1986

Effects of Gamma Irradiation on Chicken Fats

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EFFECTS OF GAMMA IRRADIATION ON CHICKEN FATS

BY

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A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science
Major in Home Economics
South Dakota State University
1986

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ON CHICKEN FATS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I. INTRODUCTION

Food irradiation is a process which may provide an alternative to existing conventional food processes. It will not completely replace or substitute for other processes, but its unique qualities offer an additional processing option. A major difficulty with food irradiation is that it can potentially affect all food, and thus it touches on all food issues - from affecting the physical characteristics of food to labeling and consumer acceptance.

Research directed toward the use of radiation for the preservation of foods began in 1945. Most of the studies have been government sponsored, at least partly because the 1985 food additives amendment to the Food, Drug and Cosmetic Act required advance approval from the Food and Drug Administration (FDA) before any particular irradiated food could be publicly sold. Currently, two events have served to renew interest in food irradiation processing.

First, recommendations have been made by the Joint Expert Committee on Wholsomeness of irradiated Foods, convened by the World Health Organization (WHO 1981). After reviewing all the food safety data available, this committee concluded that any food irradiated to an average "dose" of 1 Mrad or less is wholesome for humans and therefore should be approved without further testing. The WHO Joint Committee deferred general recombination for foods irradiated at

higher radiation levels until data from on-going studies are available for evaluation.

Secondly, the FDA has been considering new regulatory procedures regarding irradiated foods. The FDA has outlined a number of possible actions which could ultimately lead to approval for irradiation preservation of foods when the irradiation levels used are 0.1 Mrad (1 KGy) or less. The FDA has also suggested changes in the criteria for establishing the safety of foods that would be irradiated at levels between 0.1 and 1.0 Mrad (1-10 KGy) which includes levels sufficient to pasteurize foods.

The recommendations of the WHO Joint Expert Committee and the regulatory procedures under consideration by the FDA suggest that consideration for commercialization of irradiation preservation of foods is timely. However, the success of these processes will depend ultimately upon their cost, consumer acceptance, and labeling requirements, as well as evaluating whether the technology fulfills a perceived consumer need.

II. REVIEW OF LITERATURE

Preservation of food by irradiation pasteurization and sterilization has recently attracted renewed interest. The energy used in food processing and preservation in the U.S. currently amounts to about 16.5% of the total energy consumed(1). Food irradiation has been demonstrated to save large amounts of energy (1).

Radiation produces shelf-stable products that are closer to the fresh state in texture, flavor and color. Other possible advantages of radiation processing include: the product can be packed dry; there is no loss of natural juices during radiation processing; larger container sizes can be used; better portion control in packaging is possible (2,3). Radiation processing, has been shown to inhibit sprouting, kill larvae in harvested fruit and vegetables, and destroy biological hazards such as Salmonella in chicken and trichinae in pork. The treatment kills Clostridium botulinum and may eliminate the need for nitrite in bacon (2,3,4).

The A.E.C. (Atomic Energy Commission) has pointed out several factors which have served to limit industrial investment in research and development on radiation preservation of foods (5):

1) A general feeling of uneasiness that one finds, both in industry and in the general population, concerning the use of radiation in the treatment of foods.

2) Large capital investment in a radiation facility which is required to conduct meaningful product development work.

3) The lack of biologists and food technologists in private industry who are experts in understanding the effect of radiation.

4) The highly fragmented and seasonal nature of the basic food producing industry which mitigate against the establishment of

large, economic, radiation processing facilities close to the source of production (5).

Irradiation has been shown to cause some undesirable changes in foods. Fruits can undergo texture changes leading to unacceptable softening. Some foods, notably dairy products, are particularly sensitive to radiation-induced off flavors (2,3,4). Pigment changes may also occur, with red pigment of raw meat turning brown and cooked meat and poultry turning a pale pink. In general, off flavors are the major organoleptic problem in meat, fish, dairy products and poultry (6). In view of these facts, special attention will be focused on oxidative changes (formation of peroxide, carbonyl compounds and free fatty acids) which occured when fats were irradiated with varying doses of gamma rays, with varying conditions of irradiation, and if and when fats were stored after irradiation and under what conditions.

Hannan and associates studied the formation of peroxide in butter fat during irradiation and storage (7) and reported that irradiation can cause the induction of autooxidation of methyl linoliate (8,9). Chipult and Mizuno et al. found little peroxide formed during irradiation under vacuum (10,11). When beef, pork and chicken were irradiated in nitrogen atmospheres, no significant increases in the levels of peroxide were found by Groninger et al. (12,13). Hannan and Boag found that formation of peroxide in fats irradiated at low temperatures were markedly dependent upon the temperature of both irradiation and post-irradiation storage. Mead and Griffith

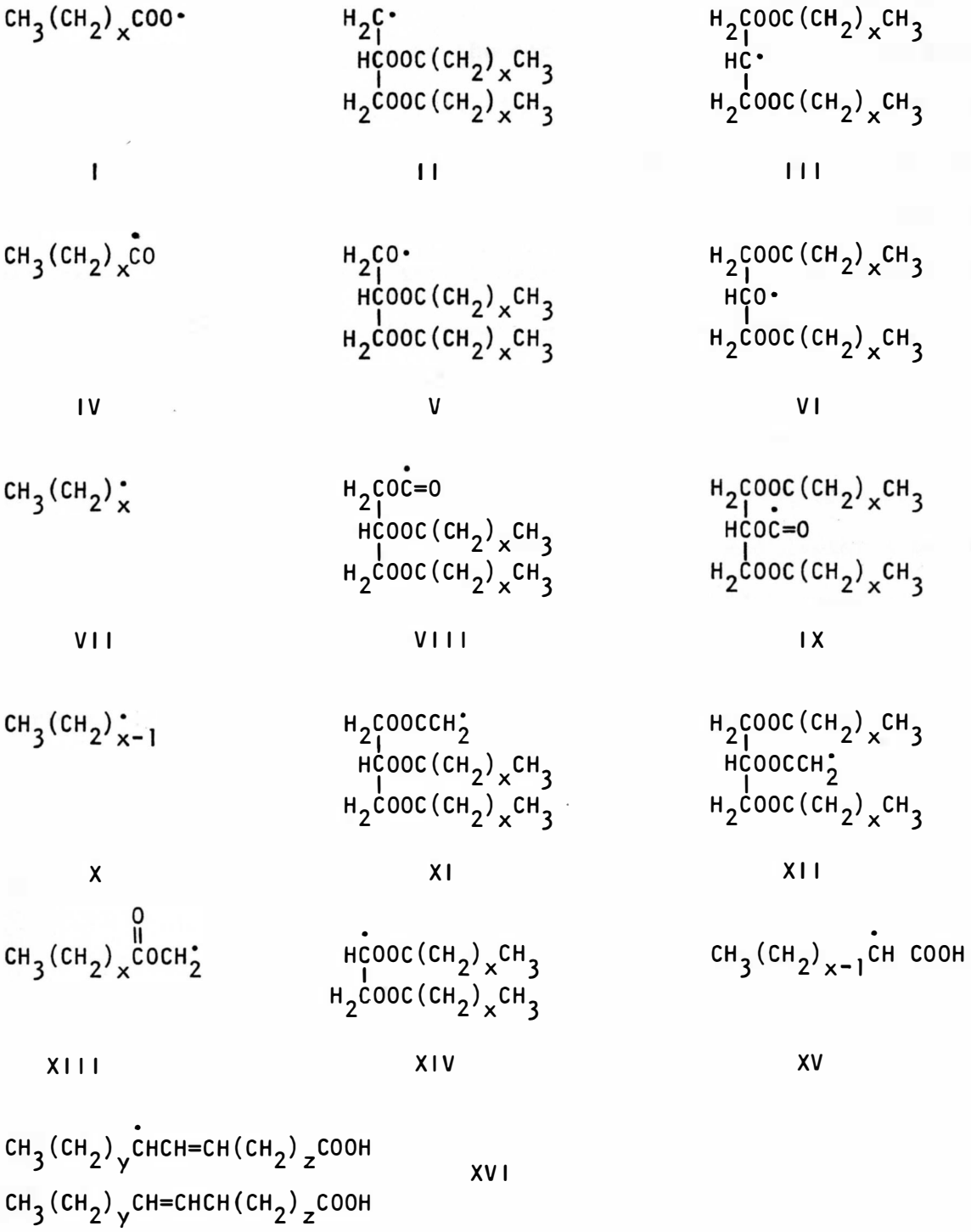
demonstrated increases in peroxide formation with degree of unsaturation (14).

Carbonyl compounds, which are known to result from degradation of hydroperoxides are thought by some researchers to be largely oxidized rancid fat (15,16). Halt and Dugan et al. indicated that alpha-irradiation at the level used in their work has no specific effect on the products of autooxidation because the initial product, the hydroperoxides, consist of the same four isomers found in non-irradiated autooxidations (17). But high energy particles or UV light (i.e., radiation) are capable of initiating or accelerating peroxide decompositions and different secondary products could be obtained from this action (14,18).

The general mechanism of the radiolysis of fats is thought to be involve primary ionization, followed by migration of positive charges toward the carboxyl groups and the double bonds, and cleavage at preferential positions near the carboxyl group. The resulting free radicals engage in various reactions leading to the formation of stable radiolytic products; these have been classified as primary, recombination and secondary products according to the mode of their formation (15,16). The free radical species that are produced preferentially from triglyceride by irradiation are shown in Figure I.

Radical types I, II and III from scission at the acyloxy-methylene, or the acyloxy-methyne bond in triglycerides and

Figure I. Free Radical Species Produced from Triglyceride by Irradiation



decarboxylation of radical type I are postulated to yield radical type VII (19). Fauncitano et al. observed radicals of the type $-CH_2C{=}O$ and suggested that cleavage at the acyl-oxy bond of fatty acids gives rise to radicals types IV, V, VI (15). Radical type IV may also lose to yield radical type VII (20, 21). Scission between carbon 1 and carbon 2 produce radical types IV, V, VI (15). Faucitano and coworkers presented experimental evidence to support the hypothesis that the participation of the reaction $\sim CH_2CH_2\dot{C}{=}O \longrightarrow CO + \sim CH_2\dot{C}H_2$ and consequently contribution of the radical anion $\sim CH_2\dot{C}(O^-)OH$ to the mechanism of radiolytic decarboxylation is of minor importance. Loss of CO_2 from radical types VIII and IX leads to the formation of radical types II and III. Cleavage between carbon 2 and carbon 3 of the fatty acid gives rise to radical types X, XI and XII. Radical types XIII and XIV result from scission between the primary and secondary carbons of the glyceryl skeleton (15,16). Radical type XVI has been shown to arise from irradiation of monounsaturated fatty acids and can be produced both by hydrogen atom abstraction and by C-H bond homolysis (15,16).

