THE EFFECT OF IONIZING RADIATIONS ON THE STORAGE STABILITY OF HYDROGENATED SHORTENING TREATED WITH CERTAIN ANTIOXIDANTS

by

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INTRODUCTION

A. Importance of the Problem.

The application of ionizing radiations for the preservation of food has opened a new horizon in the food industry. Research activities in this field, during the last fifteen years, have shown that these radiations can be used to sterilize food. This method of sterilization eliminates heat which is employed in the conventional method of canning and the drawbacks associated with it. There exists a plentiful supply of radioactive material as by-products from the atomic reactors to carry on this technique of food preservation. However, the use of these ionizing radiations in dosages sufficient to ensure sterilization, results in the production of undesirable side reactions. These may be changes in flavor, color, texture and the destruction of certain vitamins. It would be desirable to study these changes in order to find the means either of preventing or reducing them to a level where they would not be objectionable.

Actions of ionizing radiations have been the subject of recent reviews (28, 70). Many of the objectionable changes occurring in irradiated foods appear to originate in the lipid portion. A number of workers have demonstrated the formation of peroxides both during and after irradiation (20, p. 605-616; 49, p. 589; 51, p. 84-88; 55, p. 119-189). The breakdown products of these peroxides are believed to be responsible for the flavor changes in lipids. The exact nature

of these off-flavors and how they originate in irradiated lipids is not known. Attempts were made to prevent these flavor changes by introducing free radical acceptors which would prevent peroxide formation by terminating the chain reaction (2, p. 570-583). Astrack, et al. (2, p. 583) made two important observations in connection with the flavor changes: 1. Addition of the antioxidants, propyl gallate and butylated hydroxyanisole, did not prevent the development of irradiation odors. 2. The increase in peroxide value in irradiated oil did not cause a corresponding increase in off-odor and flavor. These observations cast some doubt on the role of peroxides in flavor changes. In view of this, more research is needed to establish the exact relationship between peroxides and flavor changes.

In studies on oxidative rancidity and reversion in fats and oils, aldehydes and ketones have been found among the oxidation products (9, p. 240; 34, p. 718; 35, p. 727; 36, p. 734; 66, p. 377; 69, p. 300). In some cases specific carbonyl compounds have been shown to be at least partially responsible for the off-flavors that developed (33, p. 377; 44, p. 117). These compounds are believed to be secondary products of autoxidation. It is very important, therefore, to know the extent of oxidative changes brought about in irradiated oils and fats and it is equally important to develop methods of preventing such changes.

A survey of literature showed that much work has been done on irradiated butterfat, lard, vegetable and fish oils. In all these cases, it has been found that irradiation causes oxidative changes. Since unsaturated oils are more susceptible to oxidative changes than

saturated oils, hydrogenation should help to prevent oxidative changes in irradiated fat. It is also known that the antioxidants will inhibit autoxidation in oils and fats. It is logical to assume, therefore, that both hydrogenation and incorporation of antioxidants in fats and oils might prove helpful in preventing irradiation induced autoxidation. Hydrogenated vegetable shortening is commonly used in the food industry, especially for deep fat frying purposes. Pre-cooked fish, shrimp, etcetera are processed with hydrogenated fat and stored for later use. Little or no work has been reported on the effect of ionizing radiations on the storage stability of hydrogenated vegetable shortening treated with antioxidants. In view of this, special attention was given in the present work to the determination of oxidative changes and storage stability of hydrogenated vegetable shortening treated with antioxidants and subjected to varying dosages of gamma rays.

B. Object of the Investigation.

It was the purpose of this work to investigate the oxidative changes and storage stability of hydrogenated vegetable shortening treated with antioxidants and subjected to ionizing radiations.

The oxidative changes, immediately after irradiation, after storage for three weeks and six weeks at 100°F were determined in terms of peroxide value, malonaldehyde content and total carbonyl values. Storage stability tests were conducted on several irradiated samples by the Active Oxygen Method.

REVIEW OF LITERATURE

A. Irradiation Induced Reactions in Lipids.

Radiations induce two types of effects in foodstuffs. They are distinguished as direct and indirect effects. Direct effects vary with the nature of the molecule. If it is large or stabilized by resonance, as in the benzene molecule, the effects do not occur. If the molecule has a weak bond, breakage occurs forming a free radical which can take part in a complex series of secondary reactions. These reactions are shown below.

Primary reaction:

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

Secondary reactions:

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

This irradiation of aliphatic hydrocarbons in the absence of oxygen may cause degradation into a series of hydrocarbons of lower and higher molecular weight, (reactions 1, 2 and 3) while in oxygen it may give peroxides (reaction 4). Indirect effects occur in most fresh foods in which water is a major component and takes place largely through reaction with irradiated water molecules. Reactions may be separated as follows:

$$H_2O \longrightarrow\!\!\!\!\!\!\sim\!\!\!\!\!\!\sim\!\!\!\!\!\!\sim\longrightarrow H^{\bullet} + {}^{\bullet}OH$$

The hydroxyl radical ($^{\bullet}OH$) is an oxidizing agent and the hydrogen

atom ($H^{\bullet}$) a reducing agent. A solute can, therefore, according to its chemical nature, be oxidized or reduced. If no oxidizable or reducible solute is present, these free radicals may recombine to form water again. The situation is modified in the presence of oxygen which increases the oxidative effects. Oxygen combines with a hydrogen atom to form a hydroperoxide radical ($HO_2^{\bullet}$) and hydrogen peroxide (H_2O_2), both of which are strong oxidizing agents (28, p. 16; 46, p. 160). In the presence of fat, it is also possible for the hydroxyl radical ($^{\bullet}OH$) to obtain an $H^{\bullet}$ from an active methylene group of fat giving a free radical: $- CH = CH - \overset{\bullet}{C}H - CH = CH -$. In fats, as before mentioned it is possible for both direct and indirect effects to cause irradiation damage through the formation of free radicals. The reactions involved are similar to those of autoxidation and are extremely complex. Since 1947 a torrent of literature has appeared on the elucidation of the mechanism of autoxidation. With the advent of modern instruments (polarograph, spectrophotometers) and modern isolation techniques (urea complexes, counter current distribution, chromatography, etcetera); considerable progress has been made in the study of the initial stages of oxidation of fats and separation and characterization of the reaction products. Several reviews (3, p. 147-167; 6, p. 1-21; 38, p. 48-53 and 68-69; 40, p. 1303-1309; 48, p. 126-132; 67, p. 700-703) have appeared in recent years which deal with the mechanism of autoxidation. Only a few high points concerning the present status of the problem are described in this text.

The fat or oil molecule consists of glycerol esterified with fatty acids which may be either saturated, unsaturated or both

saturated and unsaturated. Oxidation can occur only in the fatty acid portion of the glyceride molecule because the presence of a double bond is necessary for oxidation to occur readily.

Autoxidation.

The currently accepted theory of autoxidation is the radical chain theory. According to this theory oxidation takes place not at the double bond itself but at a reactive methylene group adjacent to the double bond with the production of a hydroperoxide which still retains it unsaturation. At the same time a resonance system, set up by the free radicals, leads to the production of conjugated isomers. Since geometric isomerization of a considerable proportion of double bonds from cis to trans also occurs, the product of the first stage of autoxidation is a complex mixture of hydroperoxides with a high content of conjugated and trans unsaturation (40, p. 1303). The evidence of conjugation is provided by Farmer et al. (22, p. 119-122) by measuring absorption in the ultraviolet region using a spectrophotometer. Privett et al. (54, p. 65) furnished further proof of the mechanism using infrared absorption techniques. They showed that autoxidized methyl linoleate contained at least 90 percent conjugated hydroperoxide.

At this stage it is necessary to point out the source of energy required for free radical production. A double bond is most susceptible to outside sources of energy such as light, heat or radiation energy. This energy puts the electrons in an excited state. When enough energy has been absorbed so that electrons reach a critical excitation level, the excess energy is dissipated by the electron

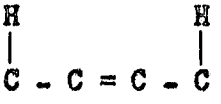
breaking away from the rest of the molecule and taking a portion with it. This leaves a fatty acid molecule with a carbon atom containing an unpaired electron which is an extremely unstable structure. This structure is called a free radical. The hydrogen atom that broke away from the molecule is also a free radical since it contains an unpaired electron.

According to Farmer (23, p. 86-93) the free radical formation from fat molecules is dependent on hydrogen lability. They have been classified into four groups according to hydrogen lability:

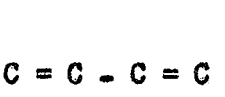
Group I

Group II

Group III



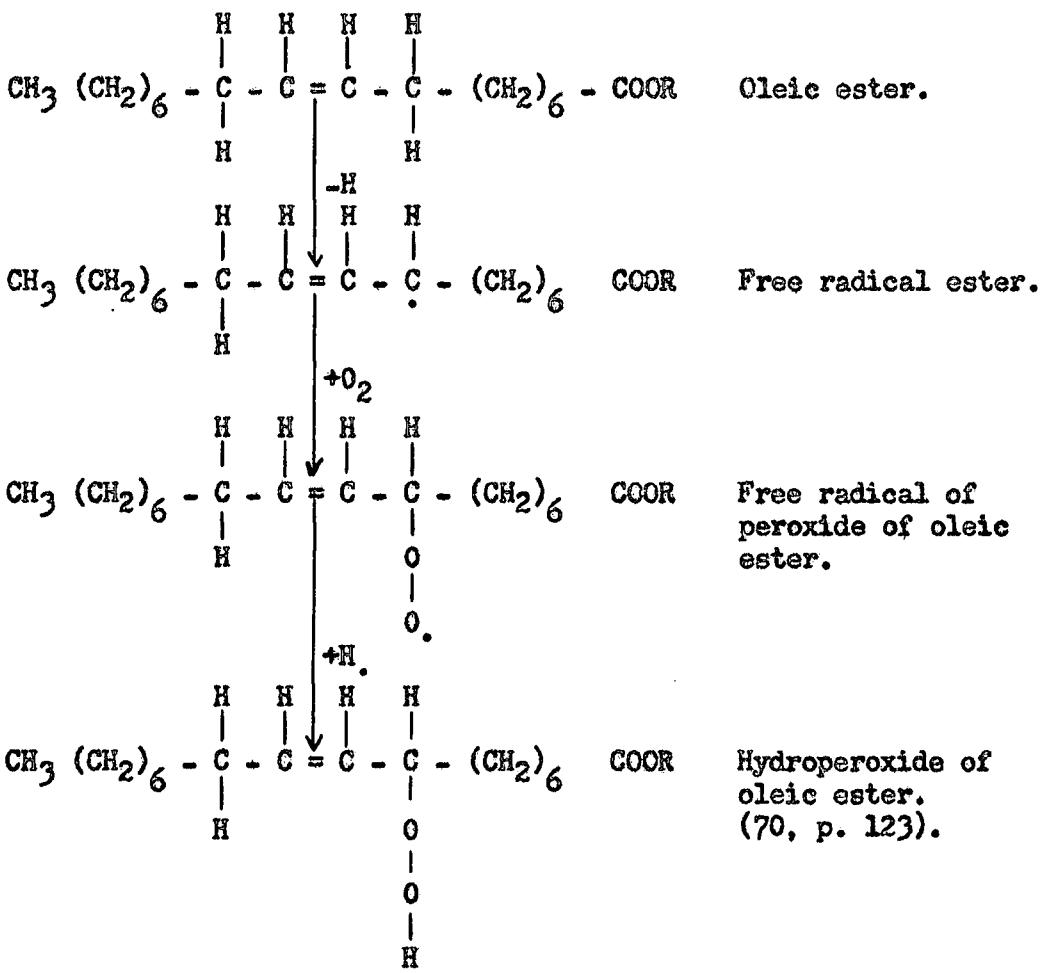
Group IV



Group I has the highest degree of hydrogen lability and Group IV the least. The least lability in Group IV is probably due to close proximity of the double bonds which give added stability by enabling the electron to dissipate excess energy through resonance. In Group I the movement of the electrons is considered to be blocked by CH_2 groups and hence an electron which has excess energy may expend it by breaking away from the remainder of the molecule. When it does so it takes a portion with it which is equivalent to the removal of a hydrogen atom leaving a free radical. Group II shows less lability because the influence of double bonds is divided between two methylene groups (23, p. 86-93; 38, p. 49).

