 10 ft. DEGS column at 175^{o} C. (See Table 11 for peak designation.)

Table 11. GLC and mass spectral identification of some high boiling components of 4.5 Mrad milk fat volatiles using a 10 ft. DEGS column at 175°C (gas chromatogram shown in Figure 7).

Peak No.	Identity	t_R/t_R [a] Irradiated Milk Fat	t_R/t_R Authentic	Mass Spectral Identification[b]	Reference
9	Butyric Acid	2.857	2.732	Positive	c
12	Hexanoic Acid	5.190	5.268	Positive	c
13	γ-Hexalactone	6.857	6.615	---	---
14	Octanoic Acid	10.286	9.756	Positive	c
15	δ-Octalactone	12.952	13.286	---	---
16	δ-Decalactone	19.762	19.637	Positive	d
17	Decanoic Acid	21.095	21.073	---	---
18	δ-Decalactone	24.048	24.000	Positive	d
19	δ-Undecalactone[b]	35.857	32.381	---	---

[a] t_R/t_R = Relative retention time with t_R of C_8 aldehyde = 1.000
10 ft. x 1/8 in. o.d. column packed with 20 percent DEGS on Celite 545; column temperature: 175°C; Barber-Colman Series-5000.

[b] This component had lactone odor. However, the identification is only tentative.

[c] ASTM (1963).

[d] McFadden, Day and Diamond (1965).

Parliment, Nawar and Fagerson, 1965). According to these workers, the lactones are formed from γ - or δ-hydroxy fatty acids which, in turn, are produced by heat-induced hydrolysis of triglycerides. It has already been shown that irradiation induces the hydrolysis of milk fat. This could explain the origin of lactones in the fat. Later, it will be shown that the lactones are also present in unirradiated control milk fat. However, the estimated amounts of these lactones seemed to be greater in irradiated milk fat than in the control. The presence of the lactones in control milk fat cannot be explained, since the milk fat was obtained from raw sweet cream and milk fat was not heated above 45° C during the separation process. The history of the cream before separation was not known. It is possible that during some stage before separation, some hydrolysis took place by milk lipase or by bacterial lipase. Since the free fatty acid analysis was not carried out on this fat, it could not be confirmed whether there were more free fatty acids present.

Many packed columns with different stationary phases were tried in an attempt to get better separation between aldehydes and hydrocarbon peaks. The Apiezon M column seemed to give satisfactory results. This column was used in conjunction with mass spectrometry for final analysis of the irradiated milk fat flavor concentrate. A 12 ft. x 1/8 in. o. d. SS column packed with 20 percent Apiezon M on 100-120 mesh Celite 545 was used. For high boiling components the separation was carried out on a 6 ft. Apiezon M column.

Figures 6 and 7 show that the acid peaks are very large and they mask many other small component peaks by tailing which made their identification extremely difficult. A base treated pre-column was developed to remove these large acid peaks. This column has already been described. The pre-column was used for most of the GLC and mass spectral analyses with Apiezon M columns. However, later it appeared that the base pre-column had some detrimental effect on lactones. When lactones were analyzed using this column, they gave GLC peaks with no change in t_R/t_R and flavor characteristics. However, the mass spectra were not of the typical lactones as obtained by McFadden, Day and Diamond (1965). No indication as to what change might be taking place, could be obtained. Because of this anomaly, the base pre-column was omitted in the analysis of high boiling components.

The gas chromatogram of the 4.5 Mrad fat steam distillate flavor concentrate obtained with the two ft. base pre-column and 12 ft. Apiezon M main column is depicted in Figure 8. The conditions for the GLC were: temperature, 75° C for six minutes, then programmed at 2° per minute to 200° C and held until the analysis was over; cell temperature, 200° C, helium flow rate, 30 ml per minute; hydrogen, 15 psig; and air, 65 psig.

Table 12 shows the results of GLC and mass spectral analysis

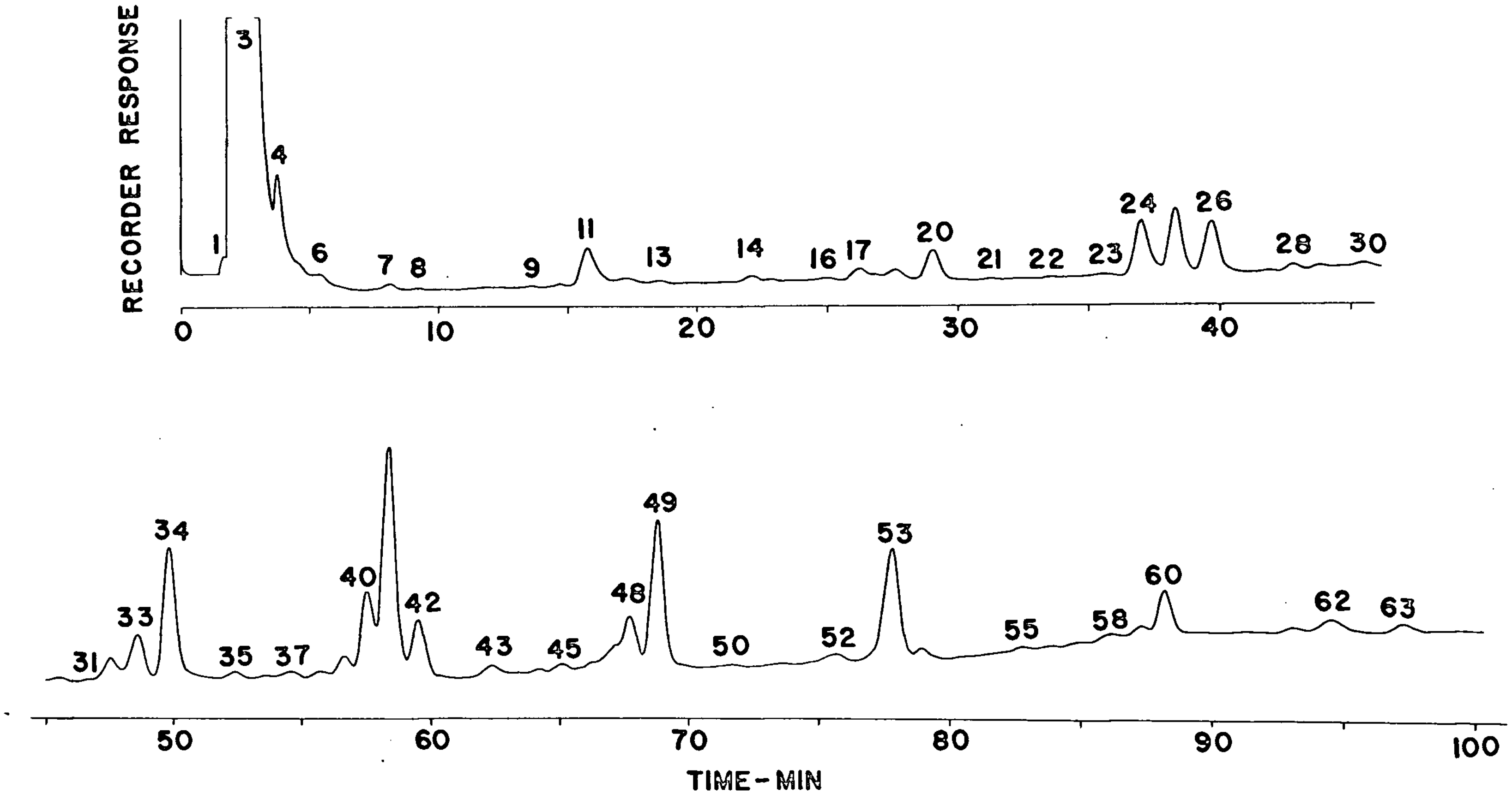


Figure 8. Gas chromatogram of 4.5 Mrad milk fat steam volatiles using a 2 ft. base precolumn + a 12 ft. Apiezon M column with temperature programming. (Peak designation given in Table 12.)

Table 12. GLC and mass spectral identification of the steam volatile components of gamma irradiated milk fat using a 2 ft. base pre-column plus 12 ft. Apiezon M column with temperature programming (gas chromatogram shown in Figure 8).