Termination of free radical formation may take place via abstraction, dissociation, radical-radical recombination, radical-radical disproportionation and radical-molecule reactions. Primary radiolytic products are formed by scission of only one bond in the parent molecule, followed by the abstraction or loss of hydrogen atom (15,16). Letellier and Nawar showed that free alkanoic acid, with a carbon number equal to that of the parent glyceride, is the most

abundant radiolytic product of triglyceride (19,22). It arises from hydrogen abstraction by radical I. Abstraction of hydrogen by radical II and III leads to the formation of propanediol diesters of the types in Figure II (22,23, respectively) while loss of hydrogen reduces the propanediol diesters of the types in Figure III. Letellier, Nawar and Meidani found that the saturated diol diesters are produced in large amounts and theorized that hydrogen abstraction is the preferential route for the termination of radical types II and III (19,22,23,24). Series of saturated and unsaturated hydrocarbons arise from termination of alkyl radicals and radical type VII are responsible for the production of the major hydrocarbons in the radiolysis of fats i.e., the decarboxylation product (25,26). In addition, radiolytic decarboxylations are influenced by temperature, physical state, and chain length of the fatty acid; the amount of CO_2 produced as well as that of the major hydrocarbon produced is inversely proportional to the fatty acid carbon number (27,28,29).

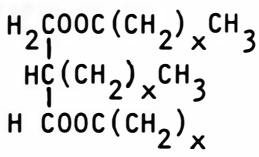
Recombination products are the compounds believed to arise by combination of the primary free radicals (15). Letellier, Nawar and Meidani succeeded in isolating and identifying these compounds from irradiated fat (Figure IV) (23,24):

A) Dimeric or recombination hydrocarbons of the type $CH_3(CH_2)_x(CH_2)_xCH_3$ which result from dimerization of radical VII and the type $CH_3(CH_2)_x(CH_2)_yCH_3$ (where y is any number smaller than x),

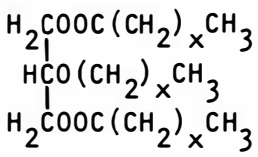
Figure II. Formation of Propanediol Diester

Figure III. Loss of Hydrogen Atom from Propanediol Diester

Figure IV. Recombination Products Arised by Combination of Primary Free Radicals



A



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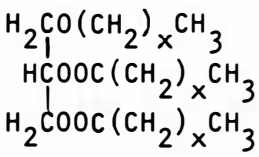

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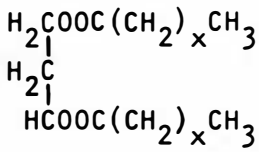

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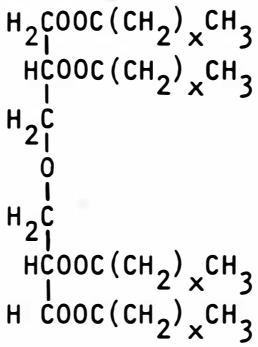

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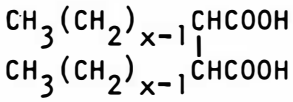

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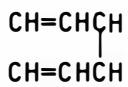

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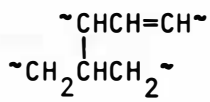

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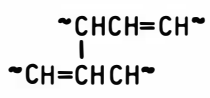

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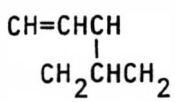

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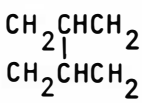


K

B) Ketones of the type $CH_3(CH_2)_xCO(CH_2)_xCH_3$ resulting from the recombination of radicals IV and VII,

C) Esters of the type $CH_3(CH_2)_xCOO(CH_2)_xCH_3$ which arise from recombination of radicals I and VII or radicals X and XIII,

D) Diketones of the types $CH_3CH_2)_xCOCO(CH_2)_3$ produced from dimerization of radicals IV,

E) Oxoalkyl esters of the type $CH_3(CH_2)_xCOOCH_2CO(CH_2)_xCH_3$ which result from recombination of radical IV and XIII,

F) 2-Alkyl-1, 3-propanediol diestesr of the type A from the recombination of radical III and VII,

G) Alkanediol diesters which can result from recombination of radicals II or XIV,

H) Glyceryl ether diesters of types B or C,

I) Erithritol tetraesters which can be produced by dimerization radical XIV,

J) Butanetriol tetraestesr type D which may form by recombination of radical II with radical XIII,

K) Glyceryl esters of type E these can result from recombination of radical II with radical V,

L) Dehydrodimers of type F which result from the dimerization of radical XV,

M) Di-unsaturated, monounsaturated and saturated dimers of type G or K. Such dimers have been shown to be major products in the radiolysis of oleic acid. Research on altered oleate demonstrated 62% was converted to dimers (30),

The physical state of the fatty acid during irradiation has a definite influence on the radiolytic formation of dimers. In solid state radiolysis, the oligomers from oleic acid were reported to have a carboxy content appreciably lower than that of the pure acid and a different degree of unsaturation. Although liquid state dimerization is believed to proceed mainly via allylic radical coupling, this mechanism in the solid state appears to account for no more than 40% of the oligomers produced, indicating that other mechanisms are involved (15,16).

Secondary products cover all radiolytic compounds which arise from more than one cleavage in the same triglyceride molecule or from decomposition of primary products (15,16). Letellier and Nawar found these compounds isolated from irradiated tricaproin they suggested were formed by double cleavage (22). The presence of other compounds in irradiated fats has also been reported, but the mechanism of their formation remains unclear.

Witting et al. suggested that principal degradation product of linoleic acid varies from fat to fat and that amino acids may influence the quantity and type of carbonyl compounds formed in irradiated lard (31). Hoff et al. demonstrated that the irradiation odor of butter fat arises from a combination of two precusors, one of which is linoleic acid and the other a terminally unsaturated acid with the same molecular weight as oleic acid but perhaps with a branched chain (37). Charles et al. described how quantitative composition of carbonyl compounds was influenced by the temperature at which irradiation takes place and by irradiation dose (16). Dugan and Landis et al. found that for equal total irradiation doses, lower dose rates resulted in higher oxidative changes (38). First, for any given dose, a low dose rate means a longer time of exposure and consequently, more oxidative changes from chain autooxidation, the second effect involves the concentration of free radicals formed during irradiation (11,14,38,39).

Groninger, et al. reported that antioxidants were not destroyed by irradiation and were effective in preventing irradiation induced changes (9,12). However, Long and Proctor found that although antioxidant added to vegetable oils reduced attack on the unsaturated center during irradiation, the total yield of carbonyl compounds was increased (40). Knapp and Tappel et al. reported a definite loss of antioxidant effectiveness and destruction of antioxidants, particularly of tocopherol (11,41,42,43). Chipault and Mizuno have shown that antioxidants are not equally sensitive to irradiation under vacuum,

the most easily destroyed being alpha-tocopherol, and the most resistant, butylated hydroxyanisol (BHA) (10). Bradshaw and Truby found that tocopherol used at a concentration of 1 percent (44): 1) accelerated the rate of decay of irradiation-induced free radicals in butter fat or tristearin, 2) had no effect on irradiated stearic acid, and 3) stabilized the free radicals of linoleic acid. From this data they concluded that alpha-tocopherol acts as an anti-oxidant by some mechanism not associated with direct combination with radiation-induced free radicals (44). Knapp and Tappel have shown that the irradiation products of tocopherol are very complex and dependent in part upon the solvent (Figure V) (41).

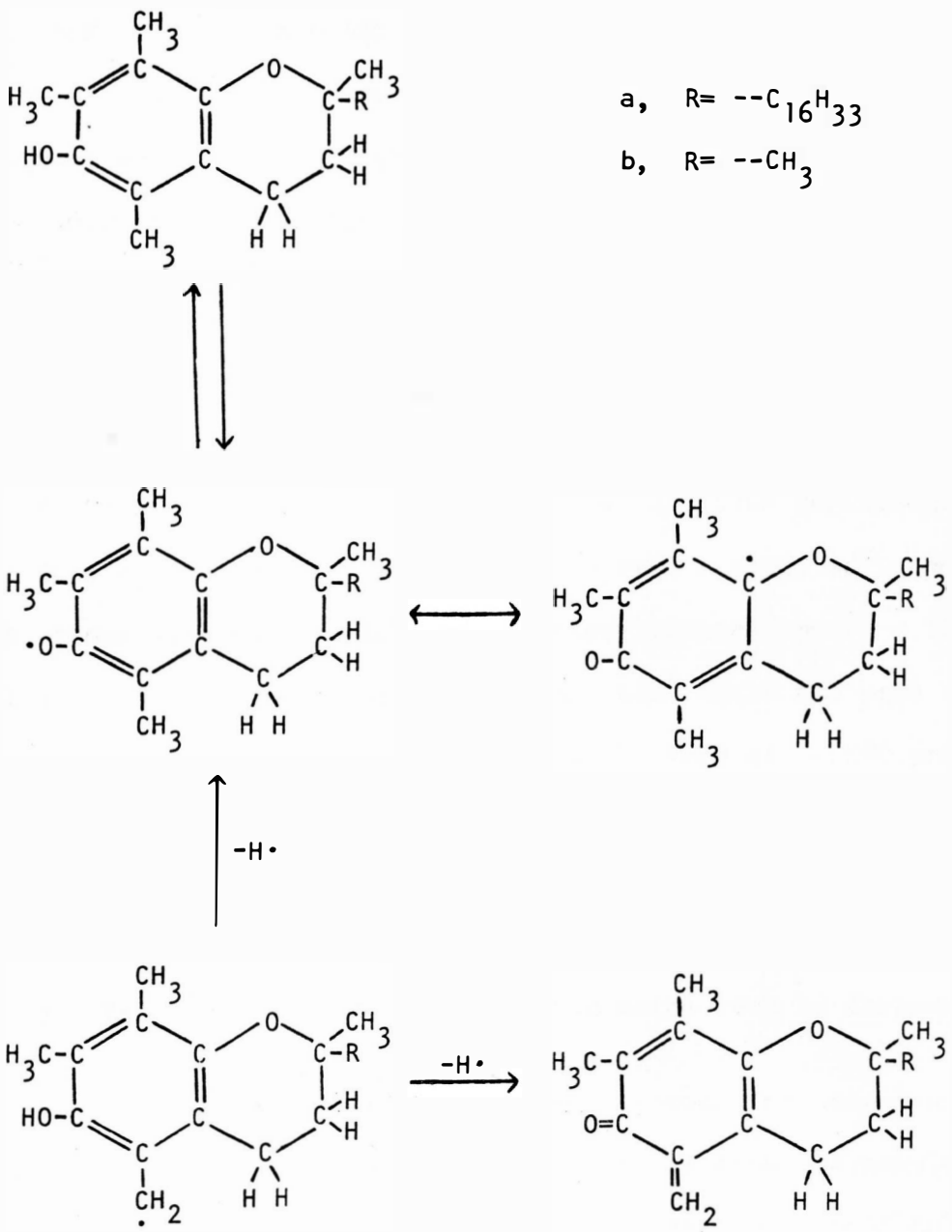
When alpha-tocopherol was irradiated in isooctane, the main product appeared to be a 5-exo-methylene-tocopherol-6-one derived by abstraction of two hydrogen atoms. In peroxidizing linoleic acid, alpha-tocopherol oxidizes to tocopheryl quinone, no radical-tocopherol addition products were detected. In tributyrin and in lard many other products were formed but were not identified (41).