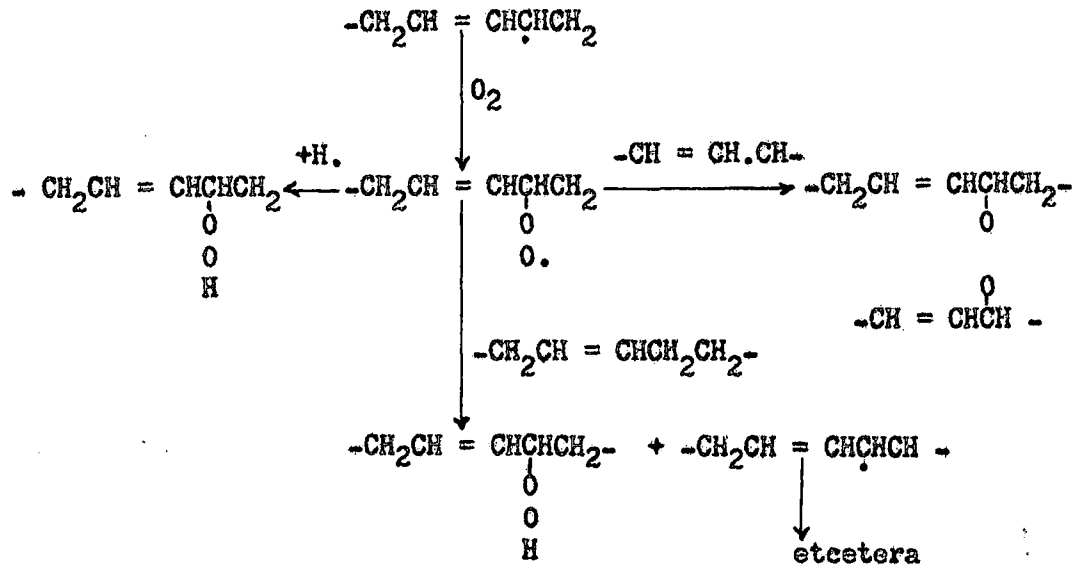
It is now generally accepted that autoxidation of unsaturated

fatty acids is initiated and propagated through an active methylene group. This is true when a methylene group is adjacent to and separates two unsaturated centers (48, p. 129). Saunder et al. (60, p. 83) recently showed that as much as 95 percent of the peroxides formed in autoxidation of methyl oleate are alpha-methylenic hydroperoxides. The overall changes that occur in the production of alpha-methylenic hydroperoxide of an oleic acid ester is presented in the following figure.



Reaction of Free Radicals.

Free radicals being extremely unstable substances will seek another electron to complete the stable paired electron structure. The fatty ester free radical can get an electron by several means as shown below.



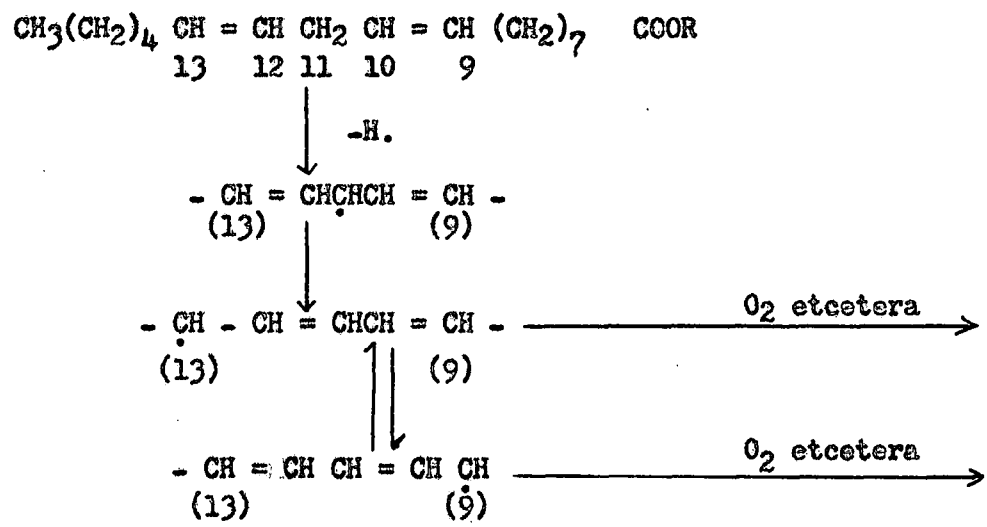
As shown in the above figure, the free radical has a high affinity for oxygen, and the oxygen, as molecular oxygen, adds on to produce a peroxide. The peroxide free radical reacts with hydrogen to produce a hydroperoxide which terminates the action of the peroxide free radical.

Another possibility is that the peroxide free radical can react with another molecule of oleic ester and remove an alpha methylenic hydrogen atom to produce a hydroperoxide. The oleic ester with which it reacted has now become a free radical similar to the one that caused its production. This is a self perpetuating reaction which is terminated only by reacting with another free radical or with

an antioxidant (38, p. 49).

Oxidation of Polyunsaturated Fatty Esters.

Oxidation of linoleate is a simple example for non-conjugated polyethenoic esters. It follows the same steps except that hydrogen which escapes is believed to come from the active methylene group. This is shown in the following figure:



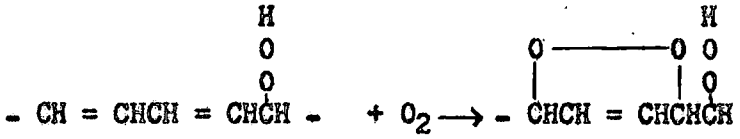
which represents the formation of free radicals with conjugated dienoic structures from a methylene separated dienoic fatty acid ester (linoleate) (38, p. 48-53).

The mechanism of oxidation of linolenate (trienoic acid) has not been studied extensively as that of oleates and linoleates. It has been assumed that oxidation of linolenate follows the same pattern as that established for olefine and diolefines.

Secondary Products of Autoxidation.

The monohydroperoxide formed is very largely conjugated and is not likely to form a dihydroperoxide on further oxidation. Some

analytical data (13, p. 450) support the view that a second molecule of oxygen can attack the linoleate molecule to form a cyclic peroxide by 1:4 addition to the monohydroperoxide, after which dimerization or polymerization takes place. The scheme of 1:4 addition is as follows:



(40, p. 1303)

A typical composition after the peak in peroxide has been passed was found to be about 30 - 35 percent peroxide, 25 - 30 percent hydroxy compounds, 20 - 25 percent oxirane compounds, 15 - 20 percent alpha, beta-unsaturated carbonyl compounds and some residual methyl oleate cleavage products and polymers (17, p. 223).

Recent investigations indicate that alpha, beta-unsaturated carbonyls are among the most important secondary products of autoxidation. Ellis (21, p. 140) isolated alpha, beta-unsaturated carbonyl compounds from autoxidized oleic and elaidic acids. These products appeared to have come directly from alpha-methylenic hydroperoxides simply by loss of water.

Swift, et al. (68, p. 39-40) have shown that fatty hydroperoxides readily decompose under suitable conditions such as high temperature, presence of catalysts, acids or alkalies to yield a complicated mixture of degradation products including aldehydes, ketones and alcohols.

Steam volatile products from cottonseed oil autoxidized at 70°C have yielded 2, 4-decadienal, 2-octanal and N-hexanal. They were isolated and identified by fractional crystallization of their

semicarbazones (69, p. 297-300).

Evidence has been accumulating that some of these hydroperoxide decomposition products, particularly the alpha, beta-unsaturated aldehydes and ketones contribute very largely to the unpleasant odor and flavor of oxidized fats. In the dairy field, the 'oil fishy flavors' are being attributed to the presence of oxidation products of unsaturated acids which are carbonylic compounds. These carbonylic compounds include 2-hydroxypropanal, a C_7-unsaturated ketone and a C_{12} alpha, beta-unsaturated carbonylic compound either aldehyde or ketone. These have been isolated by Keeney and Doan (34, p. 718).

Kawahara and Dutton (33, p. 377) have demonstrated the formation of carbonyl compounds from autoxidized soy oil. Buss and Mackinney (12, p. 487-489) have isolated carbonyl compounds from rancid corn oil. All these authors have reported that the off-flavors in rancid oil are due to these carbonyls. Recent work of Chang and Kummerow (14, p. 407; 15, p. 327) indicate that carbonyls are responsible for rancid flavor.

Influence of Fatty Acid Composition.

The breakdown products of oxidized oleic acid are believed to be responsible for most of the offensive odors in rancid fats. Linoleic with two double bonds, linolenic with three double bonds, and arachidonic with four double bonds do not produce the intense tallowy, rancid odor that comes from oleic acid oxidation. The peroxides and hydroperoxides formed during oxidation are not responsible for the

rancid odor as they are odorless (38, p. 48). In the case of linoleic acid, the hydroperoxide formed is more stable and hence does not easily produce off-flavors whereas oleic acid does although the initial tendency to oxidize is less (40, p. 1304).

B. Irradiation of Oils, Fats and Antioxidants.

The first publication concerning the influence of ionizing radiations on oils appears to stem from the work of Long and Moore in 1927 (42, p. 901-903). They studied the action of cathode rays on linseed and other drying oils and observed a decrease in iodine number, an increase in molecular weight, a bleaching of color and a reduction in drying time.

Recently Dunn et al. (20, p. 605-616) studied the action of X-rays on butter and olive oil. They noticed a slight increase in peroxide values in irradiated samples but the rate of increase was not linear. It was also noticed that the orange-yellow color of butter was progressively destroyed with an increase in radiation dosage. Mukherjee (49, p. 589) noticed peroxide formation in butterfat during, as well as after, irradiation. He also observed that butterfat was more susceptible to autoxidation when irradiated in the presence of oxygen whereas irradiation of evacuated fat did not produce this effect. Peroxide formation in mackerel tissue during irradiation was observed by Nickerson et al. (51, p. 84-88).

The effects of sterilizing doses of high intensity electron bursts upon various vegetable and fish oils have been examined by chemical and organoleptic means by Astrack et al. (2, p. 570-583).

They observed that changes in vegetable and fish oils were dependent upon the individual radiation sensitivity of the oil. Changes in fish oil were more pronounced than vegetable oils. This indicates the destruction of a naturally present protective system. The addition of antioxidants before irradiation seemed to offer a possibility for prolonging the storage life of the oil. Five-hundredths of one percent propyl gallate seemed to be quite effective for this purpose. One-hundredth of one percent butylated hydroxyanisole, although effective, caused undesirable organoleptic changes. The organoleptic changes in irradiated oils did not seem to run parallel with the chemical changes and were not influenced by the presence or absence of antioxidants. However, oxygen as well as air must play a role in the formation of radiation induced off-flavors since treatment in vacuum or inert gas inhibited the occurrence of organoleptic changes. According to these authors the radiation initiated mechanism involved polymerization, bond breakage, as well as a variety of other oxidative changes.

Hannan and Boag (29, p. 152-153) on irradiating butterfat with high energy electrons, observed that the peroxide value depended upon the temperature of the sample during irradiation. This value increased with decrease of temperature. This is believed to be due to the stable character of peroxides at lower temperatures. Further, they noticed an increase in peroxide formation with an increase of radiation dosage at any given temperature. Hannan and Shepherd (30, p. 1021-1022; 31, p. 36-41) showed that the changes which occurred during the irradiation of butterfat were followed by extensive changes after

irradiation and both were affected by temperature. The main influence of temperature was on the after-effect which showed a maximum reaction rate at -20°C. Fats irradiated at 0°C or -70°C showed a striking increase in peroxide value during the first two days of storage at -20°C. The increases in peroxide value were smaller at -20°C and barely significant at +20°C or - 70°C. No after-effect was observed after irradiation at +20°C, while molten fat at +37°C failed to show any peroxide formation. It is believed that these changes are related to the physical state of the fat. The after-effect was explained on the basis of persistence at temperatures in the neighborhood of -20°C of a free radical which reacts with oxygen as it diffuses into the fat.

Hannan and Shepherd (30, p. 1021-1022) have reported that natural antioxidants were destroyed during and after irradiation. Chipault et al. (16, p. 1715) have reported that added propyl gallate in lard samples was completely destroyed during irradiation on subjecting the samples to gamma rays with 2×10^6 rep at ambient temperature.

Chemical changes in meat fats that occur during irradiation with gamma rays and subsequent storage of the fats were investigated by Sribney et al. (65, p. 958-960). They observed marked increases in peroxide values when gamma irradiated fats were stored at 5°C in an oxygen permeable casing, but very little increase when oxygen was excluded.

In addition to peroxides, the presence of small amounts of carbonyl compounds in irradiated fats was demonstrated by Sribney et al. (65, p. 959). Batzer et al. (4, p. 705) observed an increase in

carbonyl compounds in both meat and fat with increasing irradiation dosages. They suggested that the carbonyl compounds obtained from meat were different than those obtained from irradiated fat. Lang and Proctor (39, p. 239) showed the formation of monocarbonyl compounds in irradiated refined vegetable oils by high energy cathode rays.