GLC Peak No.	Identity	t_R/t_R[a, b] Irradiated Fat	t_R/t_R Authentic	Confirmed by DEGS Column GLC[c]	Mass Spectral Identification	Reference	Odor Characteristics
1	1-Pentene	0.042	---	Yes	Positive	e	----
1	n-Pentane	0.042	---	Yes	Positive	e	----
3	Diethyl ether (solvent)	0.050	0.050	Yes	Positive	f	Ethyl ether odor
4	Ethyl acetate	0.093	0.112	Yes	Positive	g, h	----
6	Chloroform	0.135	0.152	---	Positive	e, f	----
--	Benzene	---	0.230	Yes	Positive	f	----
--	n-Pentanal	---	0.217	Yes	Positive	f, j	----
7	1-Heptene	0.204	0.248	Yes	Positive	e	Metallic-type odor
8	n-Heptane	0.232	0.272	Yes	Positive	e	----
10	2-Hexanone	0.369	0.416	---	Tentative	f	----
11	n-Hexanal	0.397	0.411	Yes	Positive	f, j	Slightly grassy odor
12	1-Octene	0.435	0.451	Yes	Positive	e	Cooked potato-like odor
13	n-Octane	0.468	0.484	Yes	Positive	e	----
14	4-Heptanone	0.556	0.589	---	Tentative	f	----
16	2-Heptanone	0.619	0.652	---	Positive	f	----
17	n-Heptanal	0.659	0.661	Yes	Positive	f, j	Aldehyde-like odor
18	Methyl hexanoate	0.675	0.692	---	Tentative	f	----
19	1-Nonene	0.694	0.703	Yes	Positive	e	----
20	n-Nonane	0.730	0.738	Yes	Positive	e	----
23	2-Methyl nonane	0.889	----	---	Tentative	e	----
24	n-Octanal	0.931	0.931	Yes	Positive	f, j	Aldehyde-like odor
24	1,?-Decadiene	0.931	---	---	Tentative	---	----
25	1-Decene	0.964	0.967	Yes	Positive	e	----
26	n-Decane	1.000	1.000	Yes	Positive	e	----
30	Dichlorobenzene	1.145	---	---	Positive	f	----
31	2-Methyl decane	1.175	---	---	Tentative	e	Candle-like odor
32	n-Nonanal	1.194	1.197	Yes	Positive	j	Aldehyde-like odor

Table 12 (Continued)

GLC Peak No.	Identity	t_R/t_R [a,b] Irradiated Fat	t_R/t_R Authentic	Confirmed by DEGS Column GLC [c]	Mass Spectral Identification	Reference	Odor Characteristics
32	1, ?-Undecadiene	1.194	---	---	Tentative	---	----
33	1-Undecene	1.224	1.224	Yes	Positive	e	----
34	n-Undecane	1.256	1.256	Yes	Positive	e	----
35	Branched Undecene	1.319	---	---	Tentative	e	Candle-like odor
37	Branched Undecene	1.373	---	---	Tentative	e	Candle-like odor
39	2-Methyl Undecane	1.427	---	---	Tentative	e	Candle-like odor
40	n-Decanal	1.450	1.455	Yes	Positive	j	Aldehyde-like odor
40	1, ?-Dodecadiene	1.450	---	---	Tentative	---	----
41	1-Dodecene	1.472	1.478	Yes	Positive	e	----
42	n-Dodecane	1.500	1.506	Yes	Positive	e	----
43	γ-Octalactone [d]	1.569	1.615	---	---	---	Lactone-like odor
46	δ-Octalactone [d]	1.667	1.732	---	---	---	Lactone-like odor
47	n-Undecanal	1.690	1.701	Yes	Positive	j	Aldehyde-like odor
48	1-Tridecene	1.704	1.711	Yes	Positive	e	----
49	n-Tridecane	1.732	1.740	Yes	Positive	e	----
53	n-Dodecanal	1.958	1.931	Yes	Tentative	j	Very slightly aldehyde-like odor
53	1-Tetradecene	1.958	1.931	Yes	Positive	e	----
54	n-Tetradecane	1.988	---	Yes	Positive	e	----
59	1-Pentadecene	2.198	2.148	Yes	Positive	e	----
60	n-Pentadecane	2.222	2.173	Yes	Positive	e	----

[a] t_R/t_R = Relative retention time calculated on the basis t_R/t_R of n-Decane = 1.000.

[b] (1) 2 ft. x 1/8 in. o.d. stainless steel pre-column packed with 20 percent Apiezon M on 100-120 mesh Celite 545 coated with 5 percent by weight of NaOH. (2) 12 ft. x 1/8 in. o.d. stainless steel main column packed with 20 percent Apiezon M on 100-120 mesh Celite 545. Barber-Colman Series-5000.

[c] 24 ft. x 1/8 in. o.d. stainless steel column packed with 20 percent LAC-3R-728 (DEGS) on 80-100 mesh Celite 545.

[d] Only tentative identification on the basis of t_R/t_R and odor characteristics of peaks.

[e] API tables (1948 to date).

[f] ASTM tables (1963).

[g] Sharkey, Shultz and Friedel (1959).

[h] Beynon, Saunders and Williams (1961).

[j] Gilpin and McLafferty (1957).

of the components shown in Figure 8. The final temperature of the GLC column, cell temperature and mass spectral EC-1 inlet valve temperature were kept below 205° C during the analysis to prevent thermal decomposition of aldehydes. Also, some of the minor components did not give strong enough mass spectra for proper interpretation. Hence, these components were concentrated by trapping on a 2 ft. x 1/8 in. o. d. packed Apiezon M column and were rechromatographed and analyzed by the mass spectrometer. The details of the method are given in the Experimental Section.

The high boiling components were analyzed using the six ft. Apiezon M column at a column temperature of 220° C. No base treated pre-column was used. The GLC conditions were: cell temperature, 250° C; helium inlet pressure, 45 psig; hydrogen, 14. 5 psig; and air, 70 psig. The gas chromatogram obtained under these conditions is shown in Figure 9, and the identification of the components, in Table 13.

The base pre-column was not used in the analysis in an effort to obtain positive mass spectral identification of lactones. The lactones could be smelled in the effluent gas of the gas chromatograph during the analysis. However, as evident from Table 13, the lactones could not be identified. When authentic γ - and δ-lactones were analyzed using the Apiezon M column, only γ -decalactone gave mass spectra comparable to those of McFadden, Day and Diamond

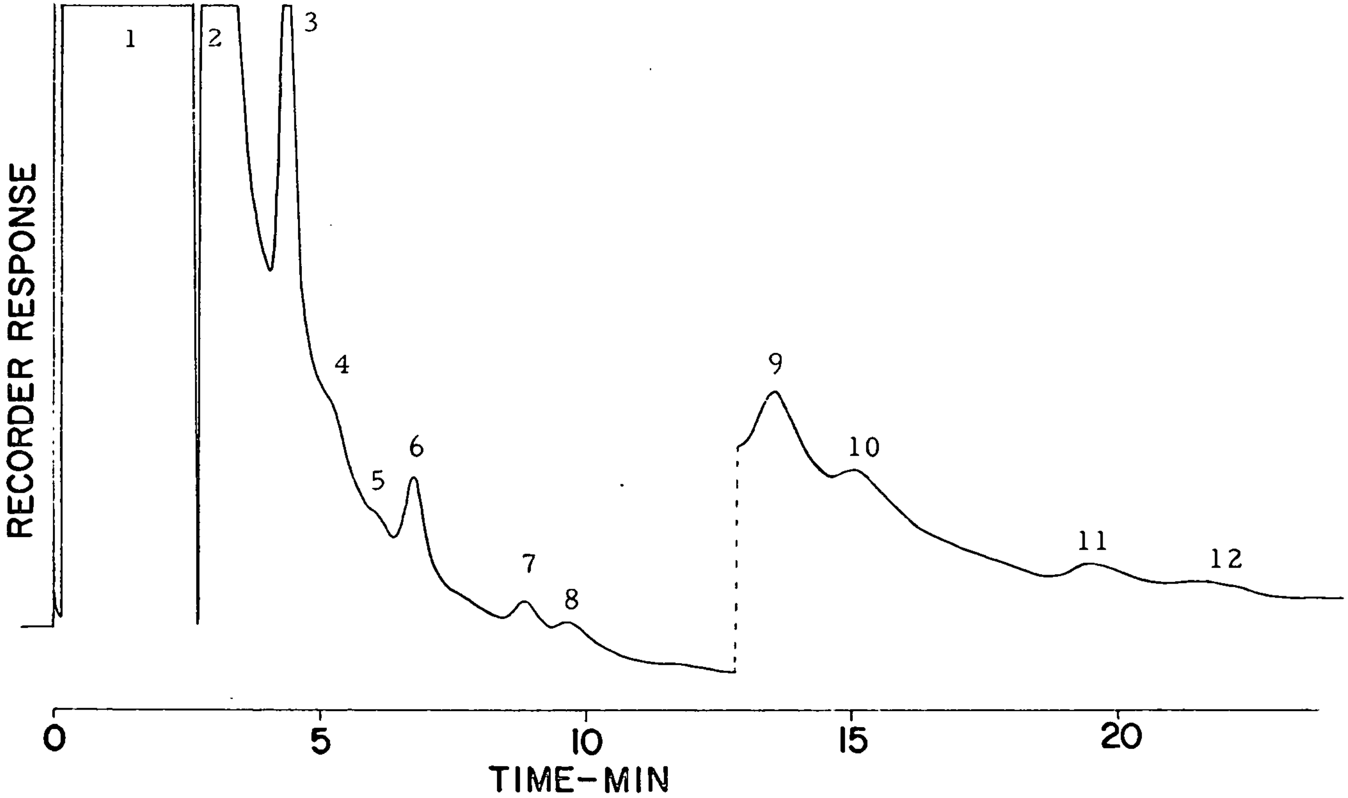


Figure 9. Gas chromatogram of high boiling components of 4.5 Mrad milk fat steam volatiles using a 6 ft. Apiezon M column at 220°C. (Peak designation given in Table 13.)

Table 13. GLC and mass spectral identification of high boiling components of 4.5 Mrad milk fat steam volatiles using a 6 ft. Apiezon M column (gas chromatogram depicted in Figure 9).