III. EXPERIMENTAL PROCEDURE

A. Sample preparation

All the chicken meat used as samples was purchased from local sources. Previous processing histories of the meat were unknown. The meat was ground finely by using an electric food chopper. After thorough grinding and mixing, aliquots were weighed out (each 10g) and

Figure V. Oxidation Products of α-tocopherol



packed in polyester-polyethylene laminate (oxygen-impermeable) casing and sealed with minimum air space, prior to irradiation. BHA and alpha-tocopherol were dissolved in 69% ethanol and were injected through the casing. Each antioxidant was added at the rate of 0.02% by weight, the maxium amount permitted by current FDA regulation. Treated meat was mixed thoroughly and resealed. All samples were held at -65°C prior to irradiation and stored with dry ice during transportation for irradiation.

B. Irradiation

An 800,000 curie, cobalt-60 source was used for irradiation with a dose rate of 20 krad/min. Samples were irradiated to dosages in the range of 0.2 x 10^6 rad (pasteurization) and 2 x 10^6 rad (sterilization). Irradiation was accomplished while the were cooled by dry ice; all irradiated samples were held at -65°C prior to analysis.

C. Malonaldehyde Assay

1) Theory of TBA (2-thiobarbituric acid) Test by Tarladgis

One of the most commonly used methods for assessing the stability or rancidity of fats has been the TBA test. Sinnhuber and Yu described a method for the quantitative determination of malonaldehyde (using 1.1.3.3.-tetraethoxypropane (TEP) as the standard) with TBA test (40). The acetal is quantitively hydrolized to malonaldehyde

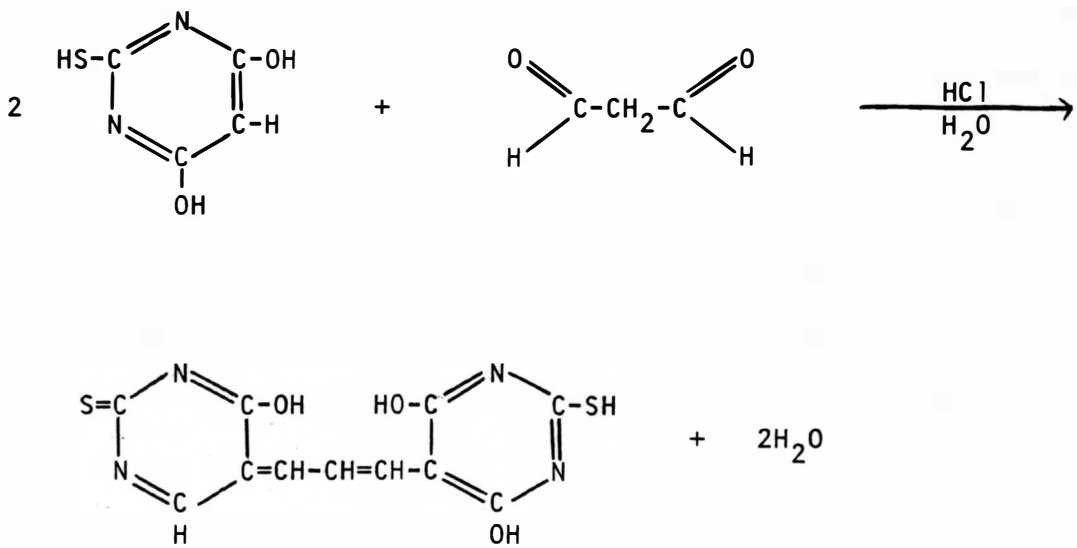
by acid; one mole of malonaldehyde is released from each mole of TEP or TMP hydrolyzed. Sinnhuber et al. proposed that the extent of oxidative rancidity may be expressed in terms of TBA number (mg malonaldehyde/kg sample) by comparing the optical density of the TBA-malonaldehyde colored complex with that of standards prepared from T.E.P. (32). Sinnhuber and Yu established that the colored complex responsible for the absorbance maximum at 532-535 nm was produced from the condensation of two molecules of TBA with one molecule of malonaldehyde (Figure VI) (33).

Two common variants of the TBA test are now used. In the Tarladgis Method the TBA reagent (in strong acid) is added to the food product and the whole mixture heated in a water bath until maximum color is developed. The color complex is extracted with a suitable solvent and measured spectrophotometrically; the malonaldehyde in the food product is first steam-distilled from an acid solution and an aliquot of the distillate is assayed. The TBA reagent mix is heated and the resulting color complex measured directly in a spectrophotometer. In this research, the TBA values were determined by the Tarladgis method (34).

2) Preparation of Malonaldehyde Standard Curve

The TEP (Tetraethoxypropane) working standards were prepared by diluting a 1×10^{-3}M standard solution (in 40% ethanol) with distilled water to give amounts ranging from 1×10^{-8} to 7×10^{-8} moles

Figure VI. Condensation of TBA and Malonaldehyde



TBA pigment

of malonaldehyde per 5ml (35). Five ml portions of working standard were mixed with 5ml of 0.02M TBA solution in 90% glacial acetic acid. These mixtures were pipeted into screw capped test tubes and immersed in a boiling water bath for 40 minutes for color development and then quickly cooled in tap water. Since standard curves were obtained by reacting the standard solutions directly with the TBA reagents, they do not give any measure of the recovery of malonaldehyde during the distillation process. The percent recovery of malonaldehyde from sample materials was estimated by adding a known quantity of TEP to samples and following the full procedure. Sample recoveries ranged generally around 64%.

3) Distillation of Meat Samples

Ten grams of meat was accurately weighed and with 50ml of distilled water blended in an osterizer blender for 2 minutes. The mixture was quantitatively transferred into Kjeldahl flasks and the blender washed with 50ml distilled water. Approximately 2.2-2.3ml of 4N HCl was added to the solution to bring the pH to 1.5. A small amount of SAG Union Carbide silicon oil was applied onto the lower neck of the flask as an antifoaming agent and a few boiling chips were added to prevent bumping.

The Kjeldahl flasks were sealed to the condensers and the distillate was driven off as rapidly as possible. The distillation was captured until 50 ml of distillate was collected. A 5ml portion of the well mixed distillate was added to 5ml of TBA solution in a screw

capped tube and immersed in boiling water for 40 minutes. Reagent blanks were prepared by pipetting 5ml of distilled water and 5ml TBA solution into tubes which were cooled in tap water for 10 minutes, transferred to a curvette, and read for optical density against the reagent blank at a wavelength of 532 nm (Beckman DB-GT Spectrophotometer was used). The TBA number (mg/kg sample) was calculated by multiplying the absorbancy by a constant K, the value of which was obtained from the standard curves and the standard distillation as shown below (36).

$$K = \frac{\text{cons in mole/5ml of distillate}}{\text{optical density}} \times \text{mol. st of malonaldehyde} \times \frac{10^7}{\text{wt. of sample}} \times \frac{100}{\% \text{ recovery}}$$

IV. Results

The purpose of this study was to determine oxidative changes (formation of Malonaldehyde as carbonyl compounds) which occured when fats were irradiated with doses of gamma rays and stored at varying temperature after irradiation. The second objective of this research was to study the effects of antioxidants on malonaldehyde formation, particularly effects of alpha-tocopherol and BHA added before and after irradiation. The study also compared how high energy radiation, such as gamma rays, affects oxidation of fats compared with oxidation of non-irradiated fats as a function of time and temperature of storage. This section describes the results obtained from the study.

Relationship between Malonaldehyde levels and Indepent variables.

Table 1 shows the mean malonaldehyde contents with antioxidant, irradiation dose, application time, storage temperature and storage period. BHA treatment was the most effective in preventing oxidation of sample meat lipids. No positive synergism was found when BHA and tocopherol were injected together. Tocopherol had the lowest effectiveness in preventing lipid oxidation as indicated by the highest value of malonaldehyde content. Higher doses of irradiation caused more oxidation than the lower doses. Samples injected with antioxidant before irradiation demonstrated higher levels of protection from oxidation. Malonaldehyde productivity was greater as storage temperature increased, but non-significantly affected by increasing storage period.

