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Changes Induced in Lipids by Irradiation

Ishak I. Shahied

To the Graduate Council:

I am submitting herewith a thesis written by Ishak I. Shahied entitled "Changes Induced in Lipids by Irradiation." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Melvin R. Johnston, Major Professor

We have read this thesis and recommend its acceptance:

Bob J. DeMott, Russell B. Stone

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

December 2, 1964

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Major Professor

We have read this thesis and recommend its acceptance:

B. J. Demott

Russell B. Stone

Accepted for the Council:

Helton A. Smith
Dean of the Graduate School

CHANGES INDUCED IN LIPIDS BY IRRADIATION

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Ishak I. Shahied

December 1964

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CHAPTER I

INTRODUCTION

The irradiation of lipids results in the formation of free radicals which may participate in a number of reactions in the irradiated medium (15). When molecular oxygen is available, the free radicals readily react with this substance to form hydroperoxides (14). Decomposition of these peroxides yields new free radicals and a chain oxidation process identical to that prevailing in autoxidation is initiated (13).

Most studies on the effects of radiations on lipids have been performed on unsaturated fats and it has been impossible to differentiate between the products formed directly as the result of irradiation and those produced through the usual autoxidative chain process (9).

Furthermore, irradiation produces a number of other chemical changes such as formation of carbonyl compounds, polymers, short-chain acids and hydrocarbons (14,8), but the formation of these compounds during irradiation and subsequent storage has not been fully investigated.

With these factors in mind, three lipid-containing foods were treated with two different types of irradiation in an attempt to:

1. Determine the effects of each treatment on the degree of unsaturation of the lipid of each sample.

2. Determine any changes induced in the molecular weight of the lipid of each sample, and

3. Elucidate the nature of any change in the composition of the lipid of each sample.

CHAPTER II

REVIEW OF THE LITERATURE

I. GENERAL CONSIDERATIONS

Despite the information from thermodynamic considerations that most organic compounds are unstable with respect to reaction with molecular oxygen (42), the activation energy for this reaction results in a very low rate under ordinary conditions. Since the oxygen molecule has some of the properties of a biradical, however, it will react readily with any organic free radical (42). The result of such a reaction is a peroxy radical, as illustrated:

$$RCH_2\cdot + O_2 \longrightarrow RCH_2OO\cdot$$

An oxidative chain reaction is initiated if the peroxy radical attacks a substrate molecule and results in the formation of another radical center which may react with oxygen (41). Autoxidation induced by ionizing radiation is unique only in the detail of its initiation and termination (40). In the usual autoxidation reaction, initiation occurs, in the presence of oxygen, by reaction of excited oxygen molecules with substrate (9). Termination is brought about

by combination of two radicals. In irradiation-induced autoxidation, however, initiation is by reaction sequences such as:

$$RCH_3 + OH\cdot \longrightarrow RCH_2\cdot + H_2O$$

$$RCH_2 + O_2 \longrightarrow RCH_2OO\cdot$$

in which a free radical is produced by either direct or indirect action (39). Thus, initiation in such cases will be a function of irradiation rate. Termination is also a function of irradiation rate in both direct and indirect action because of the higher steady-state concentration of radical moieties at higher rates (41).

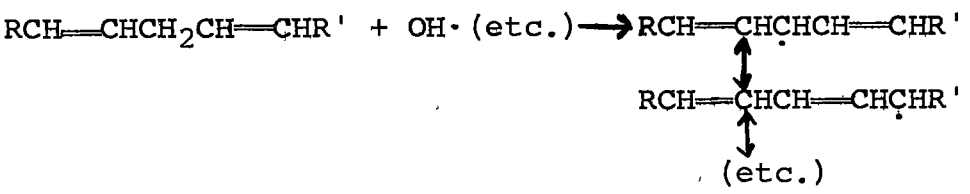
II. PRIMARY REACTION AND PRODUCTS

Polyunsaturated Fatty Acids

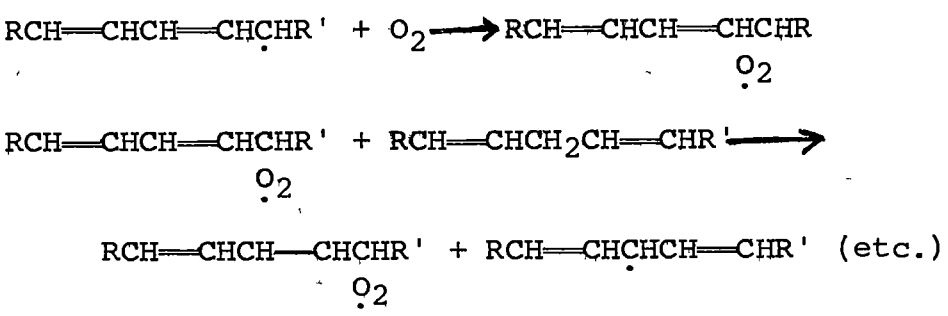
An illustration of the concept reviewed above is the irradiation-induced autoxidation of linoleic acid (40). When solutions of this acid are irradiated in the presence of air, rapid oxidation to hydroperoxide occurs. As in spontaneous autoxidation, the rate of peroxide production (or of concomitant conjugation of the diene system) is proportional to substrate concentration and a function of the oxygen

pressures, at least at low pressures. However, the rate is also directly proportional to radiation dose and inversely so to dose rate. This latter behavior is typical of radiation-induced chain reactions and is probably attributable to enhancement of the termination reactions. The reactions proposed for this autoxidation are as follows (42):

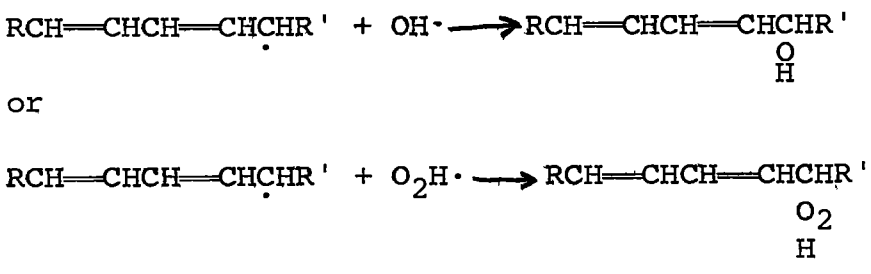
Initiation:



Propagation:



Termination:



Termination can also involve peroxyl radicals such as those shown in the above reaction.