Peak No.	Identity	t_R/t_R [a]	t_R/t_R Authentic	Confirmed by DEGS Column GLC	Mass Spectral Identification [c]
3	1-Tetradecene	0.651	0.653	Yes	Tentative
3	n-Tetradecane	0.651	---	Yes	Tentative
4	δ-Decalactone	0.767	0.801	Yes	---[b]
5	δ-Decalactone	0.907	0.914	Yes	---[b]
6	1-Pentadecene	1.000	1.000	Yes	Positive
6	n-Pentadecane	1.000	1.000	Yes	Positive
7	1, ?-Hexadecadiene	1.314	---	No	Positive
8	1-Hexadecene	1.442	1.491	Yes	Positive
8	n-Hexadecane	1.442	1.491	Yes	Positive
9	δ-Dodecalactone	1.907	2.059	No	---[b]
9	1, ?-Heptadecadiene	1.907	---	No	Positive
10	1-Heptadecene	2.232	2.270	Yes	Positive
10	n-Heptadecane	2.232	2.270	Yes	Positive

[a] t_R/t_R = Relative retention time with t_R/t_R of n-Pentadecane = 1.000
Column: 6 ft. x 1/8 in. o. d., 20 percent Apiezon M on 100-120 mesh Celite 545; column temperature: 220°C; Barber-Colman Series-5000.

[b] Odor of lactone could be distinguished, and identification only tentative; however, positively identified on DEGS column (see Table 11).

[c] Reference to mass spectrometry: API tables (1948 to date).

(1965). No spectra could be obtained for other lactones. The only possible explanation for this is that lactones were condensing somewhere in the system, probably at the EC-1 inlet valve which is cooled at the point of the capillary leak by circulating water. The identity of the lactones, except γ- and δ-decalactones, therefore, is only tentative based on GLC evidence and odor characteristics.

The identification of the series of alkanes and alkenes further confirms the previous findings with DEGS columns. Some alk-dienes also were identified, but the position of the second double bond could not be positively determined from the mass spectra.

No hydrocarbons were present in the unirradiated control. The control milk fat was steam distilled and analyzed in a similar manner as irradiated milk fat. The GLC and mass spectral analysis was carried out using the Apiezon M columns. Apart from the free fatty acids, the other compounds identified were:

1. Ethyl ether (used as solvent)
2. Ethyl acetate
3. Chloroform
4. Benzene
5. Toluene
6. 2-Heptanone
7. Ethyl benzene
8. Dichlorobenzene
9. δ-Octalactone
10. δ-Decalactone
11. δ-Dodecalactone

No γ-lactones could be found in the control milk fat. The identification of all the compounds, except δ-dodecalactone, is positive based on GLC and mass spectral evidence. The δ-dodecalactone could not be identified by mass spectra; however, sufficient t_R/t_R and odor evidence was obtained for tentative identification.

A series of n-alkanals with chain lengths C_5 through C_{12} and some ketones were identified by GLC and mass spectrometry (Table 12). The column and paper chromatography of monocarbonyl DNP-hydrazones from the irradiated milk fat volatiles, however, revealed the presence of many other n-alkanals, methyl ketones, and some alk-2-enals (Table 4). The inability to identify these compounds by GLC and mass spectrometry could be attributed to the incomplete separation from other compounds with similar retention times. For example, considerable evidence was obtained for the presence of branched chain hydrocarbons, but their exact structure could not be positively ellucidated from their mass spectra.

The compounds like ethyl acetate, benzene, chloroform, and dichlorobenzene were also detected in the control milk fat. The chloroform has been reported in milk by Wong and Patton (1962). It was suggested to have come from pesticide residues in milk. Dichlorobenzene has not been reported previously in milk fat or milk, but it may also have its origin in pesticides. The presence of ethyl acetate has been reported in milk (Lindsay, Day and

Sandine, 1965). The origin of benzene and other aromatic compounds found in control milk fat could not be visualized. These compounds may have come from the feed.

Four of the components listed in Table 12 had typical candle-like odor. These components were present in relatively minor quantities (see Figure 8). Attempts to identify these components were unsuccessful. The components were concentrated by trapping technique and analyzed by mass spectrometry. The exact nature of these components could not be determined. In an attempt to further characterize these components, the ether extract concentrate was mixed with a larger quantity of carbonyl free isopentane and passed through a 2. 5 g reaction column (1. 0 cm i. d.) of Schwartz, Haller and Keeney (1963) to remove monocarbonyls. The effluent from the column was evaporated under nitrogen to about two to three drops and made to 1/2 ml with ethyl ether. This was then gas chromatographed. In the resulting gas chromatogram, many peaks were missing indicating the presence of carbonyls. No carbonyl odor could be detected upon sniffing the effluent. From the candle-like smelling components, one (peak no. 39, Figure 8) was completely removed including its odor. In other cases the odor as well as peak sizes were not significantly affected, suggesting that one of these four candle-like components may be a carbonyl. However, attempts to elucidate its structure were not successful.

Analysis of the Mass Spectra

The positive identification of the compounds was based on the coincidence of the gas chromatographic retention times and mass spectral analysis. The interpretation of mass spectra was achieved by comparing the spectra of the unknowns with those given in the literature and assignment of fragment ions to the m/e peaks observed.

As an example, Figure 10 illustrates the mass spectra of peaks 11 through 13 shown in Figure 8. Mass spectrum (A) represents peak 11. The ion peaks at m/e 28, 29, 32, 40 and 44 are mainly due to background (see Figure 11). These ion peaks were due to traces of air present in the mass spectrometer. Spectrum (A) has its base peak at m/e 43 and its parent ion at m/e 100. There are many possible compounds which have a base peak at m/e 43, but only a few of these have a molecular weight of 100. A hydrocarbon may be excluded because there is a peak at P-18 and the spectrum does not show successive loss of m/e 14 (CH_2^+). The m/e 82 (P-18), 72 (P-28) and 56 (P-44) indicate that this compound may be an aldehyde (Silverstein and Bassler, 1963). An aldehyde with a molecular weight of 100 could only be hexanal. According to Gilpin and McLafferty (1957), hexanal and most of the shorter chain aldehydes have base peak at m/e 44. In Figure 10, the m/e 44 peak is already so big due to background, (N_2 and CO) that it is extremely difficult

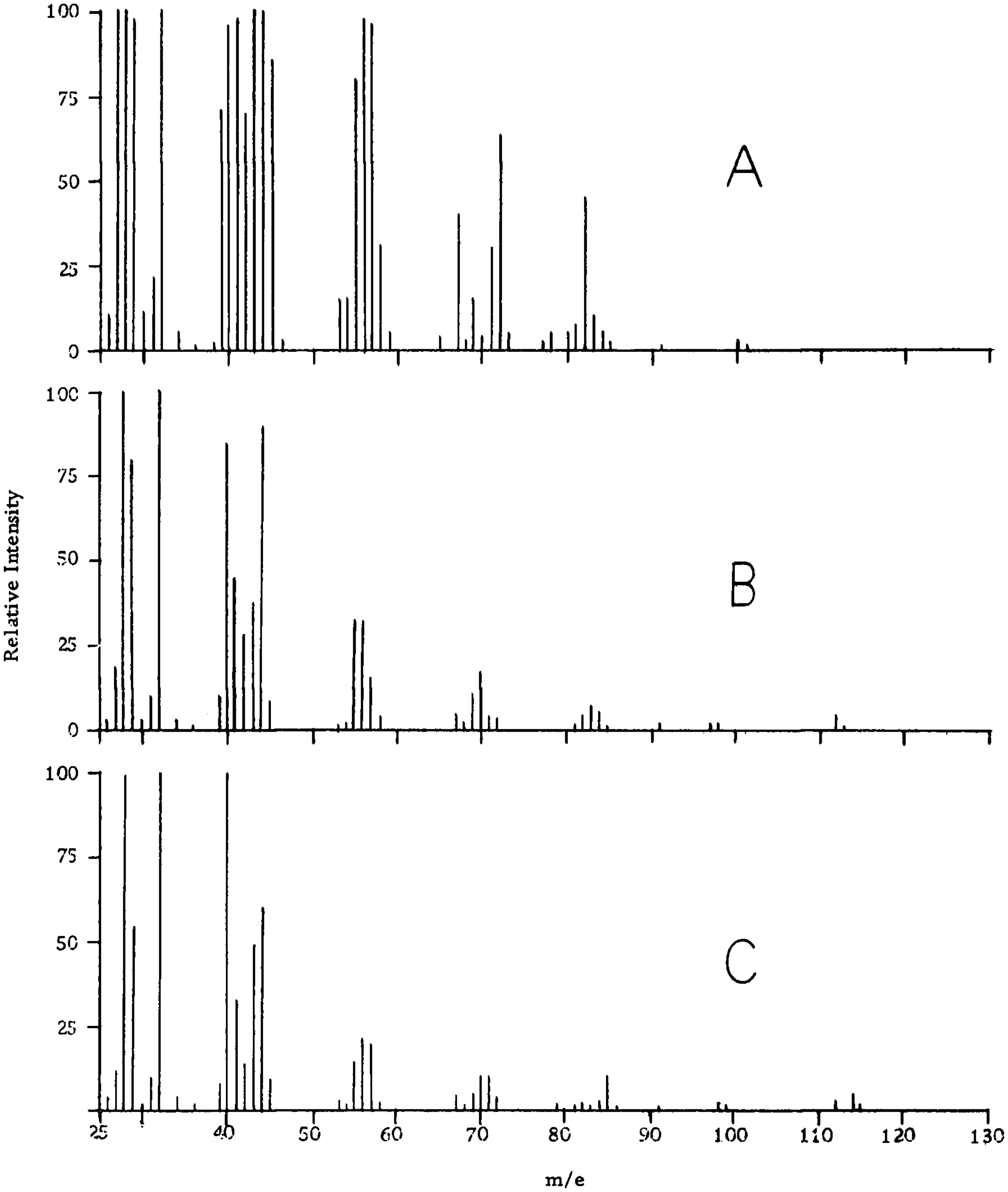


Figure 10. Mass spectral charts for chromatographic peaks shown in Figure 8: Spectra A, B, and C correspond to peaks 11, 12, and 13 respectively.