Tables 2 and 3 show mean effectiveness of antioxidants as being interrelated to application time and storage temperature. Antioxidants injected before irradiation were much more effective than those injected after irradiation. Although the dose rate was the same, higher total dose amounts caused higher levels of oxidation. Samples stored at room temperature had higher contents of malonaldehyde than those stored at -1°C; samples irradiated with 2.0×10^6 rad and stored -1°C display higher oxidation level than samples irradiated with 0.2×10^6 rad but stored at 25°C (room temperature). Malonaldehyde production in antioxidant free samples was significantly

Table 1. *Malonaldehyde Production as a Function of Irradiation Dose, Antioxidant, Application Time, Storage Temperature, and Storage Period

		Sample Number	*Malonaldehyde Contents
Antioxidant[1]	Tocopherol	48	4.51^{a}
	BHA	48	2.98^{b}
	Tocopherol + BHA	48	3.33^{c}
Dose[2] (mega rad)	2.0×10^{6}	72	4.35^{a}
	0.2×10^{6}	72	2.87^{b}
Application[3] time	before-irradiation	72	3.09^{a}
	after-irradiation	72	4.13^{b}
Storage (°C) temperature[4]	-1°C	72	3.26^{a}
	25°C	72	3.96^{b}
Storage period (day)	4	48	3.55
	9	48	3.59
	13	48	3.68

*Malonaldehyde contents of samples over 13 days of storage expressed as mg/1000g tissue.

Means in variable blocks with different superscripts are significantly different ($P<0.0001$)

Table 2. Malonaldehyde Production as a Function of Irradiation Dose, Antioxidant and Time of Application

Dose	Antioxidant	*before-irradiation ***mg malonaldehyde/1000g sample	**after-irradiation mg malonaldehyde/1000g
0.2×10^6 (rad)	Tocopherol	3.24^a	3.58^g
	Tocopherol + BHA	2.74^b	2.62^h
	BHA	2.00^c	3.03^i
2.0×10^6 (rad)	Tocopherol	5.13^d	6.10^j
	Tocopherol + BHA	3.21^e	4.75^k
	BHA	2.21^f	4.69^l

*Antioxidants added before irradiation

**Antioxidants added after irradiation

***Malonaldehyde contents of samples over 13 days of storage expressed as mg/1000g

Means in rows and columns indicated by different superscripts are significantly different ($P<0.0001$).

Table 3. Malonaldehyde Production as a Function of Irradiation Dose and Types of Antioxidant at -1°C and 25°C

Dose	Antioxidants	-1°C mg malonaldehyde*	25°C mg malonaldehyde*
0.2×10^6 (rad)	Free**	5.87^a	10.02^a
	Tocopherol	2.98^b	3.84^b
	BHA	2.13^c	2.89^c
	Tocopherol + BHA	2.60^d	2.80^d
2.0×10^6 (rad)	Free**	8.15^e	11.76^e
	Tocopherol	4.73^f	6.51^f
	BHA	3.37^g	3.52^g
	Tocopherol + BHA	3.73^h	4.23^h

*Expressed as mg/1000g tissue

**Free means controlled sample

Rows indicated by different superscripts are significantly different ($P<0.0001$).

higher than in antioxidant treated samples. Non-irradiated, antioxidant free, samples had less oxidation than irradiated, antioxidant free samples. Irradiated, antioxidant-free, samples were severely affected by temperature; malonaldehyde production at 25°C was much higher than that at -1°C. Very little difference was noted in malondaldehyde content of non-irradiated/antioxidant treated samples and sample treated with 0.2×10^6 rad/antioxidant when both were stored at 25°C.

Table 4 shows how antioxidant, application time and storage period affect the malonaldehyde production. When tocopherol was injected before irradiation, a progressive increase in malonaldehyde content was found, probably due to inactivation by irradiation. But when tocopherol injected after irradiation, it was found that malonaldehyde contents tend to be lower after 9 days storage. BHA treatment gave the most effective antioxidant protection when applied either before or after irradiation. A BHA-tocopherol mixture injected before irradiation showed progressive increases in malonaldehyde content, less than tocopherol alone, but this mixture when injected after-irradiation gave lower protection than BHA alone.

Table 5 displays the effects of antioxidants with storage temperature. In tocopherol treated samples, progressive increases in malonaldehyde content were found at both temperatures (-1°C, 25°C). But with BHA treatment, striking protection was found at both storage

Table 4. Malonaldehyde Production as a Function of Storage Period, Antioxidant and Application Time

		Storage Period			
Antioxidant	Application Time	0 day	4 day	9 day	13 day
Tocopherol	*before	2.93^{a}	3.45^{a}	4.01^{a}	5.08^{a}
	**after	4.99^{b}	4.87^{b}	4.94^{b}	4.73^{b}
BHA	*before	2.12^{c}	2.11^{c}	2.24^{c}	1.95^{c}
	**after	3.45^{d}	4.39^{d}	3.78^{d}	3.41^{d}
Tocopherol + BHA	*before	2.13^{e}	2.72^{e}	2.94^{e}	3.27^{e}
	**after	4.06^{f}	3.77^{f}	3.64^{f}	3.64^{f}

*Antioxidant added before irradiation

**Antioxidant added after irradiation

All values are expressed as mg/1000g tissue

Means in rows with different superscripts are significantly different ($P<0.0001$).

Table 5. Malonaldehyde Production as a Function of Antioxidants and Storage Temperature

Antioxidant	Storage Temperature	*Days of Storage			
		**0 day	4 day	9 day	13 day
Tocopherol	-1°C	3.96	3.67[a]	3.87[a]	4.02[a]
	25°C	3.96	4.65[b]	5.08[b]	5.79[b]
BHA	-1°C	2.78	2.85[c]	2.80[c]	2.60[c]
	25°C	2,78	3.65[d]	3.21[d]	2.76[d]
Tocopherol + BHA	-1°C	3.10	3.12[e]	3.17[e]	3.20[e]
	25°C	3.10	3.38[f]	3.41[f]	3.71[f]

*All values are expressed as mg/1000g tissue and are mean values excluding non-irradiated samples

**0 day sample was stored at -65°C before analysis

Means in rows with different superscripts are significantly different ($P<0.0001$).

temperatures. In samples treated with tocopherol and BHA mixtures treated samples, malonaldehyde production was lower than tocopherol alone. Malonaldehyde production increased strikingly for the first 4 days at both temperatures.

Table 6 shows the effect of dose, types of antioxidant, storage temperature and storage period on malonaldehyde production. Like previous data, tocopherol was the most sensitive oxidant to irradiation dose level and temperature. Malonaldehyde increased at room temperature much faster than at -1°C. Samples treated with BHA were not as sensitive to storage temperature; malonaldehyde contents approached similar levels for 25° and -1°C storage after 9 days.

Overall effects of antioxidants, and dose levels, at -1°C, before-irradiation are shown in Table 7, Figures VII and VIII. Overall effects of antioxidants and dose level at 25°C treated before irradiation are shown in Table 8, Figures IX and X. When samples were treated with BHA, malonaldehyde production was strikingly decreased at both temperatures. But, samples treated with tocopherol were more oxidized at 25°C then -1°C; in addition rates of oxidation at 25°C were much faster than -1°C stored samples at both irradiation doses. Synergism of BHA and tocopherol was not found but a BHA and tocopherol mixture was more effective at preventing oxidation than tocopherol alone at both temperatures and dose levels.

Table 6. Malonaldehyde Production as a Function of Irradiation Dose, Antioxidant and Storage Temperature over Storage Period

Dose	Antioxidants	Temperature	*Storage Period (day)			
			**0	4	9	13
2.0×10^6 rad	Tocopherol	-1°C	5.08	4.66^{a}_{b}	4.90^{a}_{b}	4.63^{a}_{b}
		25°C	5.08	5.77^{b}_{c}	6.32^{b}_{c}	7.43^{b}_{c}
	BHA	-1°C	3.07	3.46^{c}_{d}	3.44^{c}_{d}	3.22^{c}_{d}
		25°C	3.07	3.58^{d}_{e}	3.73^{d}_{e}	3.26^{d}_{e}
	Tocopherol + BHA	-1°C	3.95	3.61^{e}_{f}	3.76^{e}_{f}	3.81^{e}_{f}
		25°C	3.95	3.88^{f}	4.27^{f}	4.56^{f}
0.2×10^6	Tocopherol	-1°C	2.84	2.69^{a}_{b}	2.84^{a}_{b}	3.42^{a}_{b}
		25°C	2.84	3.53^{b}_{c}	3.84^{b}_{c}	4.15^{b}_{c}
	BHA	-1°C	2.50	2.24^{c}_{d}	2.17^{c}_{d}	1.99^{c}_{d}
		25°C	2.50	3.73^{d}_{e}	2.69^{d}_{e}	2.27^{d}_{e}
	Tocopherol + BHA	-1°C	2.25	2.62^{e}_{f}	2.58^{e}_{f}	2.59^{e}_{f}
		25°C	2.25	2.88^{f}	2.56^{f}	2.88^{f}

*All values are expressed as mg/1000g tissue

**0 day samples were stored at -65°C before analysis

Means in rwos with different superscripts are significantly different ($P<0.0001$).