Very recently Sedlacek (61, p. 547-556) has reported a number of interesting observations concerning the influence of ionizing radiations on fats. With soy oil, he reported that the samples subjected to low dosage (70,200 r) showed little increase in peroxide numbers during the first thirty days of storage. The samples radiated with higher doses showed higher peroxide numbers immediately after irradiation and also on storage. The peroxide number of soy oil samples exposed to rays increased faster on storage at 20°C than those samples stored at 4°C. This is contrary to the observation made by Hannan and Shepherd (30, p. 1022; 31, p. 36-41) who reported that peroxide formation was higher at lower temperatures. With butter and lard, Sedlacek reported that gamma rays adversely affected the organoleptic properties and caused an increase in peroxide numbers and thiobarbituric acid (TBA) values. In the case of hardened food fat, higher peroxide values were observed immediately after irradiation with gamma rays with a total dose of 76,500 r. However on storage the radiated and control samples showed the same rate of change. Results of the TBA method showed oscillating values and the acid number was low in both cases.

C. Irradiation of Simple Systems Analogous to Fats.

A basic understanding of the changes involved in irradiated oils and fats can best be sought by irradiating simpler systems. Sheppard and Burton (62, p. 1636-1639) irradiated saturated fatty acids with alpha particles in the absence of oxygen and water. They found hydrogen, carbon monoxide, carbon dioxide, water and volatile hydrocarbons in the gas phase. Saturated hydrocarbons and water soluble short chain fatty acids constituted the non-volatile products. The main reactions appear to be dehydrogenation and decarboxylation. There was some evidence that dehydrogenation did not occur in the same molecule as decarboxylation since unsaturation was not found in the nonsaponifiable products.

Burton (11, p. 4117-4119) reported the formation of stearic acid on bombarding oleic acid with deutrons. It was shown that hydrogen produced by decomposition of an organic molecule under the influence of radioactivity can react at the double bond of a neighboring molecule. This suggested that hydrogenation also took place. He also found the formation of polymers when pure oleic acid was bombarded with deutrons.

Whitehead and co-workers (71, p. 186-187) noted that as the chain length increases the relative amount of hydrogen formed over carbon dioxide increases and suggested that dehydrogenation may also occur without decarboxylation.

Mead (45, p. 470-472) irradiated linoleic acid with low doses of X-rays and showed that a chain reaction was induced similar to that of autoxidation. This study was followed by Polister and Mead

(52, p. 201) and it was shown that induced autoxidation of methyl linoleate was markedly influenced by the presence of certain antioxidants in admixture with the ester. It was found that lipid soluble antioxidants were more effective in preventing chain reactions than water soluble compounds. Tocopherol and Ionol (butylated hydroxytoluene) prevented the reaction without significant damage to themselves. Vitamin A, ascorbic acid, glutathione and cysteine, although partially effective in preventing chain reactions, were destroyed. Calciferol partially protected the esters but was not itself destroyed.

Dugan and Landis (19, p. 152-154) studied the effect of high energy radiation such as gamma rays from cobalt 60 on oxidized oleic acid and its esters. They observed that gamma radiation from cobalt 60 influences the oxidation of oleic acid and methyl oleate even at low temperatures. Determination of peroxide values and the $E^{1\%}_{1\ cm.}$ values at 224 mμ revealed that higher peroxide values could be obtained but that secondary products are formed in appreciable quantities. The products causing absorption at 224 mμ may be alpha, beta-unsaturated ketones. The level of these substances could be increased by irradiation-oxidation in the presence of metal soaps such as cobalt stearate. Irradiation-oxidation of methyl oleate through a series of temperature ranges revealed a marked thermal activation effect.

Very recently Chipault et al. (16, p. 1713-1720) reported the effects of beta and gamma radiations on methyl palmitate, methyl oleate, methyl linoleate, corn oil esters, corn oil, lard, butterfat, oleate and linoleate soaps, and methyl linoleate-urea complex. According to them, irradiation of fats with high energy ionizing

radiations in the presence of oxygen produces appreciable amounts of peroxides and carbonyl compounds. The flavor and odor changes caused did not correlate well with either peroxide values or carbonyl values. They stated that ozone and nitrogen compounds may be formed when fats are irradiated in air and some of the odor and flavor compounds may result from the action of these compounds on fats. The antioxidant, propyl gallate, in general, hindered the accumulation of peroxides and this effect was more marked with the more highly unsaturated substrates. Stability of lard containing propyl gallate was greatly reduced by irradiation under oxygen indicating that the antioxidant was largely destroyed during irradiation.

D. Method of Controlling Undesirable Changes in Irradiated Fats.

Although the typical changes characteristic of heat processing are absent in irradiated foodstuffs, other undesirable effects on taste, odor, color, and texture nevertheless can occur (7, p. 536-537). The off-flavor in irradiated foodstuffs is likely to occur due to oxidation of flavor molecules by the hydroxyl radical (.OH) formed in the product containing water. The change may be represented as follows:

$$\text{Flavor molecule} + (.OH) \longrightarrow \text{oxidized off-flavor}$$

If on the other hand, there is mixed with food some compound that acts as a free radical acceptor (FRA) and competes with the flavor molecule for (.OH) radical, it would be possible to minimize or reduce off-flavor.

$$\text{Flavor molecule} + (.OH) + \text{FRA (red)} \longrightarrow$$

$$\text{Flavor molecule} + \text{FRA (ox)} + (OH^-) \qquad (57, \text{p. } 65)$$

Proctor and Goldblith (57, p. 65) have accomplished the elimination of

off-flavors, in the case of several foods, but using ascorbic acid, d-isoascorbic acid and their salts.

Huber, et al. (32, p. 109-115) have discussed a number of methods applicable to prevent undesirable changes in irradiated foodstuffs. Those methods include freezing, vacuum packing, stripping with inert gas, regulation of dose rate, and addition of chemical protectors.

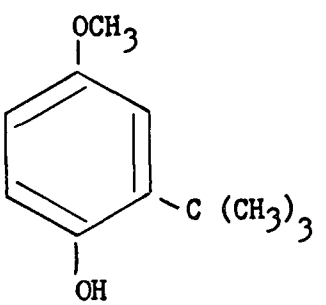
It has been shown that side effects may be minimized in the frozen state (55, p. 167). The advantages of creating vacuum and nitrogen packing on the rancidity development in various fish oils has been reported by Astrack et al. (2, p. 570-583). Dose rate has been found to have a considerable influence on irradiation changes in foodstuffs (8, p. 246). High dose rates are useful for protection of color and flavor in foodstuffs (32, p. 109-115). The undesirable effects that occur in irradiated fats are largely oxidative in nature and involve reactions with free radicals (2, p. 570; 29, p. 152; 30, p. 1021). The addition of chemical protectors to prevent the irradiation induced chain reactions due to free radicals has been reported by Proctor et al. (56, p. 237-242).

In the present work several free radical acceptors are used to prevent the undesirable effects that are likely to occur in irradiated fats. Therefore, a brief review of these free radical acceptors is made in this text.

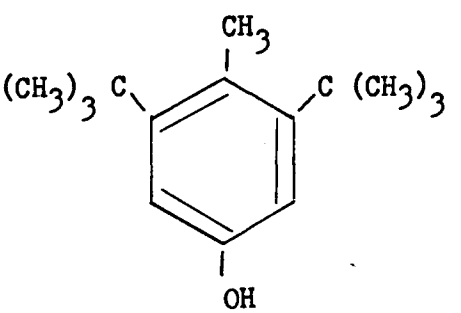
Free radical interceptors have been investigated for various foodstuffs and the use of these compounds are limited by legal considerations. Gallic esters and similar polyphenols are useful for oils and fats (2, p. 570-583; 29, p. 152; 30, p. 1021). These are

usually ortho or para substituted phenols or aromatic amines (41, p. 621). Free radical interceptors which are used in fats and oils to prevent autoxidation are also termed antioxidants. Antioxidants give up hydrogen free radicals more readily than do fatty acids. These hydrogen free radicals react with the peroxide free radical formed in oil and thereby terminate the chain reaction. Of the legally allowed antioxidants, some of the important ones are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nordihydroguaiaretic acid (NDGA) and propyl gallate (PG).

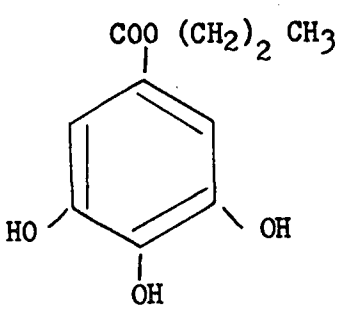
Their structures are represented below to show the similarity that exists in them.



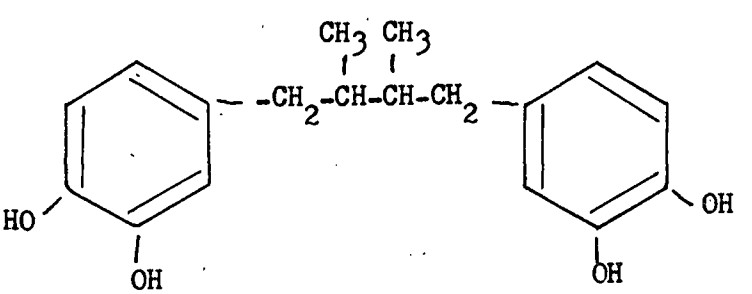
Butylated hydroxyanisole (BHA)



Butylated hydroxytoluene (BHT)

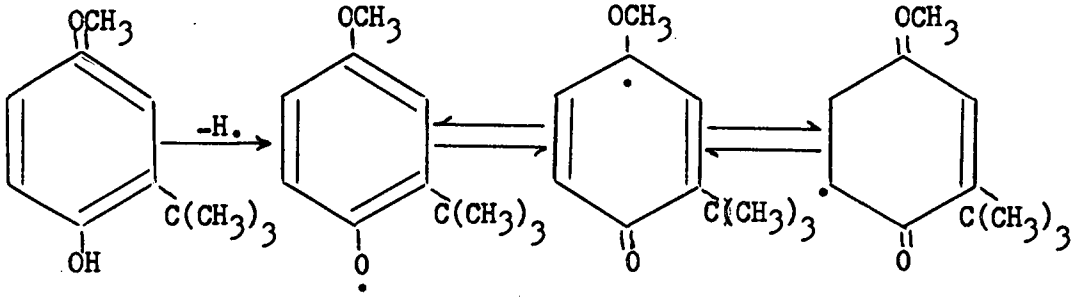


Propyl gallate (PG)



Nordihydroguaiaretic acid (NDGA)

All these are phenolic antioxidants. Thus far only phenolic antioxidants are approved for use because they are nontoxic. Their action, as described before, is due to production of hydrogen free radicals. When the antioxidant gives up a hydrogen atom it becomes a free radical as shown in the following figure:

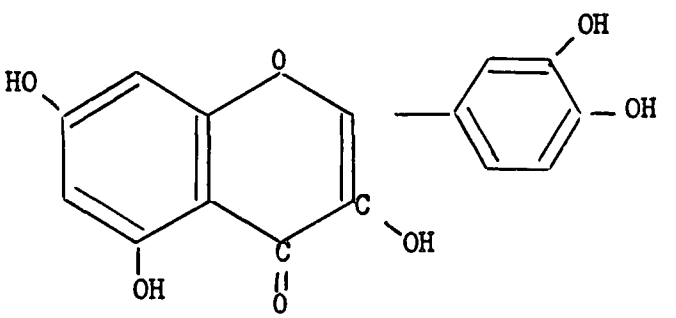


This free radical which is called a semiquinone has much greater stability than a free radical of the type produced from fatty acid because of resonance. This semiquinone does not have a strong enough attraction for a hydrogen atom to remove it from an unsaturated fatty acid molecule. However, if it encounters a free hydrogen atom the original antioxidant would be restored. Eventually all the antioxidant disappears because of other reactions (38, p. 52).

BHA and BHT differ from PG and NDGA, among other things, in the number of OH groups attached to the ring structure. This grouping has an important effect on the water solubility. BHA and BHT are completely insoluble in water, whereas PG and NDGA are slightly soluble. A general rule about the solubility of these compounds has been noted by many workers in this field. It appears that the more water soluble, the greater carry through it will have in the finished

product. This holds especially for products requiring heat such as pastry and crackers. BHA has more carry through ability than BHT. Antioxidants with slight water solubility impart the greatest AOM stability or shelf life to lard and shortening (25, p. 386-387).