to determine its increase. However, other characteristics give it away; for example, next largest peak is at m/e 43 ($^{+}CH_2CHO$); the peak at m/e 82 is due to P-H_2O; that at m/e 72 is for P-CO, and finally the peak for P-$C^{+}H_2CHOH$ (P-44) is at m/e 56. Further confirmation that this spectrum represents hexanal was obtained by comparing the spectrum with that in the ASTM Tables (1963) and the one given by Gilpin and McLafferty (1957). This mass spectral identification along with t_R/t_R evidence and the odor characteristics (Table 12) confirmed the identity of this peak.

Mass spectrum (B) has its base peak at m/e 41 and its parent at 112. It also has a fragment ion at P-29 (m/e 83) and very little fragmentation at P-15. These characteristics, and the spectrum showing a successive loss of m/e 14 ($-CH_2^{+}$) indicate that this component is an unsaturated hydrocarbon. By comparing the mass spectrum (B) with spectra in the API tables (1948 to date) it was found to represent 1- octene. This mass spectral identification together with GLC data are sufficient evidence for confirming the identification.

Peak 13 (Figure 8) gave mass spectrum (C) (Figure 10). It has a molecular weight of 114 (Parent peak at m/e 114), and its base peak is at m/e 43. Otherwise, it has characteristics quite similar to those of mass spectrum (B), that is, it shows initial loss of m/e 29 ($C_2H_5^{+}$) and then successive loss of CH_2^{+} (m/e 14) fragments.

These data suggest that this spectrum represents a saturated hydrocarbon with molecular weight of 114. It was found to be n-Octane, when the spectrum (C) was compared with that of known n-octane in the API tables (1948 to date). Again, the final confirmation was achieved by GLC t_R/t_R evidence (Table 12). Even further confirmation of the identification of aldehydes, 1-alkenes, and n-alkanes was obtained by comparing their mass spectra with those obtained with authentic compounds in this laboratory.

Another illustration of the mass spectral analyses obtained from minor components separated by GLC (Figure 8), is shown in Figure 11. Spectrum (C) represents background; and spectra (A) and (B) correspond to peaks 4 and 6 respectively, in Figure 8. Spectrum (A) in Figure 11 has its base peak at m/e 43 which does not give very much information about the compound. However, peaks m/e 70 (P-18), m/e 73 (P-15), and especially the peak at m/e 61 strongly indicate it to be an ethyl ester. The parent peak m/e 88 suggests it to be ethyl acetate. Peak 61 $(CH_3\text{-}C\genfrac{}{}{0pt}{}{\overset{+}{=}OH}{\smallsetminus OH})$ is a rearrangement ion peak typical of acetates. Also the peak at m/e 73, which is due to the fragment P-R (P-CH_3), indicates that compound (A), is ethyl acetate. The base peak at m/e 43 represents the ion $(CH_3CO)^{+}$. For further confirmation, the spectrum of (A) was compared with the spectra published by Sharkey, Shultz and Friedel (1959), and Beynon, Saunders and Williams (1961). Finally, GLC t_R/t_R data

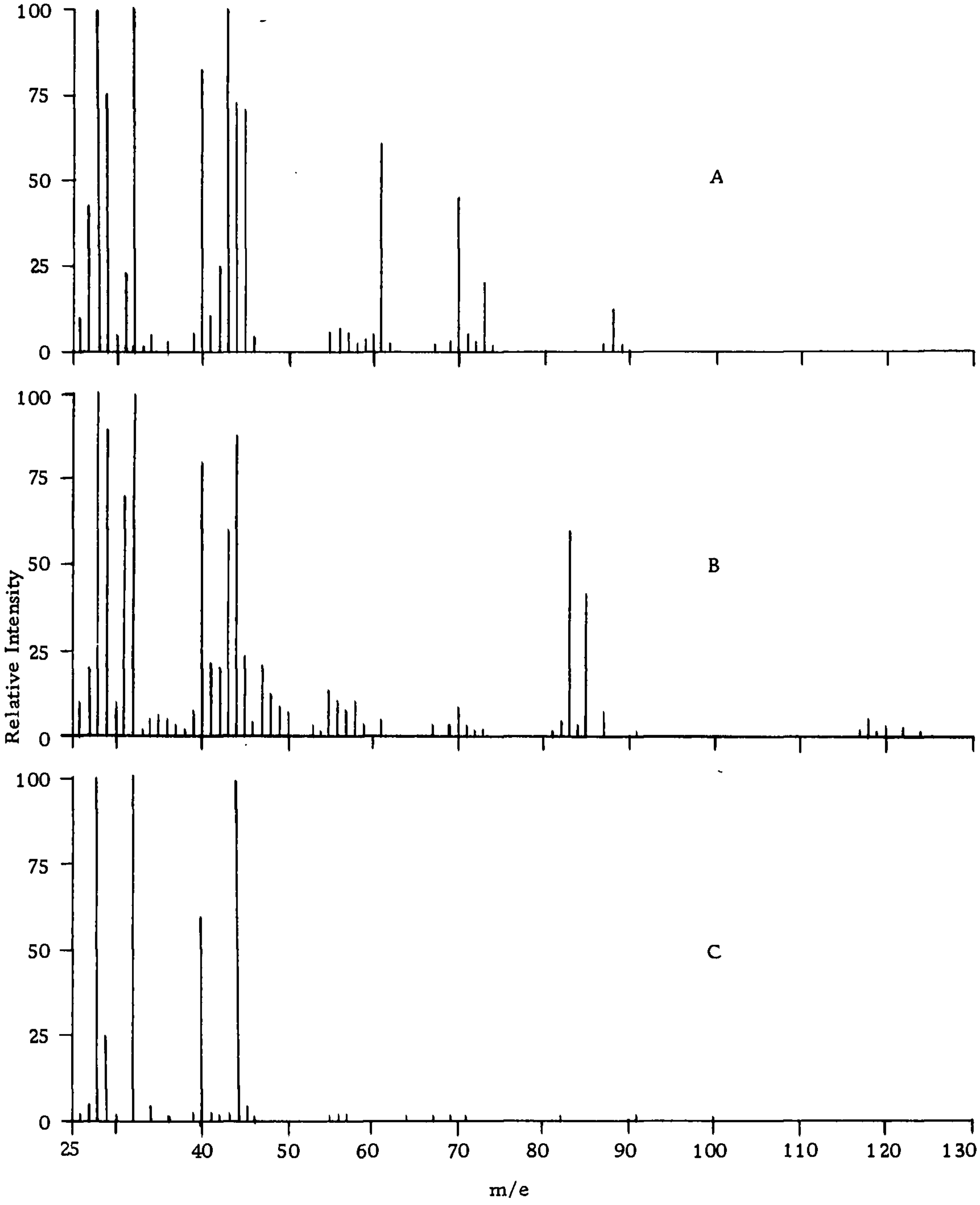


Figure 11. Mass spectral charts for chromatographic peaks shown in Figure 8: Spectra A and B correspond to peaks 4 and 6, spectrum C is background.

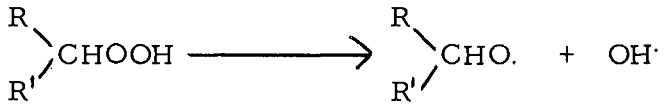
gave confirming identification.

Spectrum (B) in Figure 11 presents a very interesting situation. Ordinarily, no nitrogen, halogen or sulfur compound would be expected in milk fat, but one look at this spectrum suggests the presence of chlorine in this compound. The cluster of ion peaks in the parent region with peaks at P, P+2, P+4, and P+6 suggest a compound with three chlorine atoms. (Silverstein and Bassler, 1963). This is further supported by the ion peaks showing at m/e 35, m/e 37 and at P-35 (m/e 83). Since the parent is at m/e 118, the compound could be none other than chloroform. The verification of this identification was obtained by the spectra given for chloroform in the ASTM (1963) and API tables (1948 to date). Once again, the coincidence of gas chromatographic retention times confirms the compound to be chloroform.

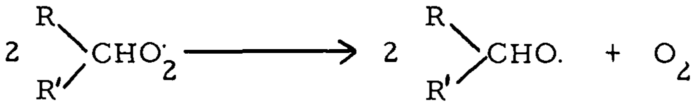
Possible Mechanisms for the Production of Some of the Compounds Identified

Various theoretical schemes which have been described in the literature have already been mentioned in the review of literature. The possible mechanisms involved in the production of free fatty acids, CO, CO_2 and methane have also been presented during the course of this discussion.