Table 7. Malonaldehyde Production as a Function of Irradiation Dose and Antioxidant at -1°C Applied Before Irradiation

		****Days of Storage			
Antioxidants	*dose	***0 day	4 day	9 day	14 day
Antioxidant free	0**	3.15	3.30	3.70	4.10
	0.2×10^6	4.40	5.54	5.89	7.65
	2.0×10^6	7.27	7.87	6.96	10.50
Tocopherol	0**	2.21	2.45	2.58	3.01
	0.2×10^6	2.66	2.48	2.75	3.35
	2.0×10^6	3.20	3.61	3.85	4.07
BHA	0**	1.69	2.04	2.57	2.57
	0.2×10^6	2.32	1.97	1.90	1.80
	2.0×10^6	1.91	2.36	2.34	2.05
Tocopherol + BHA	0**	2.23	2.62	2.81	2.94
	0.2×10^6	1.98	2.56	2.49	2.58
	2.0×10^6	2.28	2.49	2.88	3.16

*Dose unit is rad

**0 means non-irradiation

***0 day sample was stored at -65°C before analysis

****All values are expressed as mg/1000g tissue

Figure VII. Malonaldehyde Production as a Function of Antioxidant Applied Before Irradiation with 0.2×10^6 rad and Stored at -1°C

x = Tocopherol

o = BHA

✱ = Tocopherol + BHA

□ = Antioxidant free

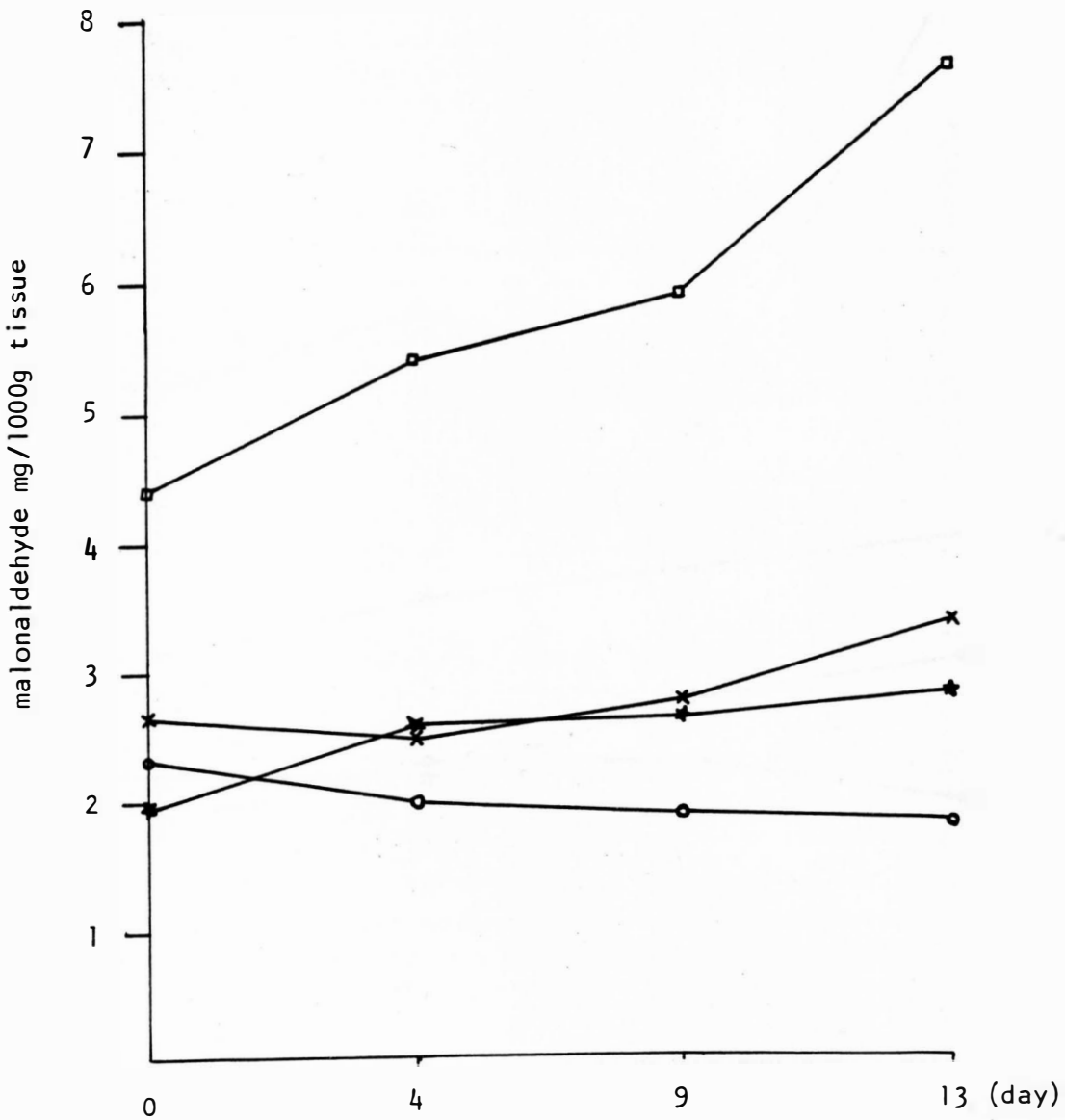


Figure VIII. Malonaldehyde Production as a Function of Irradiation Dose, Antioxidants Applied Before Irradiation with 2.0×10^6 rad and Stored at -1°C

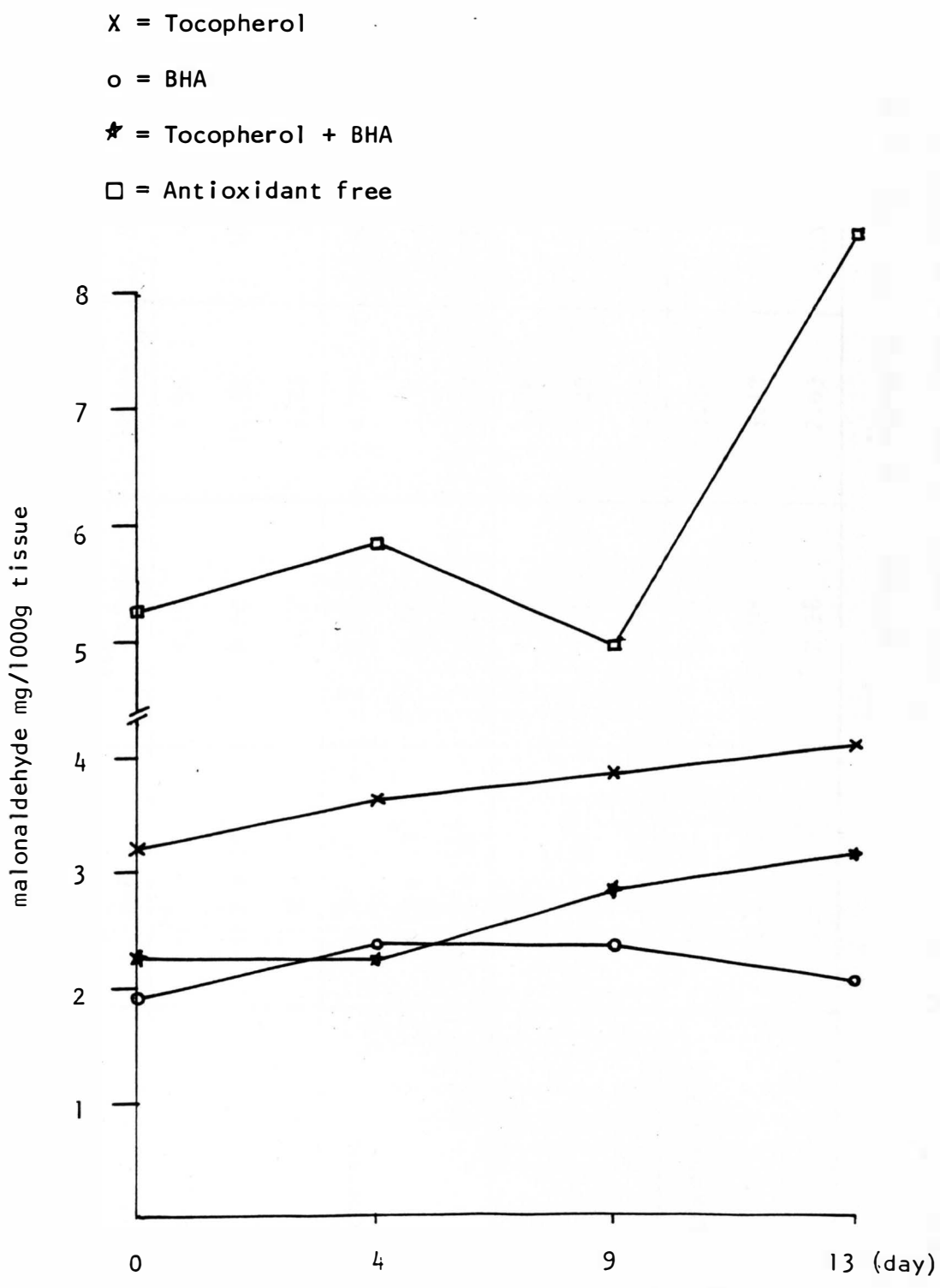


Table 8. Malonaldehyde Production as a Function of Irradiation Dose and Antioxidant at 25°C Applied Before Irradiation

Antioxidants	*dose	***0 day	****Days of Storage 4 day	9 day	14 day
Antioxidant-free	0**	3.15	3.50	4.38	4.81
	0.2×10^6	4.40	10.50	10.38	14.81
	2.0×10^6	7.27	9.83	9.84	20.10
Tocopherol	0**	2.21	2.39	2.72	3.36
	0.2×10^6	2.66	3.01	3.80	4.05
	2.0×10^6	3.20	4.72	5.64	8.87
BHA	0**	1.69	2.06	2.43	2.65
	0.2×10^6	2.32	2.20	2.25	1.84
	2.0×10^6	1.91	2.85	2.46	2.12
Tocopherol + BHA	0**	2.23	3.80	2.94	3.24
	0.2×10^6	1.98	3.12	2.66	3.05
	2.0×10^6	2.28	2.69	3.72	4.32

*Dose unit is rad

**0 means non-irradiation

***0 day sample was stored at -65°C before analysis

****All values are expressed as mg/1000g tissue

Figure IX. Malonaldehyde Production as a Function of Antioxidant Applied Before Irradiation with 0.2×10^6 at 25°C