In addition to the above mentioned antioxidants, another compound, quercetin (Q) was used as an antioxidant in the present work. It is a flavone derivative. Its structure is as follows:



Quercetin is 3, 5, 7, 3', 4' pentahydroxy flavone, slightly soluble in water and stable to heat.

Greenbank and Holm (26, p. 243) reported the effectiveness of quercetin as an antioxidant for cottonseed oil and Bradway and Mattill (10, p. 2405) for a mixture of lard and codliver oil. Richardson et al. (59, p. 410) found quercetin to be effective as an antioxidant for milkfat and lard. According to them the group $-\overset{\overset{O}{\|}}{C}-\overset{|}{C}=\overset{|}{C}-$ in the pyrone ring is responsible for antioxidant activity.

EXPERIMENTAL METHODS

A. Preparation of Samples.

The hydrogenated vegetable shortening used in this experiment was obtained from Swift and Company, Portland, Oregon in the form of a fifty pound block packed in cellophane. The commercial name of this shortening is Vream shortening. The physical and chemical constants of this shortening, as furnished by Swift and Company, are as follows: Free fatty acid 0.04; melting point 118°- 212°F; softening point 112°- 116°F; iodine number 72-77; Lovibond Color 1.5 red - 20 yellow; keeping test (AOM) sixty hours. This shortening was melted, canned in (307 x 409) "C" enameled cans and stored at 0°F. The shortening, as needed, was taken from these cans, antioxidants were incorporated at the required levels and canned in thermal death time (TDT) cans for chemical analysis and in half pound flats (307 x 200 . 25) "C" enameled cans for AOM and antioxidant recovery experiments. In each case a small head space was left in the cans. The small TDT cans in sets of four were further sealed in half pound flat cans before irradiation. This had to be done because the apparatus for irradiation was designed to hold only half pound flats, number 2 or number 10 cans. The TDT can is a special type of can, two and one-half inches in diameter and three-eighths inch deep. The small amounts of samples needed for the experiment could be conveniently filled in these cans, leaving a small head space. Glass containers were avoided because it has been found that radiations cause color changes in glass (70, p. 26 and 308). Metallic containers such as tin cans are believed to be

non-reactive.

B. Incorporation of Antioxidants.

The antioxidants were incorporated into the shortening as indicated below at concentrations of 0.02 and 0.05 percent.

1. Butylated hydroxyanisole (BHA) was directly dissolved in the melted shortening at the specified amounts.

2. Butylated hydroxytoluene (BHT) was also directly dissolved in the melted shortening at the specified amounts.

3. Nordihydroguaiaretic acid (NDGA) was dissolved as follows: A 0.5 percent solution was first prepared by dissolving NDGA in the melted shortening and then the appropriate amounts of the solution were added to the main portion to get 0.02 percent and 0.05 percent solutions.

4. Propyl gallate (PG) was treated the same way as NDGA.

5. Quercetin (Q) was first dissolved in hot absolute alcohol in a relatively high concentration and then suitable quantities were added to obtain 0.02 and 0.05 percent solutions. The alcohol was then driven off by passing nitrogen over the solution maintained at from 80° - 90°C for five hours. The complete removal of alcohol was determined as directed in Fiegel's Spot Tests (24, p. 129-130).

C. Shipping and Handling Conditions.

All of the samples except one set, which was retained at the laboratory, were packed in Shamrock carriers under dry ice conditions and shipped by Railway Express to the Materials Testing Reactor, Idaho Falls, Idaho. During the ten day interval between shipment and receipt,

the samples were kept frozen except during the irradiation period when they were maintained at ambient water temperature. When the samples were received, one set of samples required for AOM and antioxidant recovery were placed at 0°F until the analyses were made. The other two sets were placed at 100°F for three weeks and six weeks for storage stability studies. At the end of these intervals the samples were placed at 0°F.

D. Radiation Source and Dosage Levels.

Irradiation was accomplished by exposing the cans to a gamma grid. The flux, the dosage and the time of exposure were varied according to the total dose required. Each set of samples were given the total dose of 0, 1.5, 3.0 and 4.5 megarads respectively. The following table illustrates the exposure time and dose rate for each level of radiation.

Total Dose (megarads)	Dose Rate (rad/hr)	Exposure Time (hrs)
0	- - - -	None
1.5	3.10×10^6	0.483
3.0	5.45×10^6	0.550
4.5	5.00×10^6	0.900

E. Analytical.

The samples were subjected to the following determinations in order to ascertain the oxidative changes brought about by gamma irradiation and subsequent storage.

1. Determination of peroxide value (POV). American Oil Chemist's Society tentative method (1, Cd 8-53) was used for

determination of peroxide values. This method determines, all substances, in terms of milliequivalents of peroxide per 1000 grams of sample, which oxidizes potassium iodide under the conditions of the test. These are assumed to be peroxides or other similar products of fat oxidation.

2. 2-Thiobarbituric acid method (TBA number). The TBA procedure described by Yu and Sinnhuber (72, p. 105) was adopted. In this experiment a sample size of one gram was used. Instead of the reagent described in the procedure only 75 ml. of 0.6 N-hydrochloric acid was added after refluxing the fat sample and reagent for 30 minutes. The reagent described in the procedure was necessary to extract red color from fish meal. Since this reagent was unnecessary in case of fat, only hydrochloric acid was used. The rest of the procedure was exactly the same as described for fish meal. The results were expressed as TBA number or milligrams of malonaldehyde per kilogram of sample (64, p. 632).

3. Determination of total carbonyl content. The procedure described by Berry and Mckerrigan (5, p. 693-701) for determining the total carbonyl content was adopted. In this procedure instead of using a 50 ml. graduated flask for color development, a 25 ml. graduated flask was used and all the volumes of the reagents were cut into half. The contents of saturated and unsaturated carbonyls were calculated separately as described in the procedure using the formulae,

Unsaturated Carbonyls $u = 4.344\ E_{460} - 3.373\ E_{430}$ μ moles/25 c.c.

Saturated Carbonyls $s = 5.812E_{430} - 4.420E_{460}$ μ moles/25 c.c.

The saturated and unsaturated carbonyls were expressed in millimoles per kilogram of fat using the following formulae:

$U = \frac{u \times 5}{W}$ and $S = \frac{s \times 5}{W}$ in which U and S are the values calculated from the above equations and W is the weight of fat used.

The above mentioned methods were employed to determine the state of oxidation immediately after irradiation and on subsequent storage for three weeks and six weeks at 100°F.

No significant oxidative changes in irradiated fat were found after three weeks and six weeks storage at 100°F. However the small changes observed indicated the possible deterioration of irradiated fat on long storage. Therefore, in order to evaluate the oxidative changes on long storage, heat accelerated stability tests were carried out on some of the samples. This stability test was made by the AOM or Active Oxygen Method. The method has been adopted by the American Oil Chemist's Society as a tentative standard method for determing fat stability (58, p. 394-398). Briefly, the method is as follows: Clean and dry air is bubbled at a controlled rate (2.33 ml/sec) through a number of tubes of fat maintained at 97.8°C in a constant temperature bath and the number of hours required for the fat to reach an arbitrarily chosen peroxide value (POV) is recorded. The latest recommendation is a POV of 125 milliequivalents per kilogram as the 'rancid point' for all fats. The length of this period is assumed to be an index of resistance to rancidity. The exact relationships between peroxide values and such quantities as shelf life, actual rancidity, and oxidative stability have not been established. However,

the results serve as a guide to infer the possible oxidative stability of fats treated in different ways.

The AOM stability test was run on six samples. The details are given under results and discussion.

F. Quercetin Determination.

In 1947 Porter et al. (53) reported a spectrophotometric method for the determination of quercetin extracted from Douglas-fir bark. This method was abandoned primarily because a linear Beer's law relation did not exist and because of instability of quercetin in the final dilute alcoholic solution. Later workers developed direct methods involving the formation of a quercetin-aluminum chloride complex (50, p. 613-616). In these methods, the tannins and phlobaphenes interfered in the alcoholic solutions, and results were inconsistent in aqueous solutions of aluminum chloride. The critical examination of the variables and optimum conditions led to the development of a rapid, accurate and reproducible analytical procedure (18, p. 1184-1187). Modifications of the aluminum chloride method have been used to determine quercetin aglycone in several foods such as onions, strawberries, apricots and applesauce (18, p. 1186). However, such a complicated method does not seem necessary to extract and estimate quercetin in fat. The method adopted in the present work to extract and estimate quercetin in shortening is described below. The technique described here is simple, accurate and yields reproducible results.

Procedure:

1. Absorption Spectra. The spectral absorption curve for pure quercetin in 72 percent ethanol was obtained with a Beckman DK-1 Spectrophotometer, scanning time ten minutes, chart speed five inches per minute. It is shown in Figure 1 on page 51.

2. Preparation of Standard Curve. A standard solution of quercetin containing 0.015 mg/ml was prepared. A volume of each standard solution corresponding to 0.030, 0.045, 0.060, 0.075, 0.090, 0.105, 0.120, 0.135 and 0.150 mg. of quercetin per 10 ml. of alcohol was pipetted into 25 ml. volumetric flasks and made up to volume with 72 percent alcohol. The absorbance at 375mμ as measured against a reagent blank (72 percent alcohol) was plotted against weight of aglycone taken for analysis.

3. Recovery of Quercetin From Fat. Ten grams of fat containing 0.05 percent quercetin was dissolved in 50 ml. of petroleum ether (B.P. 20°- 40°C) and was extracted in a 500 ml. separatory funnel three times with 25 ml. aliquots of 72 percent ethyl alcohol by shaking three minutes per extraction. The fourth extraction was made using 60 ml. of 72 percent ethyl alcohol and shaking for one minute. The four extracts were combined and diluted to a suitable volume (200 ml.) with 72 percent ethyl alcohol and filtered through E and D folded filter paper (Grade No. 512). The clear alcoholic extract contained the quercetin. This extraction procedure was standardized by extracting in three ways as described below:

(i) Extraction with 25 ml. of 72 percent ethyl alcohol once by shaking

for three minutes and then with 60 ml. of 72 percent ethyl alcohol for one minute.

(ii) Extraction with 25 ml. of 72 percent ethyl alcohol twice by shaking for three minutes per extraction and then with 60 ml. of 72 percent ethyl alcohol for one minute.

(iii) Extraction with 25 ml. of 72 percent ethyl alcohol three times by shaking for three minutes per extraction and then with 60 ml. of 72 percent ethyl alcohol for one minute.

It was found necessary to select a suitable filter paper which did not absorb quercetin. For example, Reeve Angel-genuine American filter paper (creped surface, white paper of open texture, rapid filtering) No. 202, size 11 cm. was found to absorb quercetin. The E and D folded filter paper, grade 512, size 12½ cm. did not absorb quercetin. The results obtained using the latter filter paper agreed with the centrifuged sample.

4. Spectrophotometric Determination. Ten ml. of clear alcoholic extract recovered from shortening, as described above, was transferred to a 25 ml. volumetric flask and made up to volume with 72 percent ethyl alcohol. The absorbance of each solution was determined immediately at 375 mμ against the reagent. The blank experiment was conducted using the shortening without quercetin.

5. Stability of Quercetin in 72 Percent Ethyl Alcohol. It is pointed out by Dowd (18, p. 1184) that the spectrophotometric method of Porter et al. (53) is untenable because of the instability of quercetin in the final dilute alcoholic solution. Therefore a stability

test was made to see whether quercetin was unstable in 72 percent ethyl alcohol. This is the concentration which was used to extract quercetin from the shortening in the above procedure.

For this purpose quercetin was extracted from shortening, containing a known amount of quercetin, with 72 percent ethyl alcohol. This extracted alcoholic solution was diluted in the usual way and the absorbance was measured at 375 mμ. Considering this as the zero time, the absorbance at 375 mμ was measured at frequent intervals for one and one-half hours and the absorbance was plotted against time. Recovery results are discussed under Results and Discussion.

By adopting the procedures (3) and (4) described above and using the standard curve described in procedure (2), the quercetin content in the control and radiated samples were determined. The quercetin content in shortening, which was subjected to passage of nitrogen for five hours at 80°- 90°C in order to remove alcohol, was also determined by the same procedure.

RESULTS AND DISCUSSION

The results obtained in this investigation show the effect of gamma radiation on hydrogenated vegetable shortening alone and treated with various antioxidants. The storage stability of these irradiated samples as measured by several chemical tests is reported.

In presenting and discussing the results, the following terms are used with the meanings as indicated below.

Terms	Meaning
1. Lab. control	Samples kept in the laboratory
2. 0 Rad.	Shipped control
3. 1.5 Rad.	Samples subjected to 1.5 megarad
4. 3.0 Rad.	Samples subjected to 3.0 megarad
5. 4.5 Rad.	Samples subjected to 4.5 megarad

Results of Chemical Analyses.