Apart from these compounds, the major components that are produced in milk fat upon irradiation consist of carbonyls and saturated and unsaturated hydrocarbons. Besides the reaction mechanisms presented earlier for the production of aldehydes and ketones, Mead (1961) has suggested the formation of these as secondary products in irradiation-induced autoxidation:



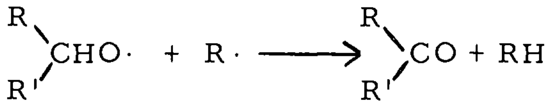
or



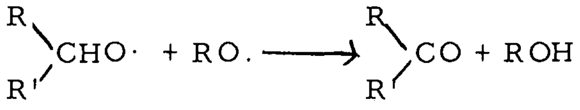
These may further react as follows:

(1) Formation of aldehydes

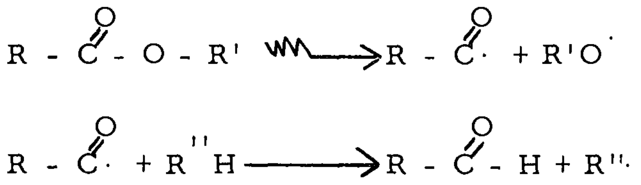
(2) Formation of ketones



or



When irradiation is carried out under vacuum, however, the carbonyl formation may take a different route:

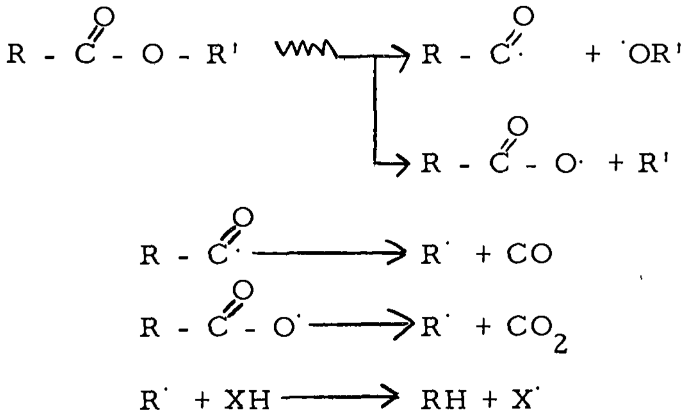


$$R-\overset{O}{\overset{/\!/}{C}}-O-R' \rightsquigarrow R-\overset{O}{\overset{/\!/}{C}}\cdot + R'O^{\cdot}$$

$$R-\overset{O}{\overset{/\!/}{C}}\cdot + R''H \longrightarrow R-\overset{O}{\overset{/\!/}{C}}-H + R''\cdot$$

This may explain the observation that no aldehydes with carbon chain less than four were observed in milk fat irradiated in evacuated glass vials, where almost complete vacuum was obtained. Since milk fat does not contain fatty acid smaller than butyric acid, the shortest aldehyde observed would be butanal.

Irradiation of milk fat under vacuum results in production of hydrocarbons as major components of the volatiles. These hydrocarbons could be formed by various pathways. One of the possible mechanisms may be similar to hydrolysis or aldehyde production mentioned above, namely:



$$R-\overset{O}{\overset{/\!/}{C}}-O-R' \rightsquigarrow R-\overset{O}{\overset{/\!/}{C}}\cdot + {}^{\cdot}OR'$$

$$\phantom{R-\overset{O}{\overset{/\!/}{C}}-O-R'} \rightsquigarrow R-\overset{O}{\overset{/\!/}{C}}-O\cdot + R'$$

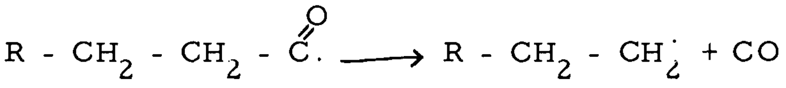
$$R-\overset{O}{\overset{/\!/}{C}}\cdot \longrightarrow R^{\cdot} + CO$$

and

$$R-\overset{O}{\overset{/\!/}{C}}-O^{\cdot} \longrightarrow R^{\cdot} + CO_2$$

$$R^{\cdot} + XH \longrightarrow RH + X^{\cdot}$$

1-Alkenes may be formed as follows:



$$R - CH_2 - CH_2 - \overset{O}{\overset{/\!/}{C}}. \longrightarrow R - CH_2 - CH_2^{\cdot} + CO$$

$$R - CH_2 - CH_2^{\cdot} \longrightarrow R - CH = CH_2 + H^{\cdot}$$

Hydrocarbons may also result from the chain scission.

Milk fat contains substantial amounts of unsaturated fatty acids. These are potential targets of irradiation effects. Like autoxidation, the first step of effect of irradiation on these acids will be hydrogen abstraction. However, where there is no air or oxygen present, these free radicals will not be able to form peroxides. Due to the high energy of irradiation, these free radicals may easily break down to form at least one alkyl radical, which in turn may either combine with available hydrogen (free or from donor molecule) or combine with another alkyl radical (among others) to form hydrocarbons. Taking oleic acid as an example, the reactions may go:

$$CH_3 - (CH_2)_6 - CH_2 - CH = CH - CH_2 - (CH_2)_6 - COOR$$

$$\Big\downarrow \text{ Hydrogen abstraction}$$

$$CH_3 - (CH_2)_6 - CH_2 - CH = CH - \dot{C}H - (CH_2)_6 - COOR \qquad (a)$$

$$+$$

$$CH_3 - (CH_2)_6 - CH = CH - \dot{C}H - CH_2 - (CH_2)_6 - COOR \qquad (b)$$

$$+$$

$$CH_3 - (CH_2)_6 - CH_2 - \dot{C}H - CH = CH - (CH_2)_6 - COOR \qquad (c)$$

$$+$$

$$CH_3 - (CH_2)_6 - \dot{C}H - CH = CH - CH_2 - (CH_2)_6 - COOR \qquad (d)$$

Each one of these free radicals may further react. For example, (c) will give:

$$CH_3 - (CH_2)_6 - CH_2 - \dot{C}H - CH = CH - (CH_2)_6 - COOR$$

$$\downarrow$$

$$CH_3 - (CH_2)_6 - \dot{C}H_2 + \dot{C}H - CH = CH - (CH_2)_6 - COOR$$

and $CH_3 - (CH_2)_6 - \dot{C}H_2 + XH \longrightarrow CH_3 - (CH_2)_6 - CH_3 + X\cdot$

(n-octane)

The formation of 1-alkenes can also be explained by these mechanisms. All types of saturated and unsaturated hydrocarbons including dienes may be expected from oleic, linoleic, and linolenic acids by these mechanisms. The branched chain compounds may also be formed as a result of irradiation. The alkyl free radicals, or an alkyl and a methyl free radical may combine to form branched chain compounds.

SUMMARY AND CONCLUSIONS

Milk fat prepared from raw sweet cream was irradiated in the presence of air and under vacuum at 4.5 Mrad with cobalt-60 gamma ray source. The effects of irradiation on free fatty acids, monocarbonyl, peroxides, TBA number and organoleptic properties were studied. The changes in the free fatty acid content of the milk fat dried over calcium hydride were determined upon irradiation. Attempts were also made to establish whether the ester linkage in methyl octanoate treated with calcium hydride was attacked by irradiation and to find out the extent of hydrolysis with increase in dosage.

The flavor volatiles were isolated from milk fat and irradiated at 4.5 Mrad under 29 inches of vacuum in 'C' enameled cans, using low temperature vacuum steam distillation. The head-space gases in the cans were analyzed with the Fisher Gas Partitioner. The volatile components in the steam distillate were extracted into ethyl ether. The ethyl ether concentrate was subsequently analyzed by GLC and GLC in conjunction with fast-scan mass spectrometry to identify various volatile compounds produced.

The following conclusions were drawn from the findings of this investigation:

1. Gamma irradiation brings about organoleptic changes in the milk fat. The golden yellow color of the fat is bleached; the fat becomes slightly rancid and a typical candle-like odor along with a waxy taste develops.
2. The changes are more drastic when irradiation is carried out in the presence of air in contrast to irradiation under vacuum. Some oxidized odor appears and a higher peroxide value and TBA number are obtained.
3. The free fatty acids are increased upon irradiation of milk fat indicating hydrolysis of the triglycerides. However, the increase is greater when the fat is irradiated in air than under vacuum.
4. When milk fat dried over calcium hydride is irradiated, the short chain fatty acids increase while long chain fatty acids are slightly decreased suggesting that the irradiation induced reactions are more complex than just simple hydrolysis.
5. Hydrolysis of the ester linkage in a methyl octanoate model system occurs upon irradiation even in the absence of traces of water. The extent of hydrolysis follows a somewhat linear relationship with radiation dosage.
6. Various n-alkanals and methyl ketones are formed upon irradiation confirming the results of Day and Papaioannou

(1963). Also some alk-2-enals (C_5, C_6, C_9, and C_{12}) are produced when milk fat is irradiated.