Of course, the decomposition of the hydroperoxide formed in this sequence can also bring about radical formation and, consequently, chain initiation but this type of initiation, which is of major importance in autoxidation is relatively minor in the irradiation reaction as shown by the greatly increased rate of the latter reaction (42).

Irradiation of Unsaturated Fatty Acids

Irradiation of unsaturated fatty acids in the presence of oxygen produces some peroxide although not usually of the same order of magnitude as is produced in aqueous solutions or emulsions equilibrated with air (1). This, in all likelihood, is due partly to the conditions of irradiation and partly to the nature of the materials irradiated. In stirred aqueous solutions or emulsions (47) equilibration with oxygen is rapid and complete. In irradiation of most pure lipids, however, efficient stirring is difficult to realize, and diffusion of oxygen into the substance is consequently slow and incomplete. Experiments by Mead (40) have shown that during the brief irradiation of unstirred lipids, only the

surface is highly oxidized, the major part of the substance coming in contact only with the oxygen already dissolved in it at the start of the irradiation.

Chipault and co-workers (14) have measured peroxide numbers produced by gamma irradiation of methyl esters of palmitic, oleic, and linoleic acids and of soap of the latter two. As in the case of aqueous solutions, peroxide production by irradiation of pure esters was more rapid at lower irradiation rates, indicating a chain reaction. Yields of peroxides were quite low, however, indicative of short reaction chains terminated by radical combination before reaction with oxygen could take place.

Triglycerides, Fats, and Oils

According to Mead (42), the autoxidation of natural fats and oils during irradiation is dependent on several factors:

(a) The presence of antioxidants which strongly inhibit peroxide formation until they themselves are destroyed.

(b) The degree of unsaturation--generally speaking, the more unsaturation the more rapid the oxidation.

(c) The amount of irradiation--generally speaking, the amount of peroxides varies directly with the amount of irradiation.

(d) The rate of irradiation--a low rate of irradiation is more efficient than a high rate, probably for two reasons: First, chains are terminated as well as initiated by irradiation-induced radicals. Second, since in the irradiation of pure substances, oxidation depends on the availability of oxygen, a lower rate allows better equilibration with air.

(e) Length of storage--the peroxide number increases rapidly during irradiation and continues to increase at a somewhat lower rate after cessation of irradiation until polymerization and fragmentation become important whereupon peroxide decreases.

(f) Temperature of irradiation and storage--there has been little agreement among the various laboratories on the exact nature of this effect except that it exerts considerable influence on the reaction (41, 39).

III. PRODUCTS EXPECTED FROM IRRADIATION OF LIPIDS (11,15)

Reaction	Products Formed
1. Oxidation:	Carbonyls, hydroxy compounds, peroxides, acids, oxygen-linked polymers, odors and flavors.
2. Polymerization:	Dimers, higher polymers.
3. Chain Scission:	Short chain hydrocarbons, short chain acids, longer chain compounds through recombination, odors and flavors.
4. Decarboxylation:	Carbon dioxide, long chain hydrocarbons, long chain carbonyls.

5. Dehydrogenation:	Compounds with new double bonds.
6. Isomerization:	Conjugation, and cis-trans isomers.
7. Hydrogenation:	More highly saturated compounds.

IV. RADIATION-INDUCED AUTOXIDATION

Formation of Free Radicals

On the basis of kinetic and chemical observations, a free radical chain mechanism was proposed (39) and which at present, is almost universally accepted as the mechanism of irradiation-induced reactions. A generalized form of the mechanism as modified in some minor details by Chipault and Mizuno (15) is:

Initiation:

$$RH + O_2 \longrightarrow \text{Free Radicals}$$

$$\left.\begin{matrix} ROOH \\ (ROOH)_2 \end{matrix}\right\} \longrightarrow \text{Free Radicals (e.g. } R\cdot,\ RO\cdot,\ RO_2\cdot,\ HO\cdot, \text{ etc.)}$$

Propagation:

$$R\cdot + O_2 \quad RO_2\cdot$$

$$RO_2\cdot + RH \quad R\cdot + ROOH$$

Termination:

$$\left.\begin{array}{l} R\cdot + R\cdot \\ R\cdot + RO_2\cdot \\ RO_2\cdot + RO_2\cdot \end{array}\right\} \text{ Stable (non-radical) end product}$$

V. SECONDARY REACTIONS AND PRODUCTS

Although the occurrence of peroxides in irradiated lipids has been well documented and explained on a firm theoretical basis, little is known with comparable certainty about the further reactions of these substances under the influence of ionizing radiation (12).

Irradiation Decomposition of Hydroperoxides

In a study of the irradiation decomposition of hydroperoxides, Mead and Griffith (41) used the readily available secondary and tertiary butyl hydroperoxide. Both substances were decomposed by exposure to gamma irradiation from the Co^{60} source at rates which indicated a chain mechanism. In the case of tertiary isomer the product could, by analogy with thermal decomposition at different temperatures, be either t-butyl alcohol or acetone. Since no acetone was found, indirect evidence thus favors the production of alcohol. Actually, good evidence has been found for the

formation of 1,2-glycol, according to Mead and Griffith (41).