The object of this experiment was to investigate the effect of ionizing radiations on hydrogenated vegetable shortening alone and stabilized with certain antioxidants. The effect of radiation was evaluated on the basis of the oxidative changes. The oxidative changes were measured in terms of peroxide value, total carbonyl value and TBA number. The results are shown in Tables 1 to 9.

The results obtained indicate that radiation has caused little or no effect on the autoxidation of the shortening. It would be erroneous to draw conclusions on the effectiveness of antioxidants used in these studies. Therefore, no attempt is made to judge the effectiveness of different antioxidants on the basis of these results. However, an attempt is made to find out the oxidative changes, if any, on radiated samples. For this purpose, the presence of antioxidants is ignored in all the samples and the mean values of all the samples at each level of radiation are calculated. These mean values are shown in the Summary Chart, Table 10.

In the Summary Chart (Table 10) it is seen that there is a small increase in peroxide values and total carbonyl values between radiated and non-radiated samples. It is evident, from the values in the chart (Table 10) that there is a slight increase in total carbonyl values with the increase in radiation dosage level.

Table 1. Peroxide Values (POV) of Samples Subjected to Different Levels of Radiation and Stored for 0 Week:

Irradiation dosage (megarads).	SAMPLES												
	Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	0.44	- -	0.61	0.29	0.44	0.65	0.40	0.39	0.77	0.20	0.41	4.60	0.46
0	0.53	1.65	0.51	0.59	0.30	0.45	0.39	0.49	0.90	0.20	0.20	6.22	0.57
1.5	0.38	0.81	1.41	1.02	o.57	0.73	1.01	1.10	1.63	1.00	0.34	11.00	1.00
3.0	0.61	0.98	4.39*	0.79	0.98	0.59	1.13	0.80	0.80	1.21	0.40	8.29	0.83
4.5	0.49	0.72	0.66	0.58	0.78	0.78	0.91	1.29	1.95	2.19	0.37	10.72	0.975

* Since it is very high, considered as an error.

Table 2. Peroxide Values (POV) of Samples Subjected to Different Levels of Radiation and Stored for Three Weeks at 100°F.

Irradiation dosage (megarads).	SAMPLES												
	Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	0.61	1.10	0.57	0.40	0.41	0.59	0.65	0.30	0.49	0.46	0.58	4.90	0.45
0	0.40	1.28	0.73	0.69	0.41	1.19	0.40	0.40	0.60	0.39	0.42	6.91	0.63
1.5	0.54	1.09	0.62	0.41	0.34	0.85	0.80	0.70	0.72	1.39	0.19	7.65	0.70
3.0	0.43	1.33	1.82	1.25	0.30	0.85	0.58	0.20	0.93	0.59	0.21	8.49	0.77
4.5	0.40	3.06	3.40	0.45	0.67	0.83	0.48	0.59	0.57	0.58	1.24	12.27	1.12

Table 3. Peroxide Values (POV) of Samples Subjected to Different Levels of Radiation and Stored for Six Weeks at 100°F.

Irradiation dosage (megarads)	SAMPLES												
	Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	1.10	1.98	1.98	0.83	1.02	0.70	0.74	0.61	0.59	0.89	0.89	11.33	1.03
0	1.02	1.93	0.76	0.63	1.42	0.61	0.62	0.59	0.60	0.99	0.55	9.72	0.88
1.5	0.40	1.03	0.57	0.40	0.40	0.59	0.20	0.48	0.31	1.37	0.62	6.38	0.58
3.0	0.55	1.19	0.52	0.44	0.61	0.67	0.18	0.69	0.61	0.49	0.38	6.33	0.58
4.5	0.35	0.49	0.21	0.20	0.20	0.50	0.56	0.38	1.86	0.63	0.20	5.58	0.51

Table 4. Carbonyl Values of Sample Subjected to Different Levels of Radiation and Stored for 0 Week.

Irradiation dosage (megarads)		SAMPLES Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	U	2.04	2.75	3.21	2.09	2.64	2.60	2.28	2.52	2.20	1.59	1.69	25.61	2.33
	S	9.92	4.59	4.22	7.06	6.21	6.45	5.72	7.20	7.64	9.71	10.55	79.27	7.21
	T	11.96	7.34	7.43	9.15	8.85	9.05	8.00	9.72	9.84	11.30	12.24	104.88	9.54
0	U	1.69	2.60	2.37	2.29	2.09	2.17	0.75	2.57	2.60	1.71	2.53	23.37	2.12
	S	9.14	9.49	6.67	7.43	8.60	7.73	7.42	6.69	6.36	8.56	6.66	84.75	7.70
	T	10.83	12.09	9.04	9.72	10.69	9.90	8.17	9.26	8.96	10.27	9.19	108.12	9.82
1.5	U	1.14	4.98	5.35	- -	1.95	2.86	3.18	2.61	3.44	3.14	2.80	31.45	3.15
	S	10.31	5.07	5.29	17.25*	10.41	7.35	7.61	8.03	6.66	8.16	6.87	93.01	8.46
	T	11.45	10.05	10.64	17.25*	12.36	10.21	10.79	10.64	10.10	11.30	9.67	124.46	11.61
3.0	U	2.29	2.94	3.97	2.15	3.29	1.83	3.88	2.79	1.51	2.39	2.14	29.12	2.65
	S	8.77	8.93	9.52	8.43	8.56	7.47	7.75	7.90	11.72	12.41	9.01	100.47	9.13
	T	11.06	11.87	13.49	10.58	11.85	9.30	11.63	10.69	13.23	14.80	11.15	129.59	11.78
4.5	U	6.04	2.41	3.09	3.09	1.25	3.21	1.75	2.88	3.74	2.75	1.99	32.20	2.93
	S	5.35	11.61	9.92	7.98	12.72	8.85	10.55	10.34	9.15	10.89	9.31	106.67	9.70
	T	11.39	14.02	13.01	11.07	13.97	12.06	12.30	13.22	12.89	13.64	11.30	138.97	12.63

* Considered as an error. U = Unsaturated S = Saturated T = Total

Table 5. Carbonyl Values of Samples Subjected to Different Levels of Radiation and Stored for Three Weeks at 100°F.

Irradiation dosage (megarads)		SAMPLES Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	U	4.45	4.57	4.61	2.97	2.56	2.58	3.32	2.20	1.72	- -	2.61	29.61	2.96
	S	4.98	4.98	4.74	6.74	7.72	6.35	4.35	8.18	9.62	16.68	8.14	82.48	7.50
	T	9.43	9.55	9.35	9.71	10.28	8.93	7.67	10.38	11.34	16.68	10.75	111.09	10.46
0	U	1.61	6.49	1.96	1.94	0.94	1.76	2.25	1.62	4.38	4.42	4.24	31.61	2.87
	S	8.69	8.65	6.06	8.79	10.45	8.11	7.39	8.72	3.17	1.14	3.49	74.66	6.79
	T	10.30	15.11	8.02	10.73	11.39	9.87	9.64	10.34	7.55	5.56	7.73	106.27	9.66
1.5	U	5.04	2.65	3.18	3.32	2.63	2.38	2.11	3.10	2.37	3.54	2.52	32.84	2.99
	S	3.52	9.95	7.80	7.99	7.19	10.00	10.39	5.70	10.81	18.45*	9.24	101.04	9.19
	T	8.56	12.60	10.98	11.31	9.82	12.38	12.50	8.80	13.18	21.99*	11.76	133.88	12.18
3.0	U	0.40	2.29	2.58	2.42	2.97	1.84	0.46	2.53	5.57	1.40	2.37	24.83	2.26
	S	10.70	8.57	10.54	9.29	7.51	10.88	13.25	10.77	8.12	12.83	10.94	113.40	10.31
	T	11.10	10.86	13.12	11.71	10.48	12.72	13.71	13.30	13.69	14.23	13.31	138.23	12.57
4.5	U	4.72	2.66	1.71	0.68	2.21	3.24	0.94	2.43	2.54	2.47	2.38	25.98	2.36
	S	9.34	8.87	11.03	12.50	11.19	9.15	11.86	10.13	11.02	10.76	13.76	119.61	10.87
	T	14.06	11.53	12.74	13.18	13.40	12.39	12.80	12.56	13.56	13.23	16.14	145.59	13.23

* Considered as an error. U = Unsaturated S = Saturated T = Total

Table 6. Carbonyl Values of Samples Subjected to Different Levels of Radiation and Stored for Six Weeks at 100°F.

Irradiation dosage (megarads)		SAMPLES Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	U	3.11	2.89	3.25	3.03	2.34	2.73	2.99	2.47	0.79	2.90	3.06	29.56	2.69
	S	6.84	7.84	6.95	7.21	7.24	7.28	7.13	14.65	12.42	5.80	6.21	89.67	8.15
	T	9.95	10.73	10.20	10.24	9.58	10.01	10.12	17.12	13.21	8.70	9.27	119.23	10.84
0	U	2.59	2.06	6.95	2.68	1.36	2.12	4.30	2.06	1.14	2.78	0.17	28.21	2.56
	S	7.19	8.43	1.59	6.61	10.19	5.54	11.37	9.13	10.80	7.91	13.24	92.00	8.36
	T	9.78	10.49	8.54	9.29	11.55	7.66	15.67	11.19	11.94	10.69	13.41	120.21	10.92
1.5	U	3.68	4.81	3.45	1.64	0.15	3.11	1.66	3.01	3.13	3.33	3.95	31.92	2.90
	S	5.06	17.37*	6.20	10.86	13.44	7.51	11.97	7.96	7.33	8.87	16.55	113.12	10.28
	T	8.74	22.18*	9.65	12.50	13.59	10.62	13.63	10.97	10.46	12.20	20.50	145.04	13.18
3.0	U	1.95	1.39	0.88	2.14	1.44	1.78	0.81	1.75	3.67	1.41	2.27	19.49	1.77
	S	10.53	13.84	13.26	9.56	10.37	11.45	13.14	10.39	9.18	11.49	9.51	122.72	11.16
	T	12.48	15.23	14.14	11.70	11.81	13.23	13.95	12.14	12.85	12.90	11.78	142.21	12.93
4.5	U	4.80	5.08	0.37	2.65	0.43	2.38	0.91	- -	2.61	2.06	1.87	23.16	2.11
	S	5.69	7.84	0.88	8.89	2.53*	9.77	14.24	17.90	15.13	11.70	12.53	114.92	10.45
	T	10.39	12.92	9.17	11.54	2.97*	12.15	15.15	17.90	17.74	13.76	14.40	138.08	12.56

* Considered as an error U = Unsaturated S = Saturated T = Total

Table 7. 2-Thiobarbituric Acid (TBA) Number of Samples Subjected to Different Levels of Radiation and Stored for 0 Week.

Irradiation dosage (megarads)	SAMPLES												
	Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	0	0	0	0.16	0.17	0.13	0.17	2.35	0.88	2.45	2.71	9.02	0.82
0	0.25	0.44	0	0.29	0.43	0.40	0.24	0.27	0.20	0.22	0	2.74	0.25
1.5	0.05	0.67	0.66	1.11	1.07	0.55	0.97	0.64	0.62	0.54	0.47	7.35	0.57
3.0	0.48	0.65	0.83	0.75	0.62	0.59	0.55	0.83	0.86	0.62	0.61	7.39	0.67
4.5	0.19	0.68	0.68	0.64	0.51	0.63	0.64	0.70	0.87	0.75	0.48	6.77	0.62

Table 8. 2-Thiobarbituric Acid (TBA) Number of Samples Subjected to Different Levels of Radiations and Stored for Three Weeks at 100°F.

Irradiation dosage (megarads)	SAMPLES												
	Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	0.36	0.28	0.04	0.35	0.19	0.17	0.09	0.46	0.33	0.39	0.48	3.14	0.29
0	0.48	0.48	0.50	0.24	0.32	0.29	0.04	0.15	0.26	0.23	0.05	3.04	0.28
1.5	0.74	0.74	0.52	0.52	0.48	0.46	0.45	0.69	0.72	0.83	0.87	7.02	0.64
3.0	0.63	0.62	0.54	0.56	0.40	0.35	0.44	0.79	0.93	0.75	0.59	6.60	0.60
4.5	0.42	0.63	0.60	0.73	0.67	0.74	0.45	0.39	0.57	0.32	0.48	6.00	0.55

Table 9. 2-Thiobarbituric Acid (TBA) Number of Samples Subjected to Different Levels of Radiation and Stored for Six Weeks at 100°F.