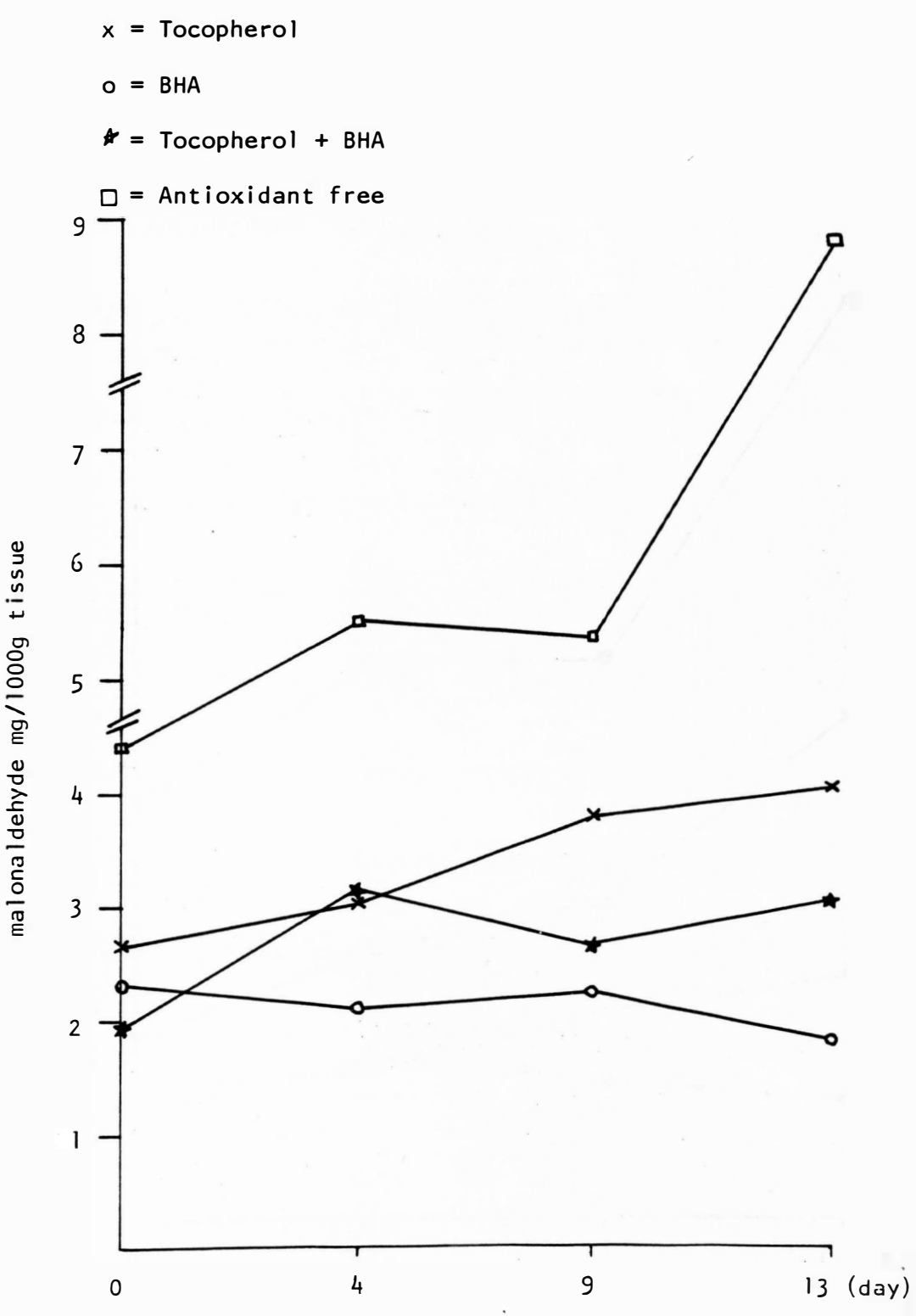


Figure X. Malonaldehyde Production as a Function of Antioxidant Applied Before Irradiation with 2.0×10^6 rad and Stored at 25°C

x = Tocopherol

o = BHA

✱ = Tocopherol + BHA

□ = Antioxidant free

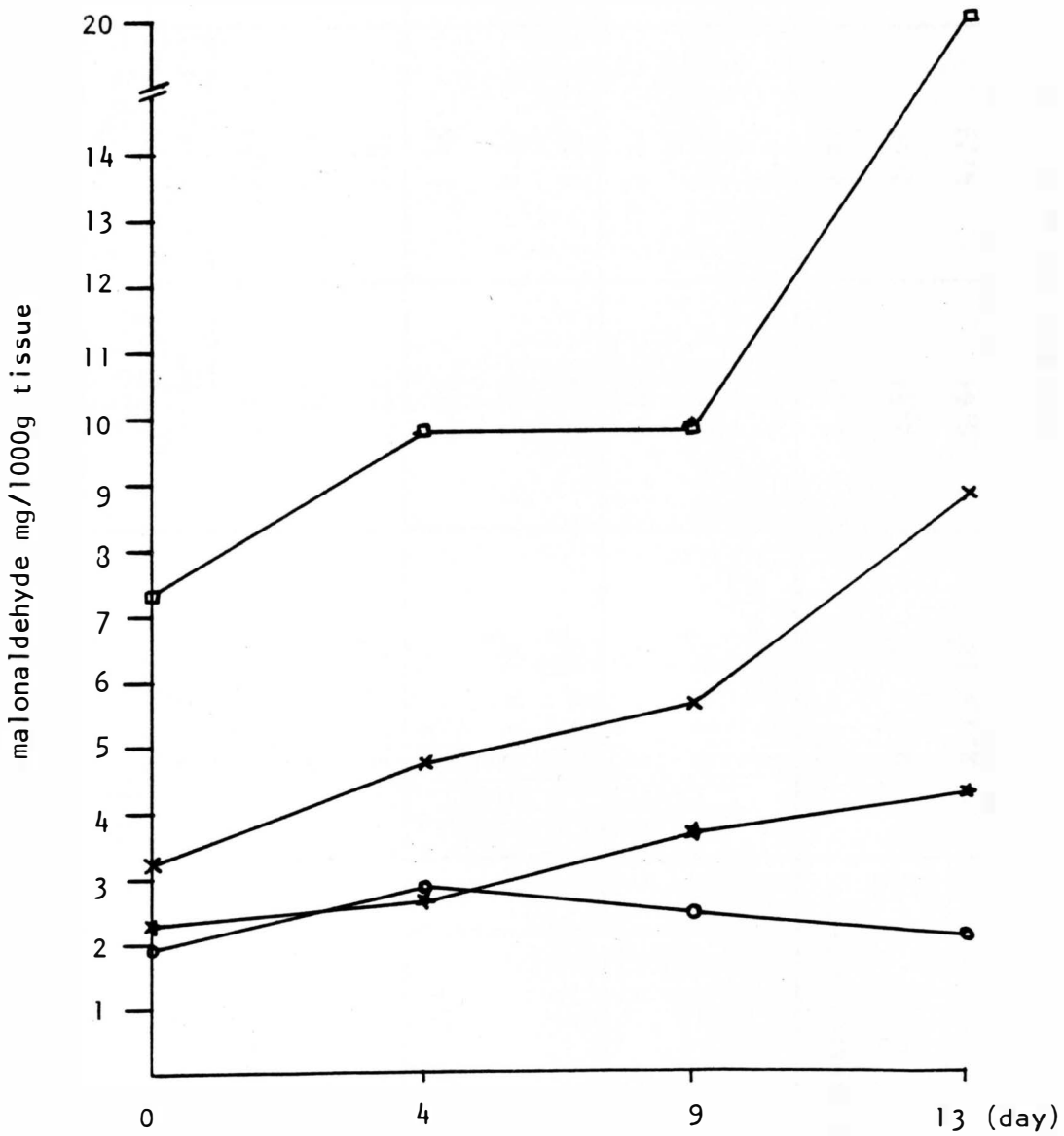


Table 9. Malonaldehyde Production as a Function of Irradiation Dose and Antioxidant at -1°C Applied After Irradiation

Antioxidant	Dose	****Days of Storage			
		***0 day	4 day	9 day	13 day
Antioxident-free	0**	3.15	3.30	3.70	4.10
	0.2×10^6	4.40	5.54	5.89	7.65
	2.0×10^6	7.27	7.87	6.96	10.50
Tocopherol	0**	2.21	2.45	2.58	3.01
	0.2×10^6	3.02	2.90	2.94	2.98
	2.0×10^6	6.96	5.71	5.95	5.20
BHA	0**	1.69	2.04	2.57	2.57
	0.2×10^6	2.68	2.50	2.44	2.18
	2.0×10^6	4.23	4.57	4.54	4.39
Tocopherol + BHA	0**	2.23	2.62	2.81	2.94
	0.2×10^6	2.51	2.68	2.67	2.62
	2.0×10^6	5.61	4.73	4.64	4.47

*Dose unit is rad

**0 means non-irradiation

***0 day sample was stored at -65°C before analysis

****All values are expressed as mg/1000g tissue

Table 10. Malonaldehyde Production as a Function of Irradiation Dose and Antioxidant at 25°C Applied After Irradiation

		****Days of Storage			
Antioxidant	*dose	***0 day	4 day	9 day	13 day
Antioxidant-free	0**	3.15	3.50	4.38	4.81
	0.2×10^6	4.40	10.50	10.38	14.81
	2.0×10^6	7.27	9.83	9.84	20.10
Tocopherol	0**	2.21	2.39	2.72	3.36
	0.2×10^6	3.02	4.05	3.89	4.25
	2.0×10^6	6.96	6.81	7.00	5.99
BHA	0**	1.69	2.06	2.43	2.65
	0.2×10^6	2.68	5.25	3.13	2.70
	2.0×10^6	4.23	5.25	5.01	4.39
Tocopheral + BHA	0**	2.23	2.80	2.94	3.24
	0.2×10^6	2.51	2.62	2.45	2.69
	2.0×10^6	5.61	5.06	4.82	4.78

