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The composition and stability of lipids in meat products and a spectrofluorometric assay for malonaldehyde in biological tissues

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THE COMPOSITION AND STABILITY OF LIPIDS IN MEAT PRODUCTS
AND A SPECTROFLUOROMETRIC ASSAY FOR MALONALDEHYDE IN
BIOLOGICAL TISSUES

Iowa State University　　　　　　　　　　　　　　　　　PH.D.　1981

The composition and stability of lipids in meat products

and a spectrofluorometric assay for malonaldehyde

in biological tissues

by

Omololu Oladele Fapojuwo

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Food Science

Approved:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1981

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

A. Statement of Problem

Malonaldehyde is known to be one of the decomposition products of autoxidized, polyunsaturated fatty acids and its assay often serves as a measure of oxidative rancidity. Malonaldehyde is also known to have carcinogenic potential (Shamberger et al., 1974; Mukai and Goldstein, 1976; Marnett and Tuttle, 1980); hence, its content in foods is of concern. The most commonly used method for the assay of malonaldehyde in rancid and nonrancid tissues is the thiobarbituric acid (TBA) test. This method involves the spectrophotometric measurement of the red pigment formed by the complexing of malonaldehyde with TBA in acid solution. However, the TBA reaction as applied to animal tissue is not specific because TBA gives a color reaction with various compounds (Landucci et al., 1955; Dugan, 1955; Baumgartner et al., 1975). Therefore, other chemical methods to determine malonaldehyde seem desirable.

A spectrofluorometric method for the determination of malonaldehyde using 4,4'-sulfonyldianiline as the reagent has been described (Sawicki et al., 1963). This method was found to be highly selective and more sensitive than the TBA test. However, the presence of water or alcoholic solvents inhibited the development of fluorescence. In spite of its greater selectivity and sensitivity, this spectrofluorometric method

has not been applied to foods. The use of the selective 4,4'-sulfonaly-
dianiline reagent for determining malonaldehyde in wet tissues should
be developed.

The use of microwave energy for the preparation and processing
of foods is becoming increasingly popular among consumers. But the
effects of the microwave energy on the stability and composition of
food lipids are still not well-defined. Many factors including heat,
light and high energy radiation are known to catalyze the oxidation of
lipids. Since microwaves are a form of radiation, they might act as a
catalytic agent. Rawls and Van Santen (1970) indicated that microwave
energy as used for cooking or pretreating foods is great enough to
activate oxygen on either a direct attack basis or to promote a reaction
to produce singlet oxygen. The activation of oxygen could lead to an
acceleration of lipid oxidation in meats treated with microwave energy.
Ke et al. (1978) found increased lipid oxidation in mackerel fillets
pretreated with microwave energy and speculated that formation of sing-
let oxygen might be responsible. However, Rosen (1972) pointed out that
microwave energy is too low to cause major chemical changes due to
direct interaction with molecules and chemical bonds. Several investiga-
tions (Berry and Cunningham, 1970; Myers and Harris, 1975; Mai et al.,
1980), showing negligible effects of microwave energy on the fatty acid
composition of meat lipids, seem to support Rosen's idea. More studies
are needed to elucidate the effect of microwave cooking on the stability
of meat lipids.

The amount of _trans_ fatty acids consumed in our diet is of current concern because of a number of physiological effects that have been attributed to _trans_ fatty acids (McMillan et al., 1963; Kummerow, 1974; Hwang and Kinsella, 1979). These physiological effects include development of arteriosclerosis and adverse effects on the fatty acid composition of vital organs. Because of these adverse physiological effects, the amount of _trans_ fatty acids from all dietary sources should be known and included in food tables for dietary advice. Amounts of _trans_ fatty acids consumed through hydrogenated fats and dairy products have been determined but data are needed on other foods where _trans_ fatty acids could be formed during processing or food preparation.