7. The differences in the monocarbonyls obtained upon irradiation in air or under vacuum are not very significant except for short chain alkanals. The important differences are in quantities of these monocarbonyls. The amounts are larger when fat is irradiated under air.

8. Head-space analysis revealed that some air is still left in the cans under 29 inches of vacuum. The oxygen from the head-space gases is consumed upon irradiation indicating slight oxidative changes. Among the gases produced as irradiation products are hydrogen, carbon dioxide, carbon monoxide, and methane.

9. Many of the volatile compounds formed in irradiated fat were identified by GLC and fast-scan mass spectrometry. Apart from the free fatty acids and monocarbonyls identified by other methods, the following compounds were identified in the stream distillate of irradiated milk fat (as determined by GLC and mass spectrometry):

n-Alkanes	C_5 through C_{17}
1-Alkenes	C_5, C_7 through C_{17}
n-Alkanals	C_5 through C_{11}
Fatty acids	C_4, C_6, C_8, and C_{10}

Other compounds	δ-decalactone, γ-decalactone 2-heptanone, benzene, ethylactate, chloroform and dichlorobenzene.

10. Tentative identification was obtained with GLC in conjunction with mass spectrometry for the following compounds: γ-hexalactone, γ-octalactone, δ-hexalactone, δ-octalactone, δ-undecalactone, δ-dodecalactone, 2-hexanone, 4-heptanone, methyl hexanoate, dodecanal, 1, ?-decadiene, 1, ?-undecadiene, 1, ?-dodecadiene, 1, ?-hexadecadiene, 1, ?-heptadecadiene, 2-methyl nonane, 2-methyl decane, 2-methyl undecane, and two branched chain undecenes.
11. Attempts to identify the candle-like smelling components failed, except that one compound probably has a carbonyl group.

BIBLIOGRAPHY

1. Albright, Lyle F., Lawrence J. Harrison and Alexander Sesonske. 1958. The effect of gamma radiation on the hydrogenation of cottonseed oil. Journal of the American Oil Chemists' Society 35:240-244.

2. American Oil Chemists' Society. 1958. Official and tentative methods of analysis. Rev. ed. Chicago. 2 vols.

3. American Petroleum Institute. 1948 to date. Research Project 44. Catalog of mass spectral data. College Station, Texas, Agricultural and Mechanical College of Texas, Chemical Thermodynamics Properties Center.

4. ASTM Committee E - 14 on Mass Spectrometry. 1963. Index of mass spectral data. Philadelphia, American Society for Testing and Materials. Various paging, loose-leaf numbers.

5. Astrack, A. et al. 1952. Effects of high intensity electron bursts upon various vegetable and fish oils. Food Research 17:571-583.

6. Ausloos, P. and C. N. Trumbore. 1959. Radiolysis of CH_3COOCH_3 and CH_3COOCD_3 by cobalt - 60 γ-rays. Journal of the American Chemical Society 81:3866-3871.

7. Bassette, Richard, Süheylâ Özeris and C. H. Witnah. 1962. Gas chromatographic analysis of head space gas of dilute aqueous solutions. Analytical Chemistry 34:1540-1543.

8. Batzer, O. F. et al. 1957. Production of carbonyl compounds during irradiation of meat fats. Journal of Agricultural and Food Chemistry 5:700-703.

9. Becker, R. R. et al. 1956. Nutritional and biochemical effects of irradiation. Food Technology 10:61-64.

10. Beynon, J. H., R. A. Saunders and A. E. Williams. 1961. The high resolution mass spectra of aliphatic esters. Analytical Chemistry 33:221-225.

11. Bierman, G. W., B. E. Proctor and S. A. Goldblith. 1956. Radiation preservation of milk and milk products. II. Off-flavors in milk and cream induced by ionizing radiations as judged by organoleptic tests. Journal of Dairy Science 39:379-390.

12. Bills, D. D., L. L. Khatri and E. A. Day. 1963. Method for the determination of the free fatty acids of milk fat. Journal of Dairy Science 46:1342-1347.

13. Boldingh, J. and R. J. Taylor. 1962. Trace constituents of butterfat. Nature 194:909-913.

14. Brasch, Arno, Wolfgang Huber and Adnan Waly. 1952. Radiation effects as function of dose rate. Archives of Biochemistry and Biophysics 39:245-247.

15. Breger, Irving A. and Virginia L. Burton. 1946. The effects of radioactivity on a naphthenic acid. Journal of the American Chemical Society 68:1639-1642.

16. Burlakova, E. B. et al. 1959. Radiolytic oxidation of fat. Izvestiya Vysshikh Uchebnykh Zavedenii, Khimiya i Khimicheskaya Tekhnologiya 2:533-540. (Abstracted in Chemical Abstracts 54:6992 e. 1960)

17. ________________. 1960. Thermal and radiolytic oxidation of methyl oleate. Izvestiya Vysshikh Uchebnykh Zavedenii, Khimiya i Khimicheskaya Tekhnologiya 3:265-271. (Abstracted in Chemical Abstracts 54:21957 h. 1960)

18. ________________. 1961. Action of γ -irradiation on the oxidation of methyl oleate in the presence of inhibitors of free-radical processes. Izvestiya Vysshikh Uchebnykh Zavedenii, Khimiya i Khimicheskaya Tekhnologiya 4:751-754. (Abstracted in Chemical Abstracts 56:9608 d. 1962)

19. Burton, Virginia L. 1949. The effect of radioactivity on oleic acid. Journal of the American Chemical Society 71:4117-4119.

20. Charlesby, A. 1955. Effect of ionizing radiations on long chain olefins and acetylenes. Radiation Research 2:96-107.

21. ________________. 1959. Ionizing radiations and organic chemistry. Scientific American 201(3):180-196.

22. Chipault, J. R. 1962. High energy irradiation. In: Symposium on Foods: Lipids and their oxidation, ed. by H. W. Schultz, E. A. Day and R. O. Sinnhuber. Westport, Connecticut, AVI. p. 151-169.

23. Chipault, J. R. et al. 1957. Effect of ionizing radiations on fatty acid esters. Industrial and Engineering Chemistry 49:1713-1720.

24. Chipault, J. R. and G. R. Mizuno. 1964. Effects of ionizing radiations on fats. II. Accumulation of peroxides and other chemical changes. Journal of the American Oil Chemists' Society 41:468-473.

25. Coleby, B. 1959. Chemical changes produced in lipids by irradiation. International Journal of Applied Radiation and Iostopes 6:71-75.

26. Cook, D. J. 1963. Radiations for the dairy industry. II. Effects of radiation on milk and milk products. Dairy Industries 28:536-540.

27. Danald, George E. 1957. Properties of electrons and beta particles. In: Radiation preservation of foods, ed. by S. D. Bailey et al. Washington, D.C., U.S. Army Quartermaster Corps. p. 58-63. (U.S. Army Research and Development Series no. 1)

28. Day, E. A., Richard Bassette and Mark Keeney. 1960. Identification of volatile carbonyl compounds from cheddar cheese. Journal of Dairy Science 43:463-474.

29. Day, E. A., D. A. Forss and S. Patton. 1957. Flavor and odor defects of gamma - irradiated skimmilk. I. Preliminary observations and the role of volatile carbonyl compounds. Journal of Dairy Science 40:922-931.

30. ________________. 1957a. Flavor and odor defects of gamma-irradiated skimmilk. II. Identification of volatile components by gas chromatography and mass spectrometry. Journal of Dairy Science 40:932-941.

31. Day, E. A. and D. A. Lillard. 1960. Autoxidation of milk lipids. I. Identification of volatile monocarbonyl compounds from autoxidized milk fat. Journal of Dairy Science 43:585-597.

32. Day, E. A. and S. E. Papaioannou. 1963. Irradiation-induced changes in milk fat. Journal of Dairy Science 46:1201-1206.

33. Desrosier, Norman W. and Henry M. Rosenstock. 1960. Radiation technology in food agriculture and biology. Westport, Connecticut, AVI. 401 p.

34. Dugan, L. R., Jr. and Paul W. Landis. 1956. Influence of high energy radiation on oxidation of oleic acid and methyl oleate. Journal of the American Oil Chemists' Society 33:152-154.

35. Dunn, Cecil G. et al. 1948. Biological and photochemical effects of high energy electrostatically produced roentgen rays and cathode rays. Journal of Applied Physics 19:605-616.

36. Egiazarov, G. M. 1959. Radiolysis of animal fat under the effect of low doses of γ - irradiation. Myasnaya Industriya S.S. S.R. 30(5):51-53. (Abstracted in Chemical Abstracts 55: 11695d. 1961)

37. Farquhar, John W. et al. 1958. The analysis of fatty acid mixtures by gas - liquid chromatography: construction and operation of an ionization chamber instrument. Nutrition Reviews 17(8, pt. 2, sup.):1-30.

38. Feigl, Fritz. 1960. Spot tests in organic analysis. 6th ed. Amsterdam, Elsevier. 675 p.

39. Fisher Scientific Company. 1962. Analysis of head-space gases with the Fisher Gas Partitioner. Technical Data TD - 171. Pittsburgh, Pennsylvania, July. 6 p. (Gas Chromatography Bulletin no. 12)

40. Fomin, A. A. 1962. Changes in the chemical indicators of food fats under the effect of ionizing radiations. Gigiena i Sanitariya 27(3):34-39. (Abstracted in Chemical Abstracts 56: 15895d. 1962)

41. Frankel, E. N. 1962. Hydroperoxides. In: Symposium on Foods: Lipids and their oxidation, ed. by H. W. Schultz, E. A. Day and R. O. Sinnhuber. Westport, Connecticut, AVI. p. 51-78.