VI. DECOMPOSITION OF FAT HYDROPEROXIDES (39)

Reaction	Products Formed
1. Polymerization:	Dimers, higher polymers
2. Further oxidation	Diperoxides → polymers
3. Fission	Aldehydes, semi-aldehydes, aldehydo-glycerides, OH-compounds → acids
4. Dehydration	Keto-glycerides
5. Oxidation of CH=CH in other molecules	Epoxides, OH-glycerides, diOH-glycerides

Production of Carbonyl

In the majority of cases studied (13,10,18,20), the irradiation of unsaturated fatty acid derivatives has resulted in the production of carbonyl compounds in varying amounts. Since such products have usually been detected only by their ultraviolet and infrared absorption spectra or colorimitrically

as dinitrophenylhydrazones, their exact nature remains unknown. However, indirect evidence has suggested that at least three types of compounds can be formed as a result of the further reaction of the initially formed hydroperoxides--aldehydes, ketones, and keto-acids. The probable intermediates in the formation of these secondary products are alkoxy radicals which may be formed by decomposition of hydroperoxides (26,18,29):

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!CHOOH \longrightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!CHO\cdot + OH\cdot$$

or by interaction of two peroxy radicals,

$$2\begin{matrix} R \\ R \end{matrix}\!\!>\!CHO_2\cdot \longrightarrow 2\begin{matrix} R \\ R' \end{matrix}\!\!>\!CHO\cdot + O_2$$

From the known reactions of such radicals, their further transformations can be postulated as shown below (4):

Formation of aldehydes:

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!CHO\cdot \longrightarrow R'\cdot + RCHO$$

Formation of ketones:

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!CHO\cdot + R\cdot \longrightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!CO + RH \qquad \text{or}$$

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!CHO\cdot + RO\cdot \longrightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!CO + ROH$$

Formation of alcohols:

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!\! CHO\cdot + RH \longrightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!\! CHOH + R\cdot$$

Further oxidation of alcohols and aldehydes to ketones and acids thus accounts for all the types of products detected in these reaction mixtures (28).

Several studies have been made in which carbonyl formation has received special attention (27,33,38,45). In the irradiation of oleic acid or of methyl oleate (19) carbonyl content was shown to increase with irradiation dose in a manner similar to that of peroxide. Increased temperature during irradiation or the presence of cobaltous ion resulted in decreased yields in peroxides and increased yields of carbonyl compounds. In these studies alpha, beta-unsaturated carbonyl compounds, as measured by the ultraviolet absorption of the mixture at 224 mu (19), did not change in a regular manner and tended to attain a maximum concentration little affected by continued irradiation.

Lang and Proctor (38) have investigated carbonyl production from cottonseed, corn, and olive oils under irradiation by 3 m.e.v. cathode rays; dinitrophenylhydrazones mixtures were partially separated by chromatography on alumina

and analyzed by means of absorption spectra. A high yield of carbonyl compounds were obtained at 0-10 C. Several others (2,21,30,34,49) have studied carbonyl formation in a wide variety of animal and vegatable oils, and it is to be concluded that these substances are inevitable end products of oxidative decomposition of fatty acid derivatives by ionizing radiation.

CHAPTER III

EXPERIMENTAL MATERIALS AND METHODS

I. MATERIALS

Whole dry milk, cottonseed oil (Wesson Oil), and butterfat samples were purchased from the local market. Most of the water was removed from the butterfat. Two different types of irradiation and irradiation-equipments were used in this experiment.

Gamma Irradiation

The materials were irradiated in the following order: butter, cottonseed oil, and whole dry milk. Each material was divided into five groups with one kept as an untreated control and the other four given the following treatments:

Sample No.	Total Dose	Time in Hours	Hours	Minutes	Seconds
1	10^6r	8.696	8	41	46
2	10^5r	0.8696	0	52	11
3	10^4r	0.08696	0	5	13
4	10^3r	0.008696	0	0	31

The radiation source was a cesium-137 device. To reduce the variation within the sample, it was decided to

use a volume of about 50 cc. in the middle of the cup where the intensity was 1.15 x 10^5r per hour plus or minus 10 per cent. The cesium is above and below the sample chamber; therefore, a rather small volume in the center is all that permits a high degree of refinement in dosage measurement.

Gas Plasma Irradiation

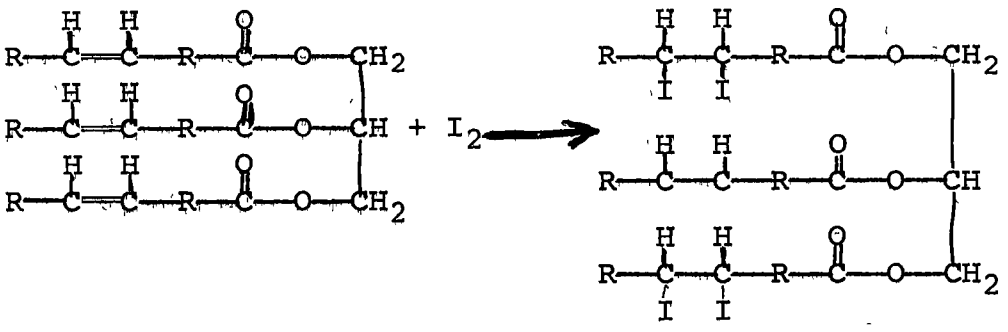
Butterfat, cottonseed oil, and whole dry milk were respectively irradiated by using the glow discharge equipment similar to that recommended by Brown et al. (7). The irradiation chamber consisted of a borosilicate glass tube, 51 mm. o.d. by 61 cm. long. At each end of the tube, a black iron coupling, 3/4 in. nominal diameter, connected to a rubber stopper by a black iron nipple, was used for an electrode. This type of metal was used to minimize sputtering of the electrodes during the treatments. A modification of the equipment was used to obtain the 200 milliampere treatment of the cottonseed oil. The system was evacuated to a predetermined pressure before applying voltage to the electrodes. High voltages were obtained from a 12,000 volt transformer. The frequency used was 60 cycles per second. The current was

regulated by connecting the primary side of the transformer to a variable transformer, such as Power-stat.