Irradiation dosage (megarads)	SAMPLES												
	Control	0.02% BHA	0.05% BHA	0.02% BHT	0.05% BHT	0.02% NDGA	0.05% NDGA	0.02% PG	0.05% PG	0.02% Q	0.05% Q	Total	Mean
Lab. Controls	0.73	0.69	0.74	0.26	0.29	0.91	0.36	0.23	0.36	0.16	0.39	5.12	0.47
0	0.32	0.66	0.48	0.19	0.29	0.16	0.14	0.08	0	0	0	2.32	0.21
1.5	0.41	0.67	0.44	0.21	0.40	0.35	0.27	0.29	0.36	0.62	0.54	4.20	0.38
3.0	0.78	0.79	0.65	0.43	0.17	0.38	0.33	0.37	0.64	0.41	0.86	5.81	0.53
4.5	0.32	0.70	0.27	0.22	0.08	0.04	0.38	0.69	0.54	0.99	0.61	4.84	0.44

Table 10. Summary Chart: Average of Peroxide Values (POV), Total Carbonyl Values, and 2-Thiobarbituric Acid (TBA) Number of Samples Subjected to Different Dosages and Stored for Different Periods (Weeks).

Irradiation dosage (megarads)	Storage Periods (Weeks)								
	Peroxide Values			Total Carbonyl Values			TBA Number		
	0	3	6	0	3	6	0	3	6
Lab. Control	0.46	0.45	1.03	9.53	10.46	10.84	0.82	0.29	0.47
0	0.57	0.63	0.89	9.82	9.66	10.92	0.25	0.28	0.21
1.5	1.00	0.70	0.58	11.61	12.18	13.18	0.67	0.64	0.38
3.0	0.83	0.77	0.58	11.78	12.57	12.93	0.67	0.60	0.53
4.5	0.98	1.12	0.51	12.63	13.23	13.56	0.62	0.55	0.44

Previously several authors have shown that oils or fats undergo oxidative changes during irradiation and are susceptible to extensive oxidative changes on subsequent storage (2, p. 570-583; 20, p. 605-616; 29, p. 152-153; 42, p. 901-903; 49, p. 589; 55, p. 167). These studies were related to vegetable and fish oils which were not hydrogenated. This effect is attributed to the relatively unstable, unsaturated fatty acid chains present in these oils. In the present work, a hydrogenated vegetable shortening with an iodine value of 72-77 was used. The investigations of Bailey and Fisher (1946) as reported by Gunstone (27, p. 96) suggest that linolenate and linoleate are more readily hydrogenated than oleate. Therefore, the unsaturation of the shortening used in this experiment is due to oleate content rather than to linoleate and linolenate. The oleate is less susceptible to oxidation than linoleate and linolenate (40, p. 1304). This is believed to be the reason why irradiation did not cause appreciable increases in oxidative changes in the shortening used in the present work. According to Sheppard and Burton (62, p. 1639) dehydrogenation takes place on irradiating saturated fatty acids with alpha particles. If dehydrogenation had occurred in the shortening upon irradiation, it would have given scope for further oxidative changes. Therefore, it appears that the stable character of the shortening has resisted such dehydrogenation at active centers.

The peroxide values remained relatively constant after three weeks storage at 100°F even with samples that had been irradiated as much as 4,500,000 rad. Although slight changes were observed in the

initially radiated samples, additional changes were not evident after storage for three weeks at 100°F. These results are contrary to the results reported by Sedlacek (61, p. 547-556). According to his report the hardened food fat which was subjected to only 76,500 rad. and storage at 20°C showed increases in peroxide values.

After six weeks storage at 100°F, there were some changes in peroxide values. In non-radiated samples the peroxide values had increased and in radiated samples the peroxide values had decreased. This is indicative of peroxide decomposition in radiated samples and peroxide accumulation in non-radiated samples. Further this is suggestive of a more rapid formation and decomposition of peroxides in radiated samples than in non-radiated samples. This sort of transitory nature of peroxides in radiated samples has been reported by Hannan and Shepherd (31, p. 36-41) in connection with their work on butterfat held at various temperatures.

The values in the fourth vertical column of Table 10 suggest that radiation has affected the samples as reflected in the increase in total carbonyl values of radiated samples. Increases in peroxide values in radiated samples have been pointed out in the first observation. It appears that there is simultaneous decomposition of peroxides and an increase in total carbonyl values. The total carbonyl values have increased on storage of samples for three weeks at 100°F as evidenced in Column 5 of Table 10. Although there is a slight increase in peroxide values of the samples in storage for three weeks at 100°F, there is a considerable increase in total carbonyl values

during this period. This may be due to the fact that peroxides formed have decomposed. This sort of transitory nature of peroxides causes the POV determination to be of questionable value for measuring small oxidative changes. However, the total carbonyl value determination is a very sensitive method of ascertaining small oxidative changes.

There has been some increase in total carbonyl values of both radiated and non-radiated samples on storage for six weeks at 100°F. This increase in total carbonyl values is possibly related to the decomposition of peroxides as evidenced in Table 10.

The TBA numbers in vertical columns 7, 8, and 9 of Table 10 indicate very little changes. According to Kenaston et al. (37, p. 35) the TBA method is a reliable method for estimating the oxidation products of linoleic and linolenic acids but it is insensitive to oxidation products of oleic acid. They suggested that the mechanism is due to condensation of TBA with aldehydes formed during autoxidation of fats. Sidwell et al. (63, p. 605) reported that the TBA test is a better indicator of fat stability than peroxide or carbonyl tests but the behaviour of the TBA test is associated with autoxidation of linolenic acid content of the fat. Recently Sinnhuber, Yu and Yu (64, p. 633) have reported that TBA reactive material is malonaldehyde. This aldehyde is likely to arise from unsaturated acids such as linolenic acid, arachidonic acid, etcetera and not from oleic acid. As pointed out in earlier discussions in this text, the fat used in this experiment mainly contains oleic acid and probably negligible portions of linoleic and linolenic acids. This seems to be the reason why TBA

values have not shown any oxidative changes.

The net result of this work discussed so far can be briefly stated as follows: Gamma radiations have very little effect on the shortening, with or without antioxidants according to the chemical tests used. The stable character of the shortening has resisted extensive oxidative changes during irradiation with dosages up to 4.5 megarads and on subsequent storage for six weeks at 100°F. A survey of literature showed that little or no work has been done on irradiation of hydrogenated vegetable shortening. When the present investigation was taken up, Sedlacek's paper (61, p. 547-556) came to the writer's knowledge. This appears to be the only work reported so far on hardened food fat. According to this paper the hardened food fat produced, immediately after irradiation, poor organoleptic properties and a higher peroxide content. On subsequent storage, the peroxide number of the radiated fat was higher than that of the control sample. However, the difference between these samples were smaller in comparison with the beginning. The radiation dosage and storage period employed by them were 76,500 r and 20°C for a period of 100 days respectively. The dosage level which ranges from 1.5 to 4.5 megarad and storage temperature 100°F for six weeks used in the present investigation were far higher than those reported by Sedlacek (61, p. 549). Naturally more deteriorative changes were expected in the samples receiving higher dosage and stored at higher temperatures, but the results obtained in the present work indicate very little oxidative changes on radiation and on subsequent storage.

The results discussed so far only indicate that the shortening used in this investigation is resistant to extensive oxidative changes on irradiation and on subsequent storage for six weeks at 100°F. However, the possibility of deterioration on long storage and with the availability of considerable amounts of oxygen is not precluded. Therefore, the Active Oxygen Method was further employed on several samples. The results and discussion of this experiment are discussed in the next section.

Another interesting observation made during the analyses of the samples was that the color of all radiated samples except the one containing quercetin were bleached. This indicated that the natural carotenoid pigments were bleached and confirms the observations of Long and Moore (42, p. 903), Hannan and Shepherd (31, p. 39) and Luckton and Mackinney (43, p. 632). The assumption that the samples containing quercetin were not bleached indicates the stable nature of the quercetin molecule and its possible resistance to irradiation destruction.

Active Oxygen Method (AOM) Results.

The Active Oxygen Method was employed on several samples to determine the effect of ionizing radiations on the stability of fat under accelerated storage conditions and to test the effect of antioxidants under these conditions. The samples used in this test were as follows:

1. The irradiated and non-irradiated control (Vream without antioxidant) samples.

2. The irradiated and non-irradiated samples with 0.02 percent BHT.
3. The irradiated and non-irradiated samples with 0.02 percent quercetin (Q).

In this work only the samples subjected to 4.5 megarads were used. Results obtained in terms of number of hours required for the fat to reach a peroxide value of 125 milliequivalents are presented in the following table.

Table 11 - AOM Value in Hours.

Radiation dosage (megarads)	SAMPLES		
	Control (without antioxidant)	0.02% BHT Vream	0.02% Q Vream
0	122	146	146
4.5	102	122	141

It is clear from Table 11 that radiated samples were less stable than the controls. Further it is evident from the data that the antioxidants BHT and Q have prevented the oxidative changes both in controls and radiated samples. In controls, the antioxidants BHT and Q have offered protection to oxidative changes to the same extent. In radiated samples, the sample treated with quercetin is more stable to oxidative changes than that treated with BHT. This indicates the possible destruction of a small amount of BHT when subjected to ionizing radiations whereas quercetin is unaffected under similar conditions.

Moore and Bickford (47, p. 1-4) have reported in their study

of 13 different antioxidants on the AOM stability of lard, cottonseed oil and cottonseed shortening. Their study did not include either BHT or quercetin. They found that propyl gallate was the most effective of the antioxidants tested. In the present work it has been shown that BHT and quercetin at 0.02 percent level exhibits equal antioxidant effect. Further the greater antioxidant ability of quercetin in radiated samples indicates that quercetin is resistant to radiation destruction and is more effective in radiated samples.

Quercetin Analysis.

1. The spectral absorption curve for pure quercetin in 72 percent alcohol at pH7 is shown in Figure 1 on page 51. It is evident from the Graph that absorption maximum is at 375 mμ.

2. The standard curve is drawn from the following values obtained from this experiment.

Mg. of quercetin in 72 percent alcohol	OD at 375 mμ
0.030	0.080
0.045	0.125
0.060	0.160
0.075	0.205
0.090	0.246
0.105	0.283
0.120	0.330
0.135	0.366
0.150	0.405

The absorbance of the samples mentioned above were measured in a Beckman DU Spectrophotometer. The standard curve shown in Figure 2 page 53 was drawn by plotting the absorbance versus weight of aglycone.

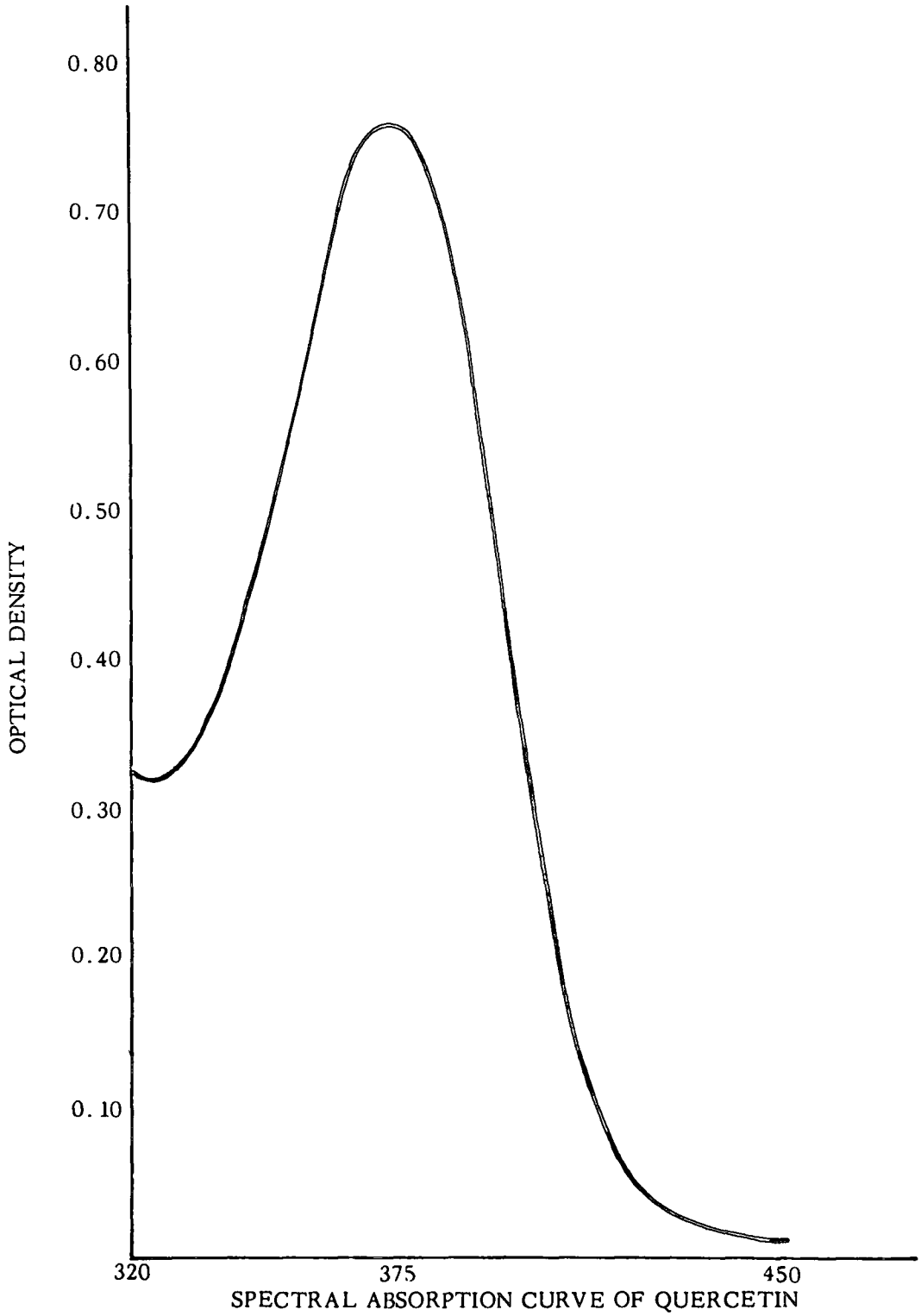

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
OPTICAL DENSITY
320
375
450
SPECTRAL ABSORPTION CURVE OF QUERCETIN
(0.001% in 72% Ethanol)