Microwave energy was found to cause the formation of _trans_ fatty acids in baked potatoes (Maga et al., 1977). The ability of microwave energy to cause isomerization of fatty acids in other foods needs to be studied. Rumen micrococci have been shown to cause isomerization of fatty acids (Mills et al., 1970). A species of _Micrococcus_ is used as a starter culture in the preparation of summer sausages. Its ability to isomerize fatty acids during this process has not been investigated. Apart from _Micrococcus_, many more microorganisms are known to be able to cause the formation of _trans_ fatty acids in the rumen. The ability of microorganisms used in the fermentation of foods to do the same should be investigated.

4

B. Objectives

The objectives of this study were:

1. To develop a procedure for the spectrofluorometric measurement of malonaldehyde in meat products using the sensitive and selective 4,4'-sulfonyldianiline reagent.

2. To compare the spectrofluorometric method with the TBA test in terms of their sensitivity and selectivity for measuring malonaldehyde in meat products.

3. To study and compare the stability of lipids in microwave and conventionally cooked meats.

4. To study the effect of microwave cooking on the composition of fatty acids in meats with particular emphasis on trans fatty acids.

5. To study the ability of microorganisms to cause isomerization of fatty acids during fermentation of selected foods.

II. LITERATURE REVIEW

A. Mechanisms and Products of Lipid Oxidation

Most fats, oils and fat-containing foods are known to be sus-
ceptible to oxidative deterioration in the presence of air, giving
rise to objectionable flavors and odors commonly referred to as
rancidity. The reactions involved in autoxidation of lipids are
complex and not entirely understood. A theory of autoxidation by a
free radical mechanism is widely accepted (Koch, 1956; Swern, 1961).
The mechanism involves three types of reactions:

1. Initiation of the chain reaction which involves the formation
of a free radical.

2. Chain propagation.

3. Chain termination.

A mechanism for the autoxidation of a methylene-interrupted
unsaturated system (e.g., linoleate) was reviewed by Holman (1954).
The various reactions which may occur are presented in Figure 1.

In the initiation step, a hydrogen atom is abstracted from the
methylenic carbon atom adjacent to a double bond. This reaction
results in the formation of a free radical (II). The free radical then
reacts with molecular oxygen to form a hydroperoxy radical (III) which
further reacts to form hydroperoxide (IV) by removing a hydrogen atom
from another fatty acid. The removal of a hydrogen atom from the
second fatty acid generates another free radical which initiates the

H   H

R   C   R

C = C   C = C

H   H H   H

Abstraction of a hydrogen
atom

(I)

-H

H

R   C

C = C   C = C

H   R'

H H

Free radical

(II)

O₂   O - O

H

R   C = C   C   R'

C = C   H

C   H

Addition of hydrogen atom
abstracted from another
molecule

Hydroperoxy radical

(III)

+H

H - O - O

H   C   R'

R   C = C   H

C = C   H

H   H

(IV)