II. METHODS

Iodine Number

Hanus Method for determination of the iodine number of all the three samples was used (59). A weighed amount of the samples (approximately 0.25 gm. in case of oil or butter, 0.800 gm. in case of whole dry milk, weighed accurately using analytical balance, was dissolved in 10 ml. of chloroform, and an excess of iodine was added (25 ml. of iodobromide solution). The flasks were allowed to stand in a dark place for 30 minutes. During this time the iodine adds across any double bonds present in the unsaturated fatty acids present in the sample.



The amount of unreacted iodine was then determined by titration with a standard solution of sodium thiosulfate using a

starch indicator. All solutions were prepared and standardized according to the United States Pharmaecopeia (57).

$$Na_2S_2O_3 + I_2 \longrightarrow 2NaI + Na_2S_4O_6$$

A blank determination was run (in triplicate) along with the samples to measure the original amount of iodine present. From the data obtained, the number of grams of iodine that reacted with 100 grams of the sample was calculated as follows:

$$\frac{(\text{Bk. tit.} - \text{Samp. tit.}) \times \text{N. } Na_2S_2O_3 \times \text{Milleq. wt. of } I_2 \times 100}{\text{Sample weight in grams}}$$

Peroxide Value

The estimation of peroxides was based primarily on their ability to liberate iodine from potassium iodide in glacial acetic acid. The peroxide value of the samples was a measure of the reactive oxygen they contained expressed in milliequivalents of oxygen per 1,000 grams of fat or as millimoles of peroxide per kilogram of sample. Wheeler's Method (58) was used in measuring the peroxide values throughout this experiment. Solutions were prepared and standardized according to the United States Pharmacopeia (57). From the

data obtained, the milliequivalents of oxygen per 1,000 grams of fat was calculated as follows:

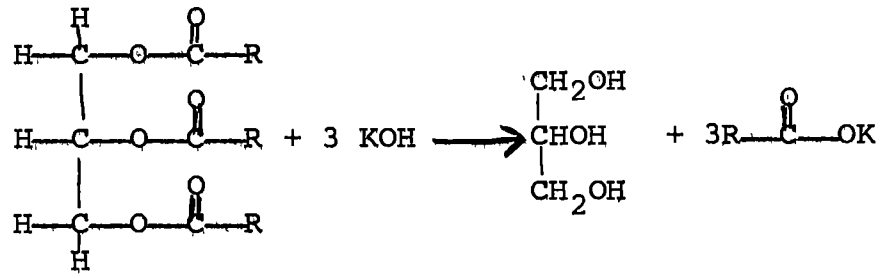
$$\frac{\text{Sample titration} \times \text{N. of } Na_2S_2O_3 \times 1000}{\text{Weight of sample used}}$$

Free Fatty Acids

The method of the United States Pharmacopeia (57) for free fatty acids was conducted, using a mixture of ethyl alcohol, 95 per cent (USSD), and ethyl ether (1:1) by volume. Free fatty acid content is expressed as: mls. of 0.1 N. NaOH used in titration of 10 or 1 gram of sample.

Saponification Number

A weighed amount of each sample (approximately two grams accurately weighed by difference using analytical balance in triplicate) was refluxed with a measured excess of 0.5 normal alcoholic potassium hydroxide solution (25 ml.). The sample reacted with some of the alcoholic potassium hydroxide solution and underwent saponification.



The amount of the alcoholic potassium hydroxide which did not react with the sample was determined by titration with standard hydrochloric acid (approximately 0.5 N.). A blank determination was run along with the samples in triplicate to determine the original concentration of KOH. From the data obtained the number of milligrams of potassium hydroxide required to saponify a one-gram sample was calculated as follows:

$$\text{Sap.\#} = \frac{(\text{Av.Bk.tit.-Samp.tit.}) \times \text{N.HCL} \times (\text{Milleq.wt.KOH})}{\text{Sample weight in grams}}$$

(59). Solutions were prepared and standardized according to the United States Pharmacopeia (57).

Refractive Index

Bausch and Lomb's Refractometer was used on all samples. Temperature of the water circulating through the refractometer was maintained at 25°C.

Percentage Nitrogen and Protein (Kjeldahl Method)

The nitrogen in the whole dry milk sample was converted to ammonium sulfate during the digestion process with concentrated sulfuric acid. This ammonium sulfate was treated with strong base and heated in a distillation rack.

The ammonia that was liberated in the reaction was distilled off and collected in 4 per cent boric acid. The boric acid solution which had absorbed the ammonia was titrated with standard acid and the per cent nitrogen was calculated from the titration data as indicated below. Since the only principle source of nitrogen in a food is protein, the per cent nitrogen was related to the per cent protein in the whole dry milk sample by a conversion factor (46).

$$\% \text{ Nitrogen} = \frac{\text{ml. HCL x N. HCL x (Milleq.wt. of N) x 100}}{\text{Weight used}}$$

$$\% \text{ Protein} = \text{Average } \% \text{ Nitrogen} \times 6.38$$

CHAPTER IV

RESULTS AND DISCUSSIONS

I. CHANGES IN THE IODINE NUMBERS

The results presented in this work indicate that the changes observed in the vegetable oil and the dairy products irradiated are very much dependent upon the individual radiation sensitivity of the particular product. The decrease in unsaturation observed in the gas plasma irradiated Wesson Oil (Table I) would indicate a partial polymerization or destruction of double bonds induced by the high intensity treatment of 200 M.A.