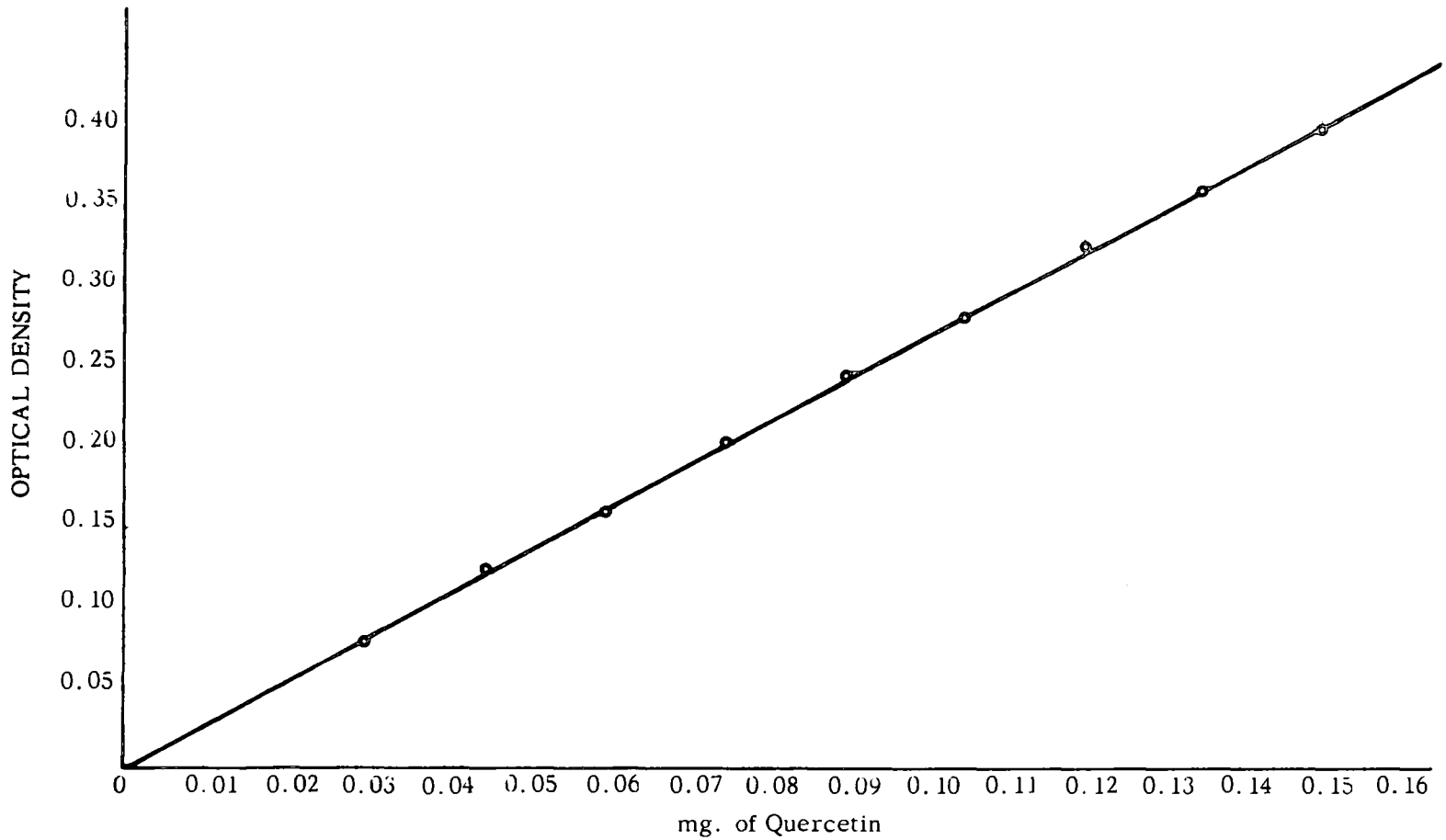
Figure 1

3. The stability curve was drawn from the following values. These values were obtained by extracting quercetin from a 0.05 percent Q sample as described before and by measuring the OD at 375 mμ for one and one-half hours at frequent intervals.

Time (in minutes)	OD at 375 mμ
0	0.337
10	0.336
20	0.336
30	0.334
40	0.333
50	0.330
60	0.330
90	0.327

The graph was drawn by plotting the absorbance versus time in minutes. The graph is shown on page 54, Figure 3. From the graph it is seen that quercetin in 72 percent alcohol, as extracted from fat, is quite stable. There is only three percent loss of quercetin in the interval of one and one-half hours. Therefore this method could be adopted to estimate quercetin in fat samples.

4. Quercetin content in control and radiated samples were determined by the method described before. The results are shown in Table 12, page 55.



STANDARD CURVE OF QUERCETIN

Figure 2

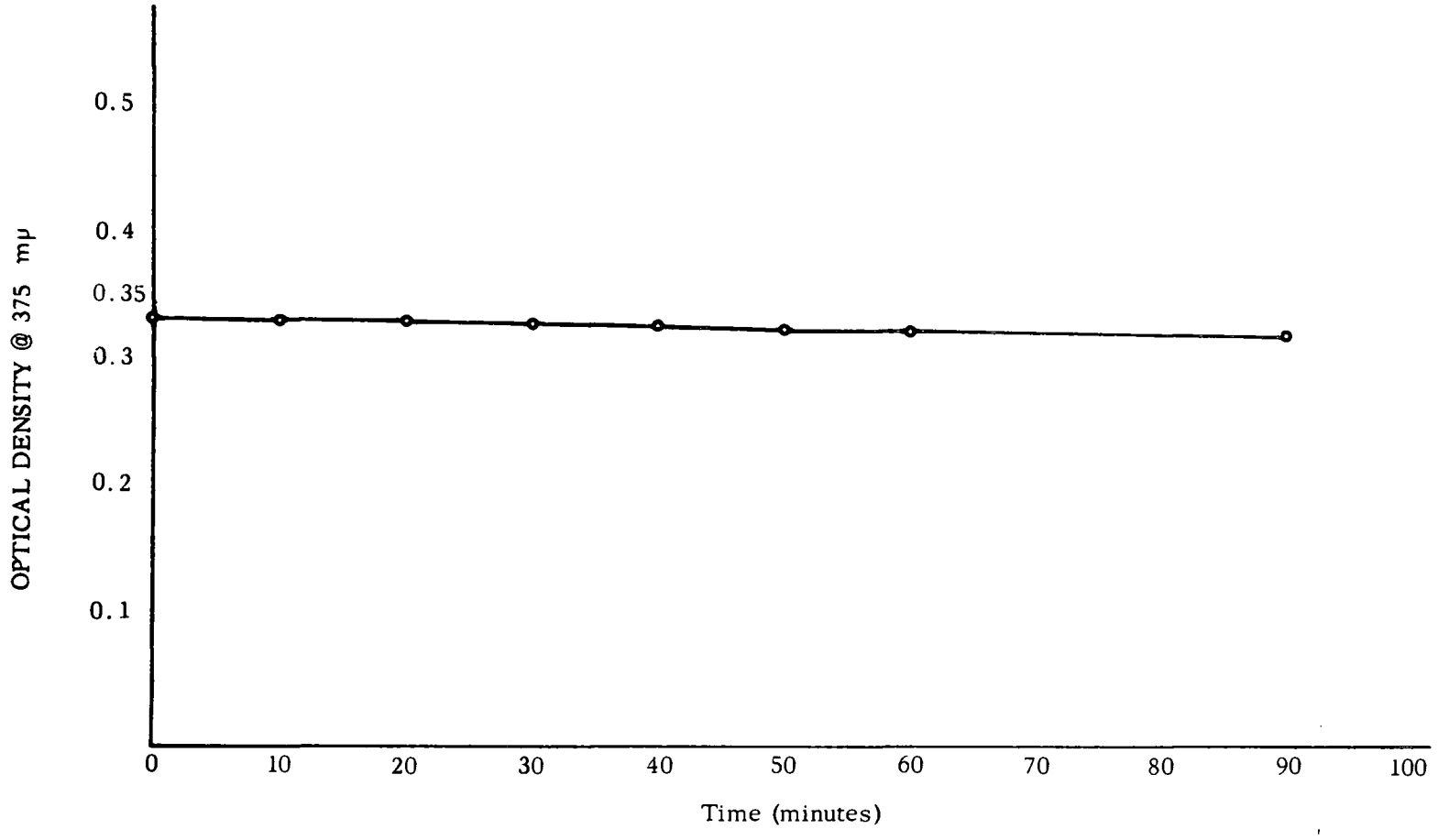


Figure 3 STABILITY CURVE OF QUERCETIN

Table 12 - Quercetin Analysis in Irradiated Samples.

Samples	Recovery in 0.02 percent sample.	Percent destruction in 0.02 percent sample.	Recovery in 0.05 percent sample.	Percent destruction in 0.05 percent sample.
Lab. Control	0.0200	nil	0.0500	nil
0 Rad.	0.0197	1.5	0.0510	nil
1.5 Rad.	0.0193	3.5	0.0492	1.6
3.0 Rad.	0.0187	6.5	0.0490	2.0
4.5 Rad.	0.0196	2.0	0.0476	4.8

The maximum destruction which occurred in 0.02 percent samples subjected to 4.5 megarads was 2.0 percent. In the case of a 0.05 percent sample subjected to 4.5 megarads, the destruction was 4.8 percent. These results suggest that quercetin is quite stable to radiation destruction.

5. The effect of passing nitrogen for five hours at 80°- 90°C on the quercetin content of Vream shortening was determined as follows: A 0.05 percent solution of quercetin in Vream shortening was prepared by dissolving the alcoholic solution of quercetin in the shortening. A portion of the sample was saved for control analysis and the other portion was subjected to the passage of nitrogen at 80°- 90°C for five hours. Recovery analysis were made in both the samples. In the control sample, the amount of quercetin recovered was 0.047 percent. In the sample subjected to the passage of nitrogen, the amount of quercetin recovered was 0.05 percent. Within the limits of experimental

error, the results indicated that the passage of nitrogen at 80°- 90°C for five hours to drive out alcohol from fat had not destroyed quercetin.

SUMMARY

The object of this investigation was to study the influence of ionizing radiations on the storage stability of hydrogenated vegetable shortening treated with several antioxidants.

The survey of literature indicated little investigation on the influence of ionizing radiations on hydrogenated vegetable shortening. Much work has been reported on vegetable and fish oils which are non-hydrogenated. A considerable amount of work has been accomplished by several investigators on irradiated butterfat. In all these investigations it has been found that oils and fats undergo oxidative changes when subjected to ionizing radiations. These oxidative changes are believed to be responsible for off-flavors associated with irradiated lipids and lipid containing foodstuffs. Attempts to control these changes during irradiation center around a relatively few techniques. These are (1) the removal of oxygen, (2) the hydrogenation, (3) use of antioxidants, (4) selection of irradiation dosage and temperature and (5) storage temperatures. Some of these techniques are employed in the present investigation. The investigations include the following.