Hydroperoxide product

Aldehydes, ketones, alcohols, acids,
hydrocarbons and epoxides
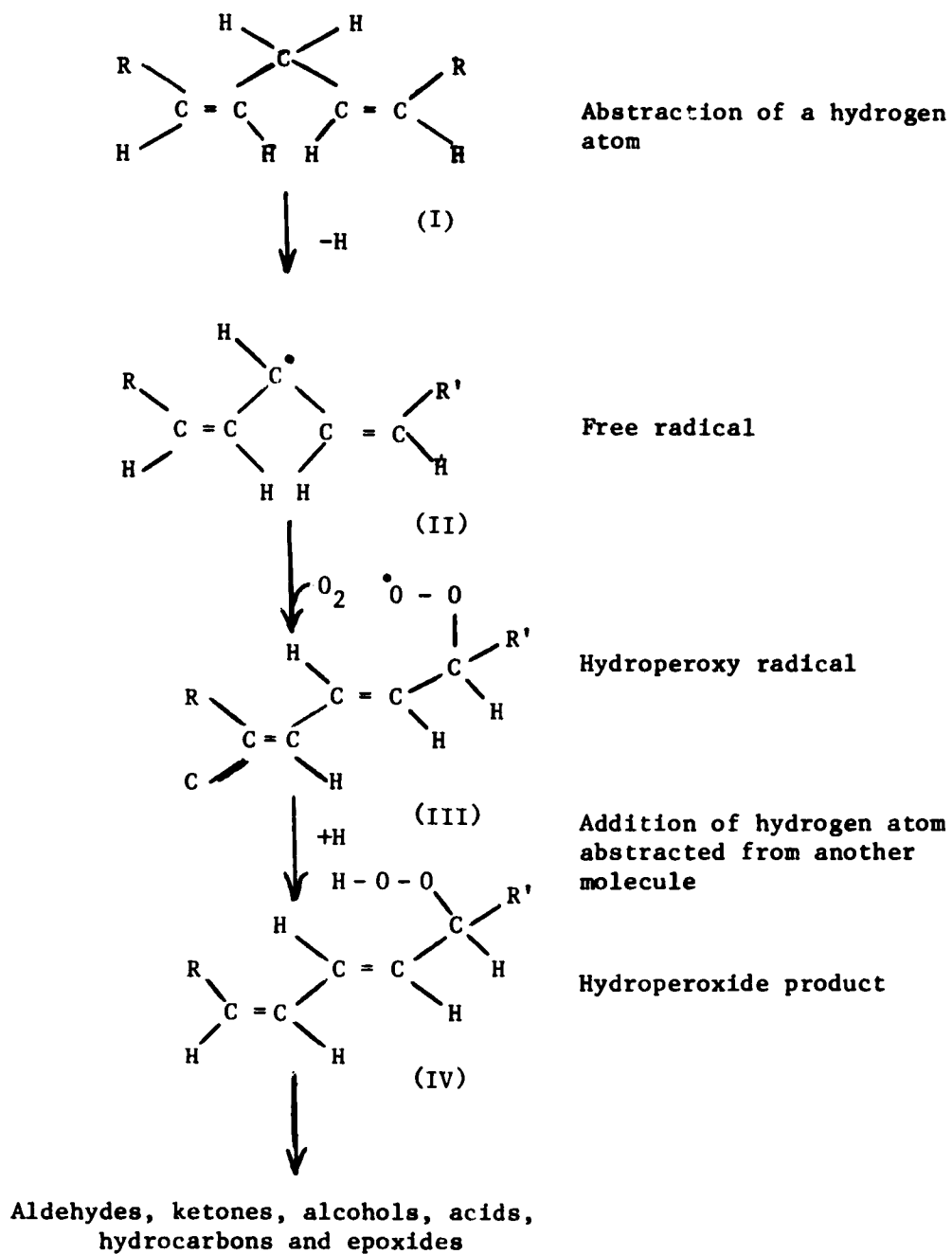
Figure 1. Reaction mechanisms for the autoxidation of an unsaturated
fatty acid

same process giving rise to a chain reaction. As the reaction continues, hydroperoxides build up and eventually decompose to form aldehydes, ketones, alcohols and acids which impart the off-flavors and odors to oxidized fat. Many of these breakdown products have very low odor threshold values. Thus, if they are objectionable in odor, only a few parts per million (ppm) or parts per billion (ppb) are needed to give the food an unacceptable odor. Hexanal, for example, has been implicated as a major breakdown product of oxidized lipids imparting off odors in various foods including dehydrated potatoes (Buttery et al., 1961; Karel and Labuza, 1967). In oil, off odors due to hexanal can be detected at 150 ppb and in milk at 50 ppb. Considering this on a molar basis, this means that less than 0.00002% of the fat has to be oxidized in order to form an objectionable off-flavor.

One of the predominant unsaturated fatty acids in foods is linoleic acid (18:2). The typical pathway for the breakdown of this acid during autoxidation is shown in Figure 2. As can be seen in the figure, the hydroperoxy radical formed from the linoleic acid has three resonance forms; thus, three major hydroperoxides could be formed. As a result of stabilization of the radical in a conjugated system, the C_9 and C_{13} peroxides account for most of the peroxides (95 to 98%) actually formed. Some typical breakdown products of oxidized lipids found in rancid foods and the hydroperoxides from which they are derived are listed in Table 1.