42. Gaddis, A. M. and Rex Ellis. 1959. Paper chromatography of 2,4 - dinitrophenylhydrazones. Resolution of 2 - alkanone, n - alkanal, alk-2-enal, and alk - 2,4 - dienal derivatives. Analytical Chemistry 31:870-875.

43. Gaden, Elmer L., Jr., Ernest J. Henley and Vincent P. Collins. 1951. Preservation of milk by radiation. Food Technology 5: 506-509.

44. Gilpin, J. A. and F. W. McLafferty. 1957. Mass spectrometric analysis. Aliphatic aldehydes. Analytical Chemistry 29:990-994.

45. Goldblith, S. A. and B. E. Proctor. 1956. Radiation preservation of milk and milk products. I. Background and problems. Journal of Dairy Science 39:374-378.

46. Groninger, H. S., A. L. Tappel and F. W. Knapp. 1956. Some chemical and organoleptic changes in gamma irradiated meats. Food Research 21:555-564.

47. Hannan, R. S. 1956. Research on the science and technology of food preservation by ionizing radiation. 1st American ed. New York, Chemical Publishing Co. 192 p.

48. Hannan, R. S. and J. W. Boag. 1952. Effects of electronic irradiation on fats. Nature 169:152-153.

49. Hannan, R. S. and H. J. Shepherd. 1952. An after-effect in butterfat irradiated with high-energy electrons. Nature 170: 1021-1022.

50. ________________. 1954. Some after-effects in fats irradiated with high-energy electrons and X-rays. British Journal of Radiology 27:36-42.

51. Hoff, J. E., J. H. Wertheim and B. E. Proctor. 1959. 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.

52. Hornstein, Irwin and Patrick F. Crowe. 1962. Carbonyl removal from nonoxygenated solvents. Analytical Chemistry 34: 1037-1038.

53. Huber, Wolfgang, Arno Brasch and Adnan Waly. 1953. Effect of processing conditions on organoleptic changes in foodstuffs sterilized with high intensity electrons. Food Technology 7: 109-115.

54. Huelin, F. E. 1952. Volatile products of apples. III. Identification of aldehydes and ketones. Australian Journal of Scientific Research 5B:328-334.

55. Hummel, R. W. 1960. Radiolysis of liquid methyl acetate with cobalt-60 γ- radiation. Transactions of the Faraday Society 56:234-245.

56. Ibragimov, A. P. and A. Mukhamedov. 1962. Action of γ-rays on the physiochemical properties of cottonseed oils. Voprosy Sovremennoi Fiziki i Matematiki, Akademiya Nauk Uzbekskoi S. S. R. p. 95-99. (Abstracted in Chemical Abstracts 58:8151 g. 1963)

57. Ibragimov, A. P. and Sh. Arifzhanov. 1962. Paper chromatographic separation of fatty acids and identification of irradiation products. Voprosy Sovremennoi Fiziki i Matematiki, Akademiya Nauk Uzbekskoi S. S. R. p. 105-111. (Abstracted in Chemical Abstracts 58:8152g. 1963)

58. Johnsen, Russel H. 1959. Some aspects of the radiation chemistry of liquid aliphatic carboxylic acids. Journal of Physical Chemistry 63:2041-2043.

59. Jurriens, G. and J. M. Oele. 1965. Detection and determination of mono-5(4)-hydroxy acid triglycerides and δ (γ)-lactones in butter. (Abstract) Journal of the American Oil Chemists' Society 42:138A.

60. Keeney, P. G. and Stuart Patton. 1956. The coconut-like defect of milk fat. I. Isolation of the flavor compound from butter oil and its identification as δ-decalactone. Journal of Dairy Science 39:1104-1113.

61. Keith, R. W. and E. A. Day. 1963. Determination of free monocarbonyl compounds in oxidizing fats and oils. Journal of the American Oil Chemists' Society 40:121-124.

62. Klein, F. and K. DeJong. 1956. Paper chromatography of 2,4-dinitrophenylhydrazones of aliphatic carbonyl compounds. Recueil des Travaux Chiniques des Pays-Bas 75:1285-1288.

63. Kliman, Phyllis G. and M. J. Pallansch. 1963. Method for the determination of water in butteroil by near infrared spectrophotometry. (Abstract) Journal of Dairy Science 46:605.

64. ________________. 1965. Method for determination of water in butteroil by near-infrared spectrophotometry. Journal of Dairy Science 48:859-862.

65. Kohn, Rudolf. 1964. Gas chromatographische Untersuchungen an Electronbestrahlten Fetten. Qualitas Plantarum et Materiae Vegetabiles 11:346-354.

66. Kohn, R., V. Leute and H. Lück. 1964. Untersuchungen zum Viskositätsverhalten γ-bestrahlter Öle und Lösungen von Ölen. Fette, Seifen, Anstrichmittel 66:824-827.

67. Kovalev, T. G. 1931. Changes in the properties of olive oil under the influence of radient energy. Izvestiya Tsental'nogo Nauchno-Issledovatel'skogo Instituta Pishchevoi i Vkusovoi Promyshlennosti S.S.S.R. p. 3-35. (Abstracted in Chemical Abstracts 26:5222^3. 1932)

68. Kung, H. C., E. L. Gaden, Jr. and C. G. King. 1953. Vitamins and enzymes in milk. Effect of gamma-radiation on activity. Journal of Agricultural and Food Chemistry 1:142-144.

69. LaFuente, B., S. A. Goldblith and B. E. Proctor. 1959. Radiation preservation of milk and milk products. IX. Comparison of bacterial effects of cathode rays, γ-rays and ultraviolet light. Food Technology 13:272-275.

70. Lang, David H. and B. E. Proctor. 1956. Some effects of ionizing radiations on lipids. I. Monocarbonyl production in vegetable oils. Journal of the American Oil Chemists' Society 33:237-239.

71. Langler, J. E. and E. A. Day. 1964. Development and flavor properties of methyl ketones in milk fat. Journal of Dairy Science 47:1291-1296.

72. Lawrence, R. C. and J. C. Hawke. 1963. Origin of methyl ketones found in steam distillates of milk fat. Nature 197: 1276-1277.

73. Lea, C. H. 1959. Chemical changes in stored foods when microbial spoilage is not limiting. International Journal of Applied Radiation and Isotopes 6:86-94.

74. LeClerc, A. M. et al. 1961. Changes induced in fatty material by irradiation. Annales de la Nutrition et de l'Alimentation 15: 65-74. (Abstracted in Chemical Abstracts 56:6431a. 1962)

75. Libbey, L. M. and E. A. Day. 1964. Reverse phase thin-layer chromatography of 2,4-dinitrophenylhydrazones of n-alkanals and n-alk-2-ones. Journal of Chromatography 14:273-275.

76. Lindsay, R. C., E. A. Day and W. E. Sandine. 1965. Identification of volatile flavor components of butter cultures. Journal of Dairy Science (In press).

77. Long, J. S. and C. N. Moore. 1927. Action of cathode rays on drying oils. Industrial and Engineering Chemistry 19:901-903.

78. Lück, H. 1961. Strahlenkonservierung anstelle von Zusatzstoffen. Die Nahrung 5:535-549.

79. Lück, Hans, Carl Ulrich Deffner and Rudolf Kohn. 1963. Nachweis von Radikalen in γ-bestrahlten Fetten durch Elektronenspinresonanzspektroskopie. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 123:200-205.

80. ________________. 1964. Einwirkung ionisierender Strahlen auf Fette. V. Mitteilung: Radikalbildung. Fette, Seifen, Anstrichmittel 66:249-256.

81. Luck, H. and R. Kohn. 1961. The effect of ionizing radiation on fats. III. Changes in the physicochemical properties. Fette, Seifen, Anstrichmittel 63:812-818. (Abstracted in Chemical Abstracts 56:669a. 1962)

82. ________________. 1961a. Zur Bildung mittelkettiger Fettsäurereste in elektronenbestrahlten Fetten. Experientia 17:109-110.

83. ________________. 1962. Zur Bildung von Carbonylverbindungen in Fetten nach Einwirkung von Elektronenstrahlen. Experientia 18:62-63.

84. ______________. 1963. Einweirkung ionisierender Strahlen auf Fette. 4. Mitt. Cis-trans Isomerisierung und Verschiebung der Doppelbindungen. Die Nahrung 7:199-211.

85. Lück, H., R. Kohn, and H. Bach. 1964. Einwirkung ionisierender Strahlen auf Fette. VI. Mitteilung: Polymerisation. Fette, Seifen, Anstrichmittel 66:1063-1071.

86. Lück, Hans and Herman Kühn. 1959. Einwirkung ionisierender Strahlen auf Fette. II. Mitteilung. Allgemeine chemische Veränderungen in elektronenbestrahlten Fetten. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 110:430-442.