*Dose unit is rad

**0 means non-irradiation

***0 day sample was stored at -65°C before irradiation

****All values are expressed as mg/1000g tissue

Figure XI. Malonaldehyde Production as a Function of Antioxidant Applied After Irradiation with 0.2×10^6 rad and Stored at -1°C

x = Tocopherol

o = BHA

✱ = Tocopherol + BHA

□ = Antioxidant free

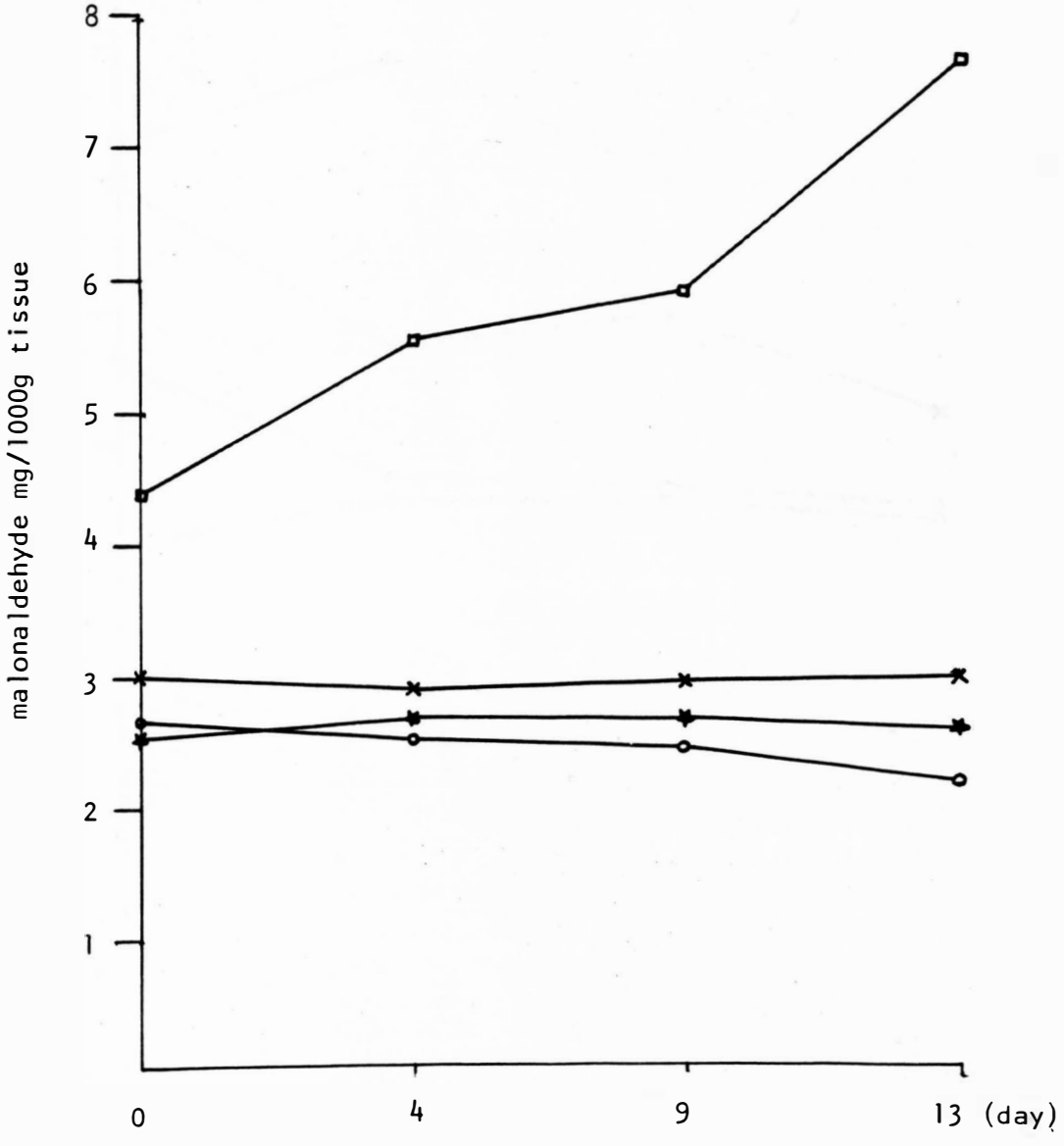


Figure XII. Malonaldehyde Production as a Function of Antioxidant Applied After Irradiation with 2.0 x 10^6 rad and Stored at -1°C

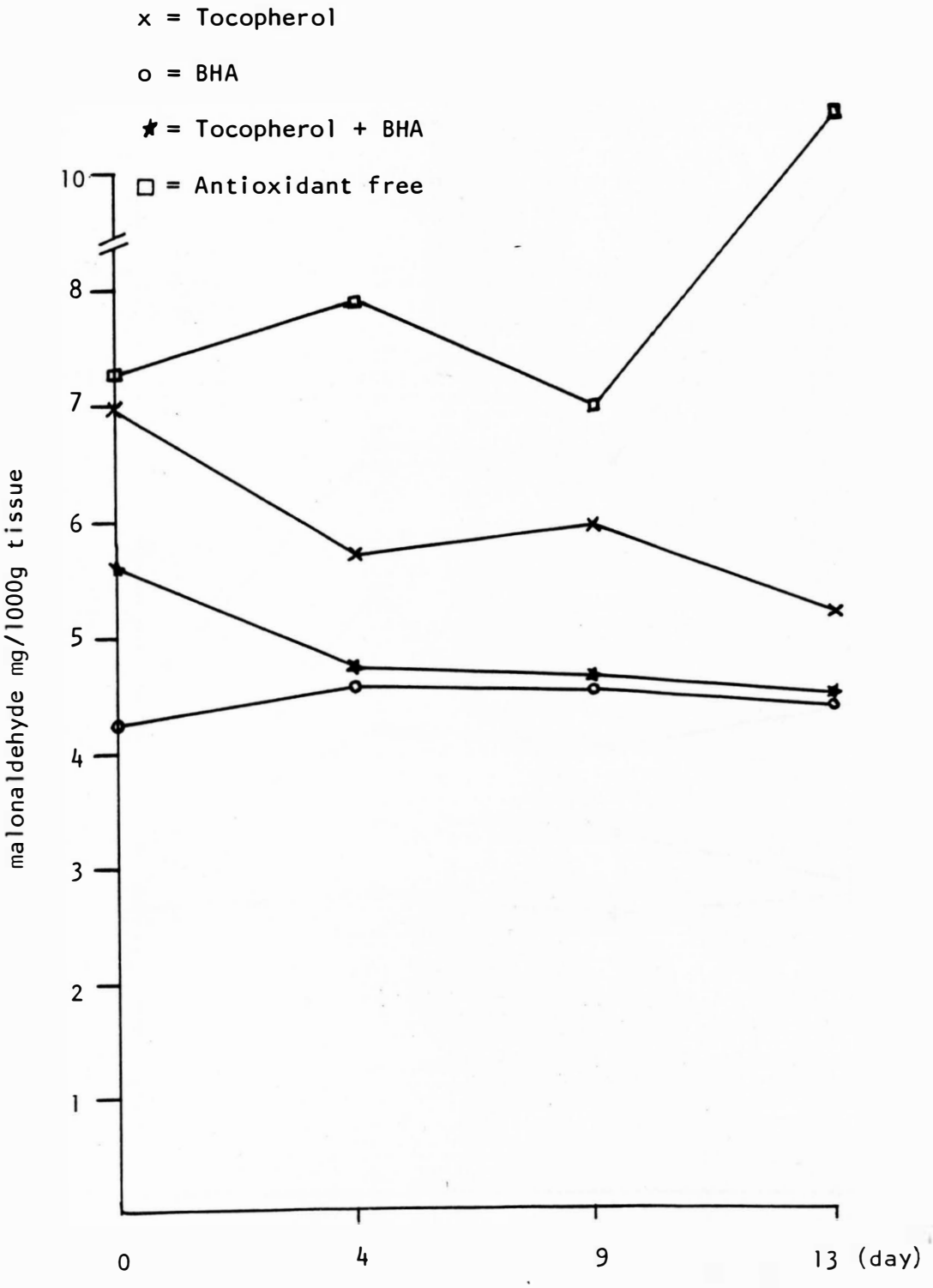


Figure XIII. Malonaldehyde Production as a Function of Antioxidant Applied After Irradiation with 0.2×10^6 rad and Stored at 25°C