LINOLEIC ACID OXIDATION WITH TYPICAL OFF ODOR COMPOUNDS

$$CH_3 - (CH_2)_4 - CH = CH - CH_2 - CH = CH - CH_2 - (CH_2)_6 - \overset{\overset{O}{\|}}{C} - O - R_o$$

Carbon # 18 17-14   13   12   11   10   9   8   7-2   1

energy $\rightarrow$ H·

$$\begin{bmatrix} R_1 - CH = CH - \overset{\bullet}{C}H - CH = CH - R_2 \\ R_1 - \overset{\bullet}{C}H - CH = CH - CH = CH - R_2 \\ R_1 - CH = CH - CH = CH - \overset{\bullet}{C}H - R_2 \end{bmatrix}$$

RESONANCE FORMS
C_9, C_{11}, C_{13}

O_2

$$R_1 - \underset{\underset{O \cdot}{O}}{CH} - CH = CH - CH = CH - R_2$$

RH

$$R_1 - \underset{OOH}{CH} - CH = CH - CH = CH - R_2 \qquad \text{(Peroxides include } C_9, C_{11}, C_{13})$$

Cleavage

Cleavage

R_1CHO
hexanal

R_1CH
O·

RH
R_1CH_2OH
hexanol

R_1·

RH
R_1H
pentane

Where:

R_o = ester linkage, i.e., to glycerol or acid

$R_1 = CH_3 - (CH_2)_4 -$

$R_2 = CH_2 - (CH_2)_6 - \overset{\overset{}{C}}{\underset{O}{\|}} - O - R_o$

Figure 2. Pathway for autoxidation of linoleic acid

Table 1. Typical breakdown products of hydroperoxides in rancid
          foods

Fatty acid	Hydroperoxide	Aldehyde formed
Oleate	C_8	un-dec-2-nal
	C_9	2-decenal
	C_{10}	nonanal
	C_{11}	ocatanal
Linoleate	C_9,	2,4-decadienal
		2-octenal
	C_{13}	n-hexanal
Linolenate	C_9	2,4,7-decatrienal
	C_{12}	2,4-heptadienal
	C_{13}	3-hexenal
	C_{16}	propanal

The simple mechanism described above (Figure 1) does not easily

explain the formation of certain carbonyls, especially malonaldehyde,

known to be present in foods. A mechanism for the formation of

malonaldehyde was described by Dahle et al. (1962). These workers

showed that β, γ unsaturated hydroperoxy radicals may undergo cyclization

to form a five-membered ring peroxide. Based on the occurrence of this

cyclic peroxide, they proposed a mechanism for the formation of

malonaldehyde as shown in Figure 3.