The effect of the gamma-radiation on the iodine number or degree of unsaturation of Wesson Oil and butterfat samples (Table II) was insignificant. In general, it can be seen that the effect of each of the low intensity gas plasma treatment (80 M.A.)(Table III) and the gamma-irradiation are only minor changes in the degree of unsaturation of both Wesson Oil and butterfat samples. However, gamma irradiation has induced a great change in the degree of unsaturation of the whole dry milk sample (Table II), which showed a great

TABLE I

IODINE NUMBER OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (200 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Grams of iodine per 100 grams of sample	
	Treated	Control
Wesson Oil	91.1	105.1
	90.3	108.9
	91.7	105.8
Average	91.0	107.0
% Deviation	0.51%	1.18%
Butterfat	32.1	34.7
	31.2	36.3
	32.0	35.6
Average	31.8	35.6
% Deviation	0.94%	1.49%
Whole Dry Milk	35.6	35.6
	35.6	36.6
	35.9	36.1
Average	35.7	36.1
% Deviation	0.28%	0.92%

TABLE II

IODINE NUMBER OF SAMPLES TREATED WITH GAMMA-IRRADIATION (CESIUM-137)

Sample	Grams of iodine per 100 grams of sample				
	Dose				
	10^6	10^5	10^4	10^3	Control
Wesson Oil	105.4	104.5	103.4	103.9	105.4
	105.3	104.9	105.9	105.0	103.8
	106.7	105.0	104.9	104.1	104.4
Average	105.8	104.8	104.7	104.3	104.5
% Deviation	0.44%	0.16%	0.79%	0.35%	0.51%
Butterfat	35.3	35.0	36.0	35.2	34.7
	34.3	36.1	35.4	35.0	36.3
	35.3	35.8	35.7	35.8	35.6
Average	34.9	35.6	35.7	35.4	35.6
% Deviation	0.95%	1.02%	0.56%	0.75%	1.49%
Whole Dry Milk	82.2	53.5	55.2	45.7	35.6
	82.9	53.5	50.0	48.3	36.6
	82.3	53.0	51.3	45.1	36.1
Average	82.5	53.3	52.1	46.4	36.1
% Deviation	0.28%	0.31%	0.33%	0.23%	0.09%

TABLE III

IODINE NUMBER OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION
(80 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Grams of iodine per 100 grams of sample	
	Treated	Control
Wesson Oil	108.3	108.3
	108.0	106.1
	107.3	104.8
Average	107.9	106.4
% Deviation	0.31%	1.09%
Butterfat	34.4	34.1
	35.0	35.5
	34.7	34.8
Average	34.7	34.8
% Deviation	0.57%	1.34%
Whole Dry Milk	23.2	23.6
	23.3	24.5
	24.7	23.7
Average	23.7	23.9
% Deviation	2.10%	1.25%

increase of the iodine number with increase of dose.

According to Mead (42), Lang and Proctor (38), and Goldblith and Proctor (22), irradiation-induced autoxidation of fatty acids with methylene interrupted unsaturation results in a double bond shift to give conjugated systems. In the case of autoxidizing methyl linoleate, a direct relationship between diene concentration and peroxide value has been established (25,39,51). However, these authors indicated that with irradiated linoleate a deviation from this relationship exists and larger amounts of diene conjugation are formed. A possible explanation is that during irradiation the conjugated free radicals are present in a relatively high concentration and can react with each other, or with compounds other than oxygen, to greater extent than during ordinary autoxidation so that a larger proportion of conjugated compounds other than hydroperoxides is formed (52,56).

II. CHEMICAL CHANGES AND ACCUMULATION OF PEROXIDES

Peroxides formed during gamma irradiation of samples are shown in Table IV, and data for the gas plasma irradiation (under vacuum) of samples appear in Table V.

After treatment, no peroxides were detected in the

TABLE IV

PEROXIDE VALUES OF SAMPLES TREATED WITH GAMMA-IRRADIATION (CESIUM-137)

Sample	MEQ. of O_2 per 1000 grams of fat				
	Dose				
	10^6	10^5	10^4	10^3	Control
Wesson Oil	3.41	2.63	1.24	1.20	0.888
	3.53	2.65	1.25	1.33	1.11
	3.71	2.54	1.52	1.21	1.04
Average	3.55	2.61	1.33	1.25	1.01
% Deviation	2.81%	1.40%	7.01%	3.46%	7.32%
Butterfat	2.35	1.78	0.502	0.301	0.289
	2.27	1.34	0.496	0.387	0.191
	2.19	1.54	0.499	0.344	0.299
Average	2.27	1.55	0.499	0.344	0.261
% Deviation	2.35%	9.46%	0.40%	8.33%	13.79%

TABLE V

PEROXIDE VALUES OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (200 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	MEQ. of O_2/1000 gm. of fat	
	Treated	Control
Wesson Oil*	22.8	7.50
	25.3	8.60
	24.0	8.10
Average	24.05	8.10
% Deviation	3.46%	2.06%
Butterfat	0.859	0.289
	0.519	0.196
	0.859	0.299
Average	0.745	0.261
% Deviation	15.21%	13.15%

*After treatment, both the control and irradiated samples were exposed to air; and were stored at refrigeration temperature before test performed.

samples irradiated under vacuum (the gas plasma irradiation); however, upon standing for about a week at refrigeration temperature, and exposed to air, peroxides were formed in appreciable quantities (Table V).

With gamma irradiation, formation of peroxides increased with the dose in both Wesson Oil and butterfat samples. The change observed in the vegetable oil when irradiated with both gamma and gas plasma irradiation was more pronounced than in the butterfat sample, and it seems that the effect in each case may be due to a destruction of the naturally present protective systems (1,36). Astrack et al. (1) also stated that the immediate effect of irradiation resulted in a partial destruction of peroxides initially present in the sample. However, the fact that there were more peroxides developed in Wesson Oil than in butterfat (Tables IV and V) emphasizes that formation of peroxides increases with unsaturation (5).

The results of this experiment on peroxides developed in unsaturated Wesson Oil and saturated butterfat are in agreement with several other workers (15,12,54,61). Chipault and Mizuno (15) and Chipault and Mizuno (12) found that small amounts of peroxides were formed in saturated fatty acid

esters, such as methyl myristate and methyl palmitate, and that larger amounts were found in samples of methyl oleate and methyl linoleate that were similarly irradiated under oxygen. The mentioned authors also reported that no peroxides were formed during irradiation under vacuum; and that water increased slightly the formation of peroxides in unsaturated compounds irradiated under oxygen; and that the presence of emulsifying agents had no additional effect.