87. Lundberg, W. O. (ed.) 1961. Autoxidation and Antioxidants. Vol. 1. New York, Interscience. 450 p.

88. Manion, J. P. and Milton Burton. 1952. Radiolysis of hydrocarbon mixture. Journal of Physical Chemistry 56:560-569.

89. McCarthy, R. D. and A. H. Duthie. 1962. A rapid quantitative method for the separation of free fatty acids from other lipids. Journal of Lipid Research 3:117-119.

90. McFadden, W. H., E. A. Day and M. J. Diamond. 1965. Correlations and anomalies in mass spectra. Lactones. Analytical Chemistry 37:89-92.

91. Mead, James F. 1952. The irradiation-induced autoxidation of linoleic acid. Science 115:470-472.

92. ______________. 1955. The effect of ionizing radiation on lipids. In: Radiation Sterilization: Reviews of the literature in selected fields. Chicago, Illinois, U.S. Army Quartermater Corps, Radiation Sterilization Project, Quartermaster Food and Container Institute for Armed Forces, Research and Development Command. p. 17-26.

93. ______________. 1961. Autoxidation by ionizing radiation. In: Autoxidation and antioxidants, ed. by W. O. Lundberg. Vol. 1. New York, Interscience. p. 299-323.

94. Merritt, C., Jr. and P. Angelini. 1965. Irradiation damage in lipids and its effects on flavor. American Chemical Society Abstracts of Papers 149:13A.

95. Merritt, Charles, Jr. et al. 1965. Hydrocarbons in irradiated beef and methyl oleate. Journal of the American Oil Chemists' Society 42:57-58.

96. Metcalfe, L. D. 1961. Gas chromatography of unesterified fatty acids using polyester columns treated with phosphoric acid. Nature 188:142-143.

97. Milch, Robert Austin and Gerald A. Klassen. 1965. Infra-red spectra and chromatographic fractions of irradiated linoleic and linolenic acids. Nature 205:1106-1108.

98. Mitchell, J. H., Jr. 1957. Action of ionizing radiations on fats, oils, and related compounds. In: Radiation preservation of foods, ed. by S. D. Bailey et al. Washington, D.C., U.S. Army Quartermaster Corps. p. 119-125. (U.S. Army Research and Development Series no. 1)

99. Monty, K. J., A. L. Tappel and H. S. Groninger. 1961. Carbonyl compounds of irradiated meats. Journal of Agricultural and Food Chemistry 9:55-58.

100. Mukherjee, S. 1950. Studies on rancidity of butterfat. Pt. VI. The action of light. Journal of the Indian Chemical Society 27: 589-598.

101. Nickerson, John T. R., Bernard E. Proctor and Samuel A. Goldblith. 1953. Public health aspects of electronic food sterilization. American Journal of Public Health 43:554-560.

102. Parliment, Thomas H., Wassef W. Nawar and Irving S. Fagerson. 1965. Delta-caprolactone in heated milk fat. Journal of Dairy Science 48:615-616.

103. Polister, Barbara Hinderer and James F. Mead. 1954. Effect of certain vitamins and antioxidants on irradiation-induced antoxidation of methyl linoleate. Journal of Agricultural and Food Chemistry 2:199-202.

104. Proctor, B. E. et al. 1952. Biochemical prevention of flavor and chemical changes in foods and tissues sterilized by ionizing radiations. Food Technology 6:237-242.

105. Proctor, Bernard E. and Samuel A. Goldblith. 1951. Food processing with ionizing radiations. Food Technology 5:376-380.

106. Revina, A. A., Sh. A. Aripdzhanov and N. A. Bakh. 1962. A study of free-radical formation in irradiation of palmitic acid and its derivatives made by the electron spin resonance (ESR) method. Doklady Akademii Nauk S. S. S. R. 145:363-365. (Abstracted in Chemical Abstracts 57:16033e. 1962)

107. Sawicki, Eugene et al. 1961. The 3-methyl-2-benzothiazolone hydrazone test. Sensitive new methods for the detection, rapid estimation and determination of aliphatic aldehydes. Analytical Chemistry 33:93-96.

108. Schultz, H. W., E. A. Day and R. O. Sinnhuber (eds.) 1962. Symposium on Foods: Lipids and their oxidation. Westport, Connecticut, AVI. 442 p.

109. Schwartz, Daniel P., Harrison S. Haller and Mark Keeney. 1959. Method for the direct quantitative isolation of the 2,4-dinitrophenylhydrazone derivatives of carbonyls from fats and oils. American Chemical Society Abstracts of Papers 136:15A.

110. ________________. 1963. Direct quantitative isolation of monocarbonyl compounds from fats and oils. Analytical Chemistry 35:2191-2194.

111. Schwartz, Daniel P., Arthur R. Johnson and Owen W. Parks. 1962. Use of ion-exchange resins in the micro analysis of 2,4-dinitrophenylhydrazones. Microchemical Journal 6:37-44.

112. Schwartz, Daniel P. and Owen W. Parks. 1961. Preparation of carbonyl-free solvents. Analytical Chemistry 33:1396-1398.

113. Schwartz, Daniel P., Owen W. Parks and Mark Keeney. 1960. Chromatographic separation of 2,4-dinitrophenylhydrazone derivatives of aliphatic carbonyl compounds into classes on magnesia. American Chemical Society Abstracts of Papers 138:15B.

114. ________________. 1962. Separation of 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes on magnesia. Analytical Chemistry 34:669-671.

115. Sharkey, A. G., Jr., Janet L. Shultz and R. A. Friedel. 1959. Mass spectra of esters. Formation of rearrangement ions. Analytical Chemistry 31:87-93.

116. Sheppard, Charles W. and Virginia L. Burton. 1946. The effect of radioactivity on fatty acids. Journal of the American Chemical Society 68:1636-1639.

117. Shriner, Ralph L., Reynold C. Fuson and David Y. Curtin. 1960. The systematic identification of organic compounds. 4th ed. New York, Wiley. 426 p.

118. Silverstein, Robert M. and G. Clayton Bassler. 1963. Spectrometric identification of organic compounds. New York, Wiley. 177 p.

119. Sinnhuber, Russell O. and T. C. Yu. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technology 12:9-12.

120. Slover, Hal T. and L. R. Dugan, Jr. 1957. 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.

121. Sribney, Michael, U. J. Lewis and B. S. Schweigert. 1955. Effects of irradiation on meat fats. Journal of Agricultural and Food Chemistry 3:958-960.

122. Täufel, K. and R. Zimmermann. 1962. Zur Bildung von Carbonylverbindungen aus gesättigten Fettesäuren unter der Wirkung von UV-sowie von ^{60}Cobalt-γ-Strahlung. Journal für Praktische Chemie, ser. 4, 15:127-138.

123. Täufel, K., R. Zimmermann and R. Huber. 1960. Über die Bildung von Carbonylverbindungen aus gesättigten Fettesäuren durch ^{60}Co-γ-Strahlung. Die Naturwissenschaften 47:355.

124. Tharp, B. W. and Stuart Patton. 1960. Coconut-like flavor defect of milk fat. IV. Demonstration of δ-dodecalactone in the steam distillate from milk fat. Journal of Dairy Science 43:475-479.

125. Toma, Sam Z. and William H. Hamill. 1964. The mechanism of hydrogen formation in γ-irradiated hydrocarbons. Journal of the American Chemical Society 86:4761-4764.

126. Uri, N. 1959. 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.

127. Valseth, Alm. 1953. Purification of ether containing peroxides. Meddelelserfra Norsk Farmaceutisk Selskap 15:21-30. (Abstracted in Chemical Abstracts 47:16655f. 1953.)

128. Weiss, Joseph. 1944. Radiochemistry of aqueous solutions. Nature 153:748-750.

129. ________________. Effects of radiations on aqueous systems under aerobic and anaerobic conditions. International Journal of Applied Radiation and Isotopes 6:52-58.

130. Wertheim, John H. et al. 1957. Irradiation preservation of milk and milk products. Journal of Agricultural and Food Chemistry 5:944-950.

131. Whitehead, Walter L., Clark Goodman and Irving A. Breger. 1951. The decomposition of fatty acids by alpha particles. Journal de Chimie Physique 48:184-189.

132. Wick, Emily L. 1963. Volatile components of irradiated beef. In: Exploration in future food-processing techniques, ed. by Samuel A. Goldblith. Cambridge, Mass., M.I.T. Press. p. 5-18.

133. Witting, L. A. and B. S. Schweigert. 1958. A characterization of volatile carbonyl compounds isolated from meat fat subjected to gamma-radiation. Journal of the American Oil Chemists' Society 35:413-416.

134. Wong, Noble P. and Stuart Patton. 1962. Identification of some volatile compounds related to flavor of milk and cream. Journal of Dairy Science 45:724-728.

135. Zhuravlev, A. I. 1961. Effect exerted by γ-radiation on sunflower seed oil. Voprosy Pitaniya 20(2):65-69. (Abstracted in Chemical Abstracts 57:15562a. 1962)

136. Zhuravlev, A. I., M. A. Lomova and V. N. Benevolenskii. 1961. Toxicity of irradiated and oxidized fat. Meditsinskaya Radiologiya 6(2):46-51. (Abstracted in Chemical Abstracts 56:10667f. 1962)