Of the hydroperoxy radicals formed from linolenate, only two

possess unsaturation β, γ with respect to the peroxy function. Such

a configuration is significantly absent in the isomeric radicals of

linoleate. Thus, the ring peroxide appears only in the oxidation of

methylene-interrupted systems with three or more double bonds. It
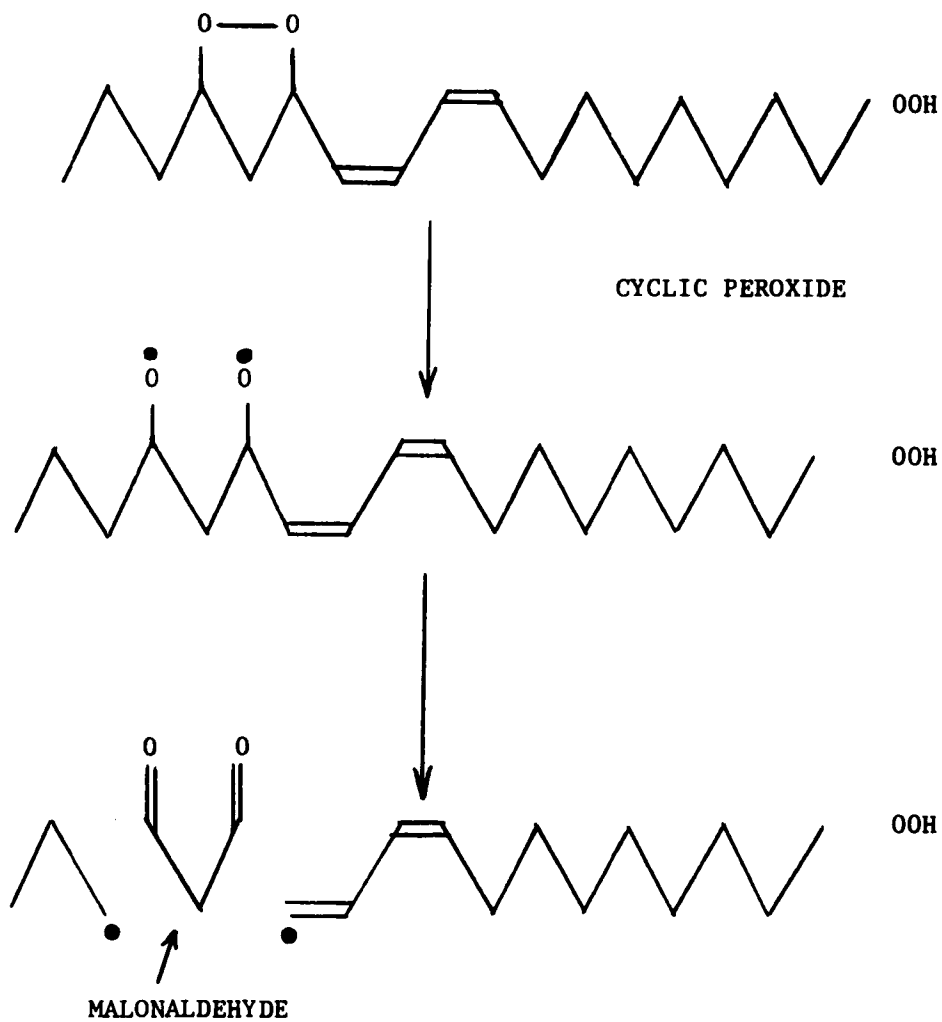
Figure 3. Mechanism for the formation of malonaldehyde (from Dahle et al., 1962)

was concluded by Dahle and co-workers (1962) that, in addition to the

generally accepted mechanism for lipid oxidation, the mechanism of

oxidation of linolenic acid and more highly unsaturated fatty acids

may involve an additional step of intramolecular rearrangement of

the hydroperoxide free radicals. Lillard and Day (1964) also showed

that malonaldehyde and other carbonyls found in autoxidized lipid

systems which are not theoretically expected from the decomposition of

lipid hydroperoxides, could be formed by the degradation of mono-

carbonyls which are initial degradation products of lipid hydro-

peroxides.

The kinetics of lipid oxidation in foods has been thoroughly

reviewed by Labuza (1971). It is known that the rate of reaction is

not a direct function of the number of double bonds but that it in-

creases drastically as the unsaturation increases. Thus, linoleate

oxidizes 10x faster than oleate and linolenate 20 to 30x faster. Small

amounts of various compounds either accelerate or inhibit the reaction

in a drastic way. For example, heme compounds may act to either accelerate

or inhibit the reaction depending on the ratio of heme to lipid, while

antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxy-

toluene (BHT) generally cause inhibition. Lipid oxidation is also

known to be catalyzed by metals, light and various biocatalysts

(Schultz, 1962).

The possible role of singlet oxygen in the primary initiation

reaction of lipid oxidation has been studied (Ingold, 1961; Rawls and

van Santen, 1971; Terao and Matsushita, 1977; Thomas and Pryor,

1980). Singlet oxygen can be formed through photochemical reactions
in the presence of a sensitizer as follows:

$$'S + hv \rightarrow {}^1S^* \rightarrow {}^3S^*$$

$$^3S^* + {}^3O_2 \rightarrow {}^1O_2^* + {}^1S$$

$$^1O^* + RH \rightarrow ROOH$$

$$ROOH \rightarrow Free\ radicals$$
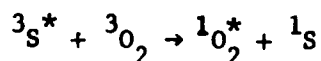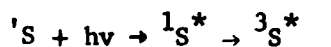
where ^1S = singlet state sensitizer

$^1S^*$ = excited singlet state sensitizer

$^3S^*$ = excited triplet state sensitizer

$^3O_2$ = normal triplet oxygen

$^1O_2^*$ = excited singlet state oxygen