According to Chipault and Mizuno (9), the saturated molecult has no site with a bond of low enough energy to be activated by a secondary energy transfer. He argues that the peroxides he found in irradiated methyl myristate represented the primary reaction products with oxygen and the free radicals initially formed as a result of irradiation; and that the site of attack is possibly at the methylene group adjacent to the carboxyl.

Electron paramagnetic resonance has been used by Bradshaw and Truby (6) to correlate the formation of peroxides with changes in the type and amount of free radicals present in irradiated fats. The results of their work lead the mentioned authors to the conclusion that the build-up of peroxides depends on the type of free radicals produced and

on the rates of decay of the free radicals relative to their reactivity with oxygen. Since the type of free radicals produced varies with substrate and temperatures of irradiation and storage, the accumulation of peroxides is also dependent on these factors (16). The effect of substrate is in agreement with the results of this experiment that peroxide formation increases with unsaturation (Tables IV and V) , also that peroxide formation observed in the butterfat is probably due to the destruction of the natural antioxidants present (1,17).

III. CHANGES IN THE FREE FATTY ACIDS CONTENT

The results of this experiment also show that with gas plasma irradiation there was an increase of the free fatty acids formed, which was in proportion to the dose increase (Tables VI and VII). In the case of the two gas plasma treatments of 200 M.A. and 80 M.A., there were more free fatty acids formed in butterfat and whole dry milk than in Wesson Oil.

The gamma irradiation has resulted in development of free fatty acids that increased with the dose, with the exception of the highest dose where low free fatty acids was

TABLE VI

FREE FATTY ACID CONTENT OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (200 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Mls. of 0.1 N. NaOH used in titration of 10 gm. sample	
	Treated	Control
Wesson Oil	0.40	0.15
	0.40	0.15
	0.40	0.15
Average	0.40	0.15
Butterfat	3.0	1.30
	3.0	1.20
	3.0	1.25
Average	3.0	1.25
% Deviation		2.67%
Whole Dry Milk	20.0	17.2
	20.0	17.2
	20.0	17.5
Average	20.0	17.3
% Deviation		0.84%

TABLE VII

FREE FATTY ACID CONTENT OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (80 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Mls. of 0.1 N. NaOH used in titration of 10 gm. sample	
	Treated	Control
Wesson Oil	0.25	0.15
	0.25	0.15
	0.25	0.15
Average	0.25	0.15
Butterfat	1.80	1.30
	1.80	1.20
	1.80	1.25
Average	1.80	1.25
% Deviation		2.70%
Whole Dry Milk	1.40	1.70
	1.50	1.70
	1.45	1.70
Average	1.45	1.70
% Deviation	4.96%	

the case (Table VIII). Here again, butterfat and whole dry milk samples gave proportionally more acids than Wesson Oil sample. In all cases of irradiation, the whole dry milk sample gave the highest free fatty acids yield.

The results shown in the tables suggest that with irradiation, some cleavage of fatty acid peroxides might have occurred with the resultant formation of fatty acids split products (37,45).

Whole dry milk sample showed less free fatty acids content when treated with gas plasma irradiation of 80 M.A. than the controls (Table VII), while with the intense treatment of 200 M.A., the amount of free fatty acids present has surpassed that of the controls (Table VI. Jenness and Patton (32) and Krukovsky (35) suggested that when water is removed from systems containing milk lipids, as in the case of whole dry milk, it seems reasonable to expect that water soluble constituents having a bearing on lipid oxidation might be rendered largely inactive. He added that this should be true at least to the extent that these substances cannot dissolve in or contact the lipids. Thus, one might expect that ascorbic acid, copper proteinate and various water soluble pro-and antioxidants would be less effective in a "dry" system (23,31).

TABLE VIII

FREE FATTY ACID CONTENT OF SAMPLES TREATED WITH GAMMA-IRRADIATION (CESIUM-137)

Sample	Mls. of 0.1 N. NaOH used in titration of 1.0 gram sample				
	Dose				
	10^6	10^5	10^4	10^3	Control
Wesson Oil	0.10	0.15	0.05	0.05	0.05
	0.10	0.15	0.05	0.05	0.05
	0.10	0.15	0.05	0.05	0.05
Average	0.10	0.15	0.05	0.05	0.05
Butterfat	0.25	0.25	0.25	0.20	0.20
	0.25	0.24	0.25	0.20	0.20
	0.25	0.25	0.25	0.20	0.20
Average	0.25	0.25	0.25	0.20	0.20
Whole Dry Milk	1.70	1.85	1.40	1.45	1.75
	1.70	1.85	1.41	1.45	1.74
	1.71	1.84	1.40	1.44	1.75
Average	1.70	1.85	1.40	1.45	1.75
% Deviation	0.19%	0.18%	0.24%	0.23%	0.19%

It was also suggested (20,36,53), that when phospholipids are present in the aqueous phase of milk the triglycerides are relatively stable and the phospholipids are preferentially oxidized due to irradiation. When water is absent, such as in whole dry milk, the triglycerides are relatively susceptible to oxidation whereas the phospholipids are more stable and when present in the triglycerides, they serve as antioxidant (48). With butter, which represents an aqueous concentrate of phospholipid dispersed in fat, both fat and phospholipid are susceptible, the latter being most readily oxidized (60).

The fact that with high dose of gamma irradiation, the free fatty acids were decreased (Table VIII), indicates that the high dose might have caused some destruction of the fatty acids; and although irradiation induced oxidation may account in part for such loss, hydration of double bonds in unsaturated acids should also be considered (24,43).

In view of the evidence from Goldblith and Proctor's work on irradiated dairy products (22), that irradiation-induced oxidation flavor of butterfat was produced most strongly in the unsaturated glycerides, it was therefore concluded by the mentioned authors that linolenic acid was a precursor to irradiation-induced oxidation flavor in butterfat.