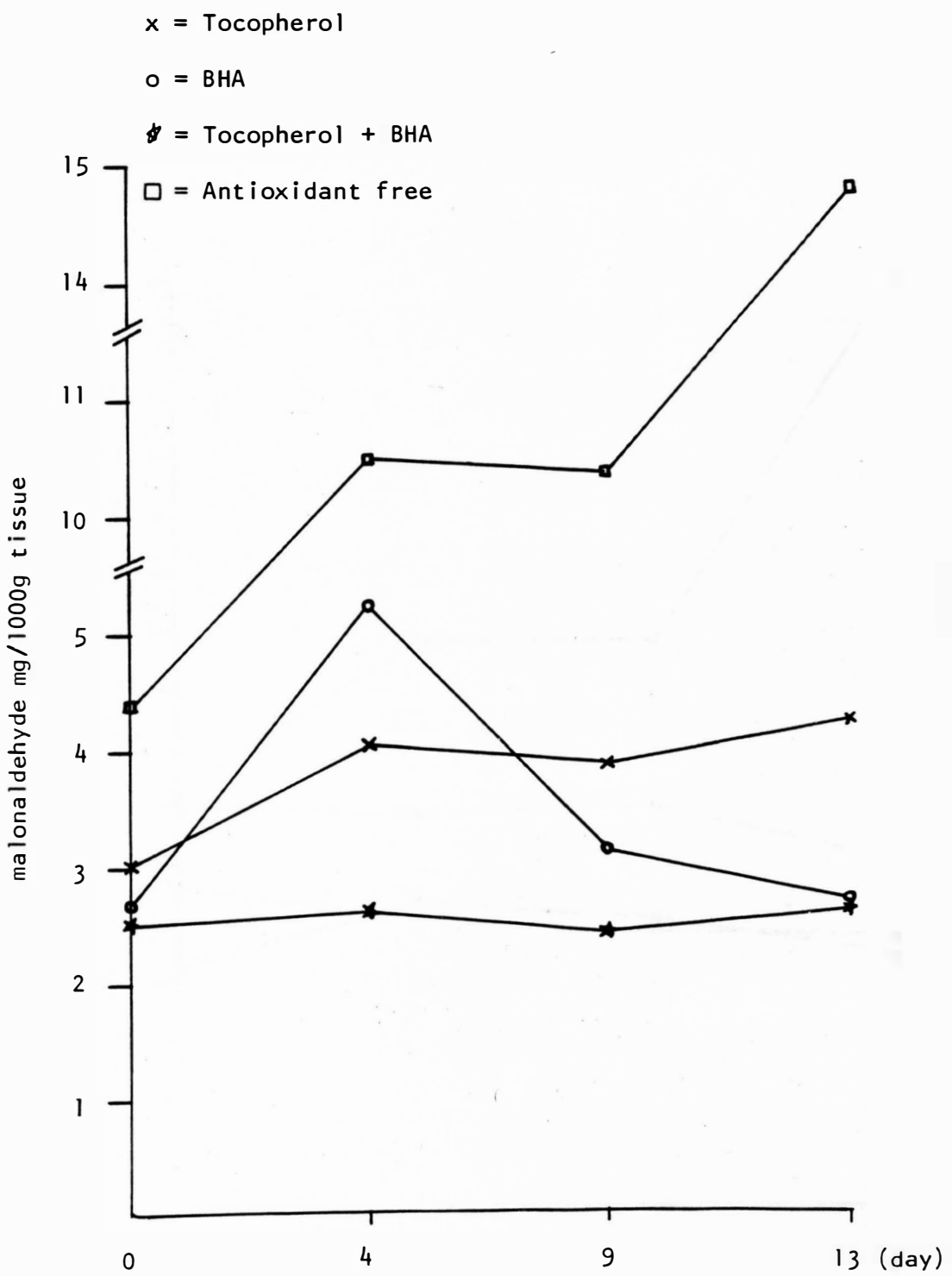


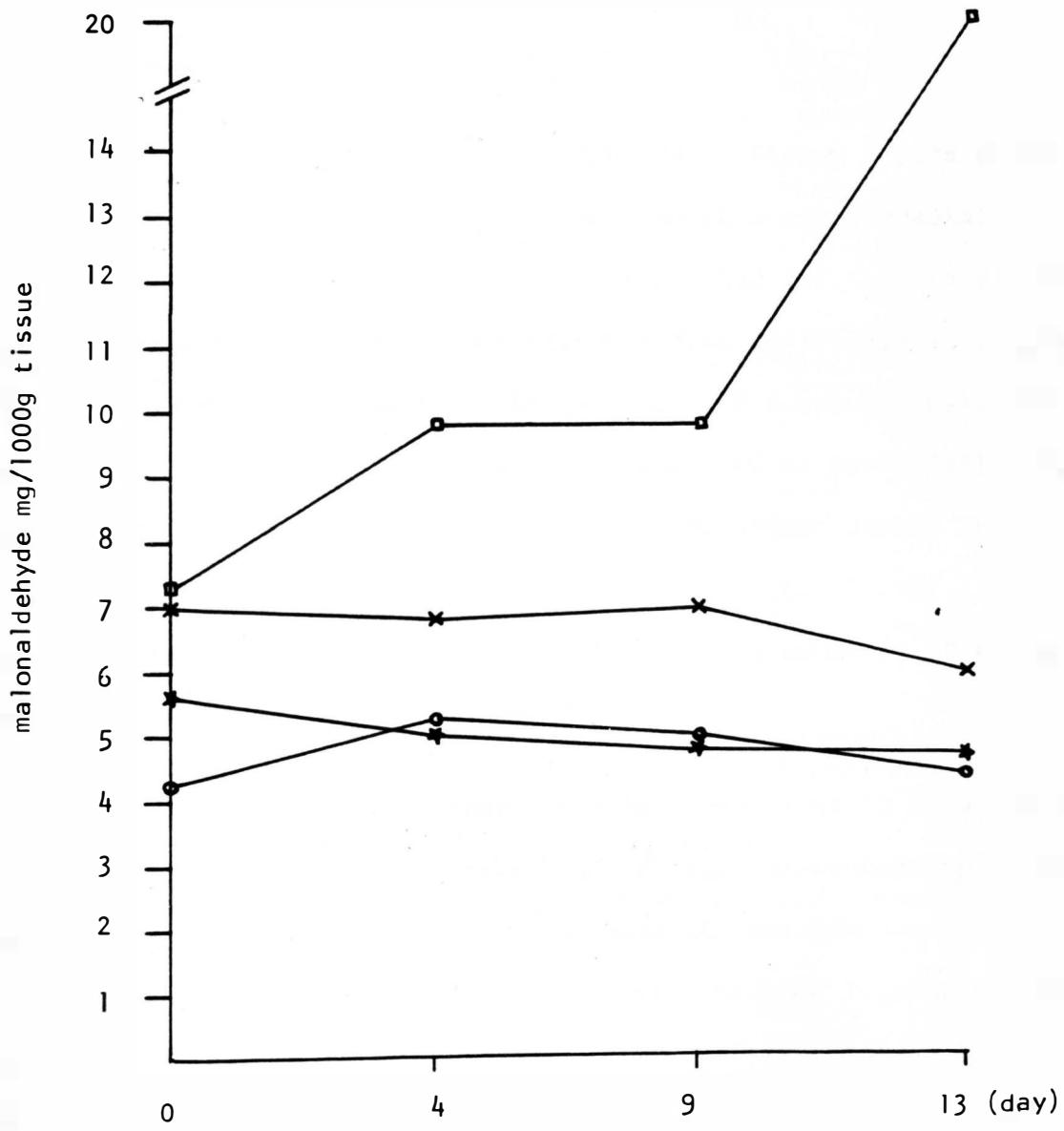
Figure XIV. Malonaldehyde Production as a Function of Antioxidant Applied After Irradiation with 2.0×10^6 rad and Stored at 25°C

x = Tocopherol

o = BHA

✱ = Tocopherol + BHA

□ = Antioxidant free



Overall effects of antioxidants, dose levels, and storage temperatures at -1°C, 25°C on samples treated after-irradiation are shown in Tables 9 and 10 and Figures XI through XIV. In samples treated with tocopherol, rates of oxidation were slower than that of samples treated before-irradiation At both temperatures; malonaldehyde contents were higher than that of before-irradiation treated samples. Analysis of variance data can be found in Appendix A.

V. Discussion

It was apparent that the oxidation of tissue lipids proceeds at a very rapid rate when chicken meat samples were irradiated. In general, antioxidant-free irradiated samples had 1.5 to 2 fold higher malonaldehyde contents than the antixidant-free un-irradiated samples. This is thought to be due to a higher level of polyunsaturated fatty acid [22% of fat as 18:2, 1.5% as 18:3, and 1.5% as 20:4] (45), which makes it more susceptible to attack by molecular oxygen than beef [2.5% of fats as 18:2, 1.3% as 18:3 and 0.3% as 20:4] (46). Chicken fat contains a great amount of phospholipid which consists of a higher degree of polyunsaturated fatty acid.

The oxidation of chicken lipid by irradiation is also thought to be related to the inactivation of the Se-containing enzyme (glutathion peroxidase) which is present in chicken muscle and may serve a function in breakdown of lipid hydroperoxide to nonfree radical components. Thus, it would serve the same function as the added antioxidant in preventing myoglobin induced oxidation in muscle.

Meat pigment of chicken may be converted rapidly to an active oxidative catalyst, trivalent iron hemochromagen, during irradiation. These catalytic activators may cause a higher rate of oxidation in irradiated samples than in non-irradiated samples. But the most important factor is though to be due to ionization of unsaturated fatty acid and initiation or acceleration of peroxide decomposition by high energy particles.

In the antioxidant free sample, higher dose rates of irradiation caused more oxidative change than lower dose rates of irradiation. This is thought to be due to a longer time of exposure which may form a higher concentration of free radicals during irradiation. Non-irradiated samples stored at 25°C were severely deteriorated but irradiated samples with 2.0×10^6 rad were fresh at the level of the 13th day. Malonaldehyde production of non-irradiated samples was much lower than irradiated samples, therefore, little influence of bacterial action on oxidative changes occuring in chicken meat lipid was thought to have occured.

Samples innoculated with BHA showed the lowest malonaldehyde production. This is thought to be due to strong resistance of BHA to gamma-irradiation and effective inhibition of an enzymic system for the oxidation of microsomal lipids in the presence of Fe^{+3}, ADP and NADPH or NADH. Tocopherol action was the most sensitive to gamma-irradiation. This is thought to be due to the destruction of the

chemical structure of tocopherol but the actual mechanism is not understood.

Malonaldehyde has been reported to be a mutagen and has been reported to initiate skin cancer in mice; (47) it has been shown to cross-link the amino groups of DNA in solution, presumably through the formation of schiff bases. Cutler and Hayward (48) have summarized the effects of oxidized unsaturated fatty acids in rats, these include: damage to the intestinal mucosa with necrosis, edema, increased numbers of cytoplasmic vacuoles, inhibition of enzyme systems, oxidation of sulphydryl compounds, malabsorption syndrome, decreased body weight gain, and an impaired absorption of fat and an increased caloric requirement. Malonaldehyde levels can be significantly reduced in meat by addition of antioxidants and low storage temperature.

VI. SUMMARY

The effects of several antioxidants, application times, irradiation dose and storage period on oxidative changes were investigated in irradiated chicken. On an overall basis, marked increases in malonaldehyde production was observed when chickens were irradiated and stored with 25°C. A lower malondaldehyde production was observed when treatment at 0.2×10^6 rad was used as compared to 2.0×10^6 rad.

Samples injected with BHA before irradiation gave higher levels of protection from oxidative changes. The BHA treatment gave

striking protection at both -1°C and 25°C. BHA was most effective in preventing oxidation of chicken meat lipids. Tocopherol had the lowest effectiveness in preventing lipid oxidation when treatment was applied before irradiation. Synergism of the BHA and tocopherol mixture was not found at both temperature and irradiation dose used in this study.

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Appendix A

Relationships Between Variables Affecting Malonaldehyde Production

Analysis of Variance

Variable	D.F.	F	Prob. F
Irradiation	1	239.06	0.0001
Antioxidant	2	93.99	0.0001
Irradiation X Antioxidant	2	15.65	0.0001
Application Time	1	119.23	0.0001
Irradiation X Application Time	1	42.54	0.0001
Antioxidant X Application Time	2	14.01	0.0001
Storage Temperature	1	54.01	0.0001
Irradiation X Storage Temperature	1	1.25	0.2661
Antioxidant X Storage Temperature	2	10.40	0.0001
Application Time X Storage Temperature	1	0.42	0.5206
Storage Time	2	0.64	0.5314
Irradiation X Storage Time	2	1.92	0.1508
Antioxidant X Storage Time	4	5.36	0.0005
Application Time X Storage Time	2	10.93	0.0001
Storage Temperature X Storage Time	2	0.34	0.7128