1. Effect of different levels of ionizing radiations on Vream shortening alone and treated with different antioxidants.

2. Effect of ionizing radiations on the shortening on subsequent storage for three weeks and six weeks at 100°F.

3. Effect of different antioxidants in preventing the oxidative changes during and after irradiation of Vream shortening.

4. Active Oxygen Method stability test on radiated and non-radiated samples.

5. Comparison of effectiveness of different antioxidants such as BHT and quercetin on radiated and non-radiated samples using AOM Stability Test.

CONCLUSIONS

1. Ionizing radiations caused very small oxidative changes in Vream shortening during irradiation as well as on subsequent storage for six weeks at 100°F. This shows that the fat used in this investigation is relatively stable and is little affected by irradiation.

2. There was no perceptible increase in peroxide values with the increase in radiation dosage.

3. The peroxide values of samples remained relatively constant after storage for three weeks at 100°F.

4. The peroxide values increased in non-radiated samples and decreased in radiated samples on storage for six weeks at 100°F.

5. There was a slight increase in total carbonyl values with the increase in radiation dosage and on subsequent storage for three and six weeks at 100°F.

6. Changes in TBA values were negligible with the increase in radiation dosage as well as on subsequent storage.

7. AOM Stability Tests showed that radiated samples were less stable than the non-radiated samples.

8. Comparison of BHT and quercetin on irradiated and non-irradiated samples by AOM Stability Tests showed that BHT and quercetin were equally effective in preventing oxidative changes in control samples whereas in radiated samples quercetin was more effective than BHT.

9. The recovery tests showed that quercetin was unaffected by ionizing radiations.

BIBLIOGRAPHY

1. American Oil Chemists Society. Official and tentative methods. 2nd ed. Chicago, 1946-1960. 2 vols.

2. Astrack, A. et al. Effects of high intensity electron bursts upon various vegetable and fish oils. Food Research 17:570-583. 1952.

3. Bateman, L. Olefin oxidation. Chemical Society, London, Quarterly Reviews 8:147-167. 1954.

4. Batzer, O. F. et al. Production of carbonyl compounds during irradiation of meat and meat fats. Journal of Agricultural and Food Chemistry 5:700-705. 1957.

5. Berry, N. W. and A. A. Mckerrigan. Carbonyl compounds as a criterion of flavor deterioration in edible fats. Journal of the Science of Food and Agriculture 9:693-701. 1958.

6. Bolland, J. L. Kinetics of olefin oxidation. Chemical Society, London, Quarterly Reviews 3:1-21. 1949.

7. Brasch, A. and W. Huber. Reduction of undesirable by effects in products treated by radiation. Science 108:536-537. 1948.

8. Brasch, A., W. Huber and A. Waly. Radiation effects as functions of dose rate. Archives of Biochemistry and Biophysics 38:245-247. 1952.

9. Brekke, J. and G. Mackinney. Aldehydes from rancid corn and avocado oils. Journal of the American Oil Chemists' Society 27:238-240. 1950.

10. Bradway, E. M. and H. A. Mattill. The association of fat-soluble vitamins and antioxidants in some plant tissues. Journal of the American Oil Chemists' Society 56:2405-2408. 1934.

11. Burton, V. L. The effects of radioactivity on oleic acid. Journal of the American Chemical Society 71:4117-4119. 1949.

12. Buss, C. D. and G. Mackinney. Carbonyl compounds in rancid corn oil. Journal of the American Oil Chemists' Society 32:487-489. 1955.

13. Cannon, J. A. et al. Analysis of fat acid oxidation products by counter-current distribution methods. IV. Methyl linoleate. Journal of the American Oil Chemists' Society 29:447-452. 1952.

14. Chang, S. S. and F. A. Kummerow. The isolation and characterization of the polymers formed during the autoxidation of ethyl linoleate. Journal of the American Oil Chemists' Society 30:403-407. 1953.

15. Chang, S. S. and F. A. Kummerow. The relationship between the oxidative polymers of soybean oil and flavor reversion. Journal of the American Oil Chemists' Society 31:324-327. 1954.

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

17. Coleman, J. E. and Daniel Swern. Reaction of fatty materials with oxygen. XVIII. Catalytic hydrogenation of autoxidized methyl oleate and oleic acid. Preparation of monohydroxy-stearic acids. Journal of the American Oil Chemists' Society 32:221-224. 1955.

18. Dowd, L. E. Spectrophotometric determination of quercetin. Analytical Chemistry 31:1184-1187. 1959.

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

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

21. Ellis, G. W. Autoxidation of fatty acids. The oily products from elaidic and oleic acids. The formation of monoacyl derivatives of dihydroxy stearic acid and γB-unsaturated keto acids. Biochemical Journal 46:129-141. 1950.

22. Farmer, E. H. and D. A. Stutton. The course of autoxidation reactions in polyisoprenes and allied compounds. IV. The isolation and constitution of photochemically formed methyl oleate peroxide. Journal of the Chemical Society 119-122. 1943.

23. Farmer, E. H. Certain fundamental concepts relating to nonpolar mechanisms in olefinic systems. Journal of the Society of Chemical Industry 66:86-93. 1947.

24. Feigl, F. Spot tests. Organic applications. Vol. II. New York, Elsevier Publishing Company. 1954. 436 p.

25. Gearhart, W. M. and B. N. Stuckey. A comparison of commercially used phenolic antioxidants in edible animal fats. Journal of the American Oil Chemists' Society 32:386-390. 1955.

26. Greenbank, G. R. and G. E. Holm. Antioxidants for fats and oils. Industrial and Engineering Chemistry 26:243-245. 1934.

27. Gunstone, F. D. An introduction to the chemistry of fats and fatty acids. New York, Wiley. 1958. 161 p.

28. Hannan, R. S. Scientific and technological problems involved in using ionizing radiations for the preservation of food. London. 1955. 192 p. (Great Britain Department of Scientific and Industrial Research. Food Investigation Board. Special Report No. 61).

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

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

31. Hannan, R. S. and H. J. Shepherd. Some after-effects in fats irradiated with high energy electrons and x-rays. British Journal of Radiology 27:36-41. 1954.

32. Huber, W., A. Brasch and A. Waly. Effect of processing conditions on organoleptic changes in food-stuffs sterilized with high intensity electrons. Food Technology 7:109-115. 1953.

33. Kawahara, F. K. and H. J. Dutton. Volatile cleavage products of autoxidized soybean oil. Journal of the American Oil Chemists' Society 29:372-377. 1952.

34. Keeney, M. and F. J. Doan. Studies on oxidized milk fat. I. Observations on the chemical properties of the volatile flavor material from oxidized milk fat. Journal of Dairy Science 34:713-718. 1951.

35. Keeney, M. and F. J. Doan. Studies on oxidized milk fat. II. Preparation of 2, 4-dinitrophenyl-hydrazones from the volatile material from oxidized milk fat. Journal of Dairy Science 34:719-727. 1951.

36. Keeney, M. and F. J. Doan. Studies on oxidized milk fat. III. Chemical and organoleptic properties of volatile material obtained by fractionation with various solvents and Girard's reagent. Journal of Dairy Science 34:728-734. 1951.

37. Kenaston, C. B. et al. Comparison of methods for determining fatty acid oxidation produced by ultraviolet irradiation. Journal of the American Oil Chemists' Society 32:33-35. 1955.

38. Koch, R. B. Mechanism of fat oxidation. The Baker's Digest 30(2): 48-53, 68-69. 1956.

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

40. Lea, C. H. Recent developments in the study of oxidative deterioration of lipids. Chemistry and Industry 1303-1309. Dec. 5, 1953.

41. Lea, C. H. Some nutritional and allied problems confronting the food manufacturers. Journal of the Science of Food and Agriculture 9:621-632. 1958.

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

43. Luckton, A. and G. Mackinney. Effect of ionizing radiations on carotenoid stability. Food Technology 10:630-632. 1956.

44. Martin, C. J., A. I. Schepartz and B. F. Daubert. Flavor reversion in soybean oil. III. Isolation of reversion compounds in soybean oil. Journal of the American Oil Chemists' Society 25:113-117. 1948.

45. Mead, J. F. The irradiation induced autoxidation of linoleic acid. Science 115:470-742. 1952.

46. Miller, W. C. Jr., B. E. Proctor and S. A. Goldblith. Recent developments in radiation sterilization of foods. Journal of Milk and Food Technology 17:159-163. 1954.

47. Moore, R. N. and W. G. Bickford. A comparative evaluation of several antioxidants in edible fats. Journal of the American Oil Chemists' Society 29:1-4. 1952.

48. Morris, S. G. Recent studies on the mechanism of fat oxidation in its relation to rancidity. Journal of Agricultural and Food Chemistry 2:126-132. 1954.

49. Mukherjee, S. Studies on rancidity of butterfat. Journal, Indian Chemical Society 27:589. 1950.

50. Naghski, J., C. C. Fenske, Jr. and J. F. Couch. Use of paper chromatography for the quantitative estimation of quercetin in rutin. Journal of the American Pharmaceutical Association (Scientific Edition) 40:613-616. 1951.

51. Nickerson, J. T. R., S. A. Goldsblith and B. E. Proctor. A comparison of chemical changes in mackerel tissue treated by ionizing radiation. Food Technology 4:84-88. 1950.

52. Polister, B. H. and J. F. Mead. Effect of certain vitamins and antioxidants of methyl linoleate. Journal of Agricultural and Food Chemistry 2:199-202. 1954.

53. Porter, W. L. et al. Tentative spectrophotometric method for the determination of rutin in various preparations. Washington, July 1947. (U.S. Dept. of Agriculture. Bureau of Agricultural and Industrial Chemistry. AIC 159).

54. Privett, O. S. et al. Structure of hydroperoxides obtained from autoxidized methyl linoleate. Journal of the American Oil Chemists' Society 30:61-66. 1953.

55. Proctor, B. E. and S. A. Goldsblith. Electromagnetic radiation fundamentals and their application in food technology. Advances in Food Research 3:119-189. 1951.

56. Proctor, B. E. et al. Biochemical prevention of flavor and chemical changes in food. Food Technology 6:237-242. 1952.

57. Proctor, B. E. and S. A. Goldsblith. Prevention of side effects in sterilization of foods and drugs by ionizing radiations. Nucleonics 10(4):64-65. 1952.

58. Report of the Fat Stability Subcommittee of the Fat Analysis Committee 1956. Active oxygen method for determining the fat stability. Journal of the American Oil Chemists' Society 34:394-398. 1957.

59. Richardson, G. A., El-Rafey and M. L. Long. Flavones and flavone derivatives as antioxidants. Journal of Dairy Science 30:397-413. 1947.

60. Saunders, D. H., C. Riccuiti and D. Swern. Reactions of fatty materials with oxygen. XVI. Relation of hydroperoxide and chemical peroxide content to total oxygen absorbed in autoxidation of methyl oleate. Journal of the American Oil Chemists' Society 32:79-83. 1955.

61. Sedlacek, B. A. J. Studie über den Einflussionisierender Strahlen auf Fette Sonderdruck aus, Die Nahrung 2:547-556. 1958.

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

63. Sidwell, C. G. et al. The use of thiobarbituric acid as a measure of fat oxidation. Journal of the American Oil Chemists' Society 31:603-606. 1954.

64. Sinnhuber, R. O., T. C. Yu and Techang Yu. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Research 23:626-634. 1958.

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

66. Stapf, R. J. and B. F. Daubert. Flavor reversion in soybean oil. VI. Isolation and identifications of reversion compounds in unhardened soybean oil. Journal of the American Oil Chemists Society 27:374-377. 1950.

67. Swern, D. and J. E. Coleman. Reactions of fatty material with oxygen. XX. Recent developments in the autoxidation of methyl oleate and other monosaturated fatty materials. Journal of the American Oil Chemists' Society 32:700-703. 1955.

68. Swift, C. E. et al. Decomposition of methyl hydroperoxide oleate. Journal of the American Oil Chemists' Society 25:39-40. 1948.

69. Swift, C. E. et al. The aldehydes produced during the autoxidation of cottonseed oil. Journal of the American Oil Chemists' Society 26:297-300. 1949.

70. U. S. Army. Quartermaster Corp. Radiation preservation of food. Washington, D. C., 1957. 461 p.

71. Whitehead, W. L., C. Goodman and I. A. Breger. Decomposition of fatty acids by γ-particles. Journal de Chimie Physique 48:184-189. 1951.

72. Yu, T. C. and R. O. Sinnhuber. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technology 11:104-108. 1957.