Linolenic acid has previously been reported (27,50) to be present in butterfat in amounts ranging from 1 to 3 per cent.

It was also reported (29,60), that irradiation of milkfat and milk products resulted in three types of flavor fractions: hydrolytic rancidity, oxidative rancidity, and candle like; and that the short-chain aldehydes (less than C_{11}) were the cause for the oxidized type flavor. The authors also mentioned that ketones and long chain aldehydes usually found in the irradiated fat were not produced in appreciable quantities in autoxidized lipids. The authors' findings on the origin of methyl ketones and long chain aldehydes in milk fat points to hydrolytic cleavage of ester and enol-ether linkages as the mechanisms accounting for the compounds. Thus, in their views, the long chain aldehydes that exist as natural constituents of milk fat bound to glycerol through the enol-ether linkage; and that irradiation-induced hydrolytic cleavage of this linkage, rather than oxidative degradation of fat, was suggested as the mechanism accounting for the ketones and long-chain aldehydes found in irradiated samples.

IV. CHANGES IN THE SAPONIFICATION NUMBER

The data obtained from the determination of the saponification numbers for the different samples are presented in Tables IX, X, and XI. With the gamma irradiation, at the highest dose of 10^6r, there has been a lowering of the saponification number which correlates with a high molecular weight, indicating a possible polymerization (Table IX). Gamma irradiation of doses lower than 10^6r did not show much difference in the saponification number. Doses of 10^3r and 10^4r resulted in rather higher saponification numbers, corresponding to lower molecular weights in the whole dry milk sample treated at these two doses (Table IX). Some chain scission might have taken place. However, at the high dose of 10^6r for the same sample, again a low saponification number, corresponding to a high molecular weight, was indicated. Here again a possible polymerization might have occurred at that high dose.

In the case of gas plasma irradiation, there was only a slight difference in the saponification numbers between irradiated and the controls. Only in the cases of butterfat and whole dry milk there was a little increase of the saponification numbers at the intense treatment of 200 M.A.

TABLE IX

SAPONIFICATION NUMBER OF SAMPLES TREATED WITH GAMMA-IRRADIATION (CESIUM-137)

Sample	Mgs. of KOH per 1 gram of sample				
	Dose				
	10^6	10^5	10^4	10^3	Control
Wesson Oil	185.4	193.1	192.2	190.5	191.5
	186.4	193.9	193.8	190.6	190.7
	185.5	190.5	193.0	190.0	191.3
Average	185.9	192.5	193.0	190.4	191.2
% Deviation	0.18%	0.59%	0.28%	0.10%	0.14%
Butterfat	231.5	232.5	226.3	230.8	231.7
	232.6	233.5	229.4	226.3	225.3
	229.8	229.1	230.8	227.2	218.9
Average	231.3	231.7	228.8	228.1	225.3
% Deviation	0.40%	0.63%	0.65%	0.66%	1.89%
Whole Dry Milk	258.0	271.3	266.8	273.7	262.5
	259.0	270.9	270.0	273.5	262.4
	258.0	271.7	266.0	274.0	262.6
Average	258.5	271.3	267.6	273.7	262.5
% Deviation	0.13%	0.09%	0.49%	0.061%	0.002%

TABLE X

SAPONIFICATION NUMBER OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (200 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Mgs. of KOH per 1 gram of sample	
	Treated	Control
Wesson Oil	193.7	191.7
	197.8	195.8
	195.1	194.1
Average	195.5	193.8
% Deviation	0.70%	0.70%
Butterfat	227.1	231.7
	229.6	225.3
	232.2	218.9
Average	229.6	225.3
% Deviation	0.74%	1.89%
Whole Dry Milk	279.9	262.4
	280.0	262.5
	278.0	262.6
Average	279.3	262.5
% Deviation	0.24%	0.02%

TABLE XI

SAPONIFICATION NUMBER OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (80 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Mgs. of KOH per 1 gram of sample	
	Treated	Control
Wesson Oil	192.4	197.4
	196.6	198.6
	197.5	197.0
Average	195.5	197.7
% Deviation	0.87%	0.27%
Butterfat	230.2	207.9
	230.5	213.7
	228.1	217.7
Average	229.6	213.1
% Deviation	0.35%	1.53%
Whole Dry Milk	270.8	271.1
	270.2	270.8
	268.9	272.8
Average	270.0	271.8
% Deviation	0.23%	0.24%

(Table X), which corresponds to some lowering of the molecular weight at that treatment. The gas plasma irradiation of 800 M.A., however, did not show any significant difference in the saponification number of any of the samples (Table XI).

In discussing some of the above-mentioned changes in the saponification numbers induced by irradiation, some authors' views (4,44,55), may apply to the results of this experiment. These authors suggested that cleavage of the molecule may be caused by irradiation, which subsequently may give rise to short chain volatile monomers; some of which have very distinct odors characterized as acrid and/or rancid. The non-volatile cleavage products may be monomeric, e.g., short chain semi-dicarboxylic acids, with carbonyl as second function, such as azelaic acid semi-aldehyde. They also suggested that the non-volatile cleavage products may be dimers or polymers.

V. CHANGES IN THE REFRACTIVE INDEX

The data obtained for the refractive index determination of gamma irradiated samples showed no significant difference between the treated and the controls (Table XII). Gas plasma irradiation of the intense treatment of 200 M.A.

TABLE XII

REFRACTIVE INDEX OF SAMPLES TREATED WITH GAMMA-IRRADIATION (CESIUM-137)

Sample	Refractive Index at 25°C. Dose				
	10^6	10^5	10^4	10^3	Control
Wesson Oil	1.4713	1.4714	1.4713	1.4712	1.4710
	1.4713	1.4714	1.4711	1.4712	1.4710
	1.4713	1.4714	1.4712	1.4711	1.4710
Butterfat	1.4600	1.4600	1.4600	1.4600	1.4602
	1.4600	1.4600	1.4600	1.4600	1.4602
	1.4600	1.4600	1.4600	1.4600	1.4602
Whole Dry Milk	1.4475	1.4473	1.4470	1.4471	1.4474
	1.4475	1.4473	1.4470	1.4471	1.4471
	1.4475	1.4473	1.4470	1.4471	1.4473

did show but a very slight increase in the refractive index with irradiation; so did the low treatment of 80 M.A., with the exception of the butterfat sample, which after treatment and standing for several days at refrigeration temperature showed a much higher increase in the refractive index compared to the control (Tables XIII and XIV).

Since the refractive indices of both fats and fatty acids increase with increase in the length of the hydrocarbon chains and the number of double bonds in the chain (3), and while the refractive index is related to molecular structure and unsaturation, for the same type of sample variations due to the latter are greater than variations from all other causes. The data obtained for refractive index in this experiment correlates, to good extent, with data for iodine and saponification numbers.

VI. CHANGES IN THE NITROGEN AND PROTEIN CONTENT

It is noted from the results of this experiment, that both gamma and gas plasma irradiation did not induce much of a change in the total nitrogen and protein content of whole dry milk. Although there was a tendency of increase of the nitrogen in the irradiated samples in all cases of

TABLE XIII

REFRACTIVE INDEX OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (200 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Refractive Index at 25°C.	
	Treated	Control
Wesson Oil	1.4712	1.4698
	1.4711	1.4698
	1.4711	1.4698
Butterfat	1.4603	1.4602
	1.4603	1.4602
	1.4603	1.4602
Whole Dry Milk	1.4470	1.4471
	1.4470	1.4471
	1.4470	1.4471

TABLE XIV

REFRACTIVE INDEX OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (80 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Refractive Index at 25°C.	
	Treated	Control
Wesson Oil	1.4695	1.4698
	1.4696	1.4694
	1.4696	1.4695
Butterfat	1.4704	1.4602
	1.4704	1.4602
	1.4704	1.4602
Whole Dry Milk	1.4580	1.4578
	1.4580	1.4578
	1.4579	1.4578

irradiation. This slight increase was even more profound in case of the intense gas plasma irradiation of 200 M.A. (Tables XV and XVI).

Goldblith and Proctor (22) have suggested that irradiation does, in some unknown manner, disturb the inherent stability of the casein micelles, and that the lability produced is then manifested in different ways, depending on the further treatment given to the milk. This author added that a subsequent storage gelation would, on the other hand, come about through a disturbance of the electrostatic balance of the micellar system that has occurred due to irradiation, and that is easily influenced by changes in the ionic composition of the milk. According to the mentioned author, the sites at which the disturbances have occurred are seemingly restricted sterically to the approach of cations such as Ca^{++} while permitting the interaction with other cations such as Mg^{++} and Mn^{++}. Goldblith and Proctor (22) further suggested that these sites could be pyro-phosphate linkages essential for the stabilization of the casein micelles; and which linkages that would be expected to react preferentially when subject to ionizing radiations.

TABLE XV

TOTAL NITROGEN AND PROTEIN OF WHOLE DRY MILK TREATED WITH GAMMA-IRRADIATION (CESIUM-137)

	Percent per dry weight				
	Dose				
	10^6	10^5	10^4	10^3	Control
% Nitrogen	4.26	4.19	4.23	4.23	4.15
	4.26	4.19	4.15	4.15	4.15
Average	4.26	4.19	4.19	4.19	4.15
% Protein	27.2	26.7	27.0	27.0	26.5
	27.2	26.7	26.5	26.5	26.5
Average	27.2	26.7	26.7	26.7	26.5

TABLE XVI

TOTAL NITROGEN AND PROTEIN OF SAMPLES TREATED WITH GAS PLASMA IRRADIATION (80 AND 200 MILLIAMPERS; 5 MINUTES; 3 MM. Hg.)

Sample	Percent per dry weight			
	Treated		Control	
	Nitrogen	Protein	Nitrogen	Protein
I. 80 milliampers; 5 minutes; 3 mm. Hg.				
Whole Dry Milk	4.16	26.6	4.14	26.3
	4.33	27.6	4.29	27.4
Average	4.24	27.1	4.15	26.5
II. 200 milliampers; 5 minutes; 3 mm. Hg.				
Whole Dry Milk	4.50	28.7	4.15	26.5
	4.38	27.9	4.15	26.5
Average	4.44	28.3	4.15	26.5

CHAPTER V

SUMMARY

A study was made on the effect of gamma and gas plasma irradiation on samples of Wesson Oil, butterfat, and whole dry milk.

Under conditions of the experiment reported in this paper it was found that:

1. Intense gas plasma irradiation of 200 M.A. has induced a decrease in the degree of unsaturation of Wesson Oil, while causing an insignificant change in the unsaturation of butterfat and whole dry milk.

2. Gamma irradiation has induced an insignificant change in the degree of unsaturation of the samples, with the exception of whole dry milk, where a significant increase in the degree of unsaturation was observed with increase of the dose.

3. A low intensity gas plasma-treatment of 80 M.A. induced a very insignificant change in the degree of unsaturation of all samples.

4. The primary effects of irradiation on all samples was the formation of free radicals, which produced a significant amount of peroxides that increased proportionally with the dose in gamma irradiation, and upon exposure to air, of the gas plasma irradiated.

5. Gamma irradiation caused increase of the free fatty acids content with dose increase; a decrease with the highest dose used.

6. Gas plasma irradiation, followed by exposure to atmosphere, caused an increase of the free fatty acids content with increase of intensity of treatment.

7. Some polymerization effected by high dose irradiation, which was insignificant in case of low dose treatment.

8. The two types of irradiation caused but an insignificant change in the refractive index of all samples, with the exception of gas plasma treated butterfat, which was exposed to the atmosphere for longer time after being irradiated.

9. Irradiation of both types used did but very slightly change the nitrogen and protein content. There was only a very slight increase with treatment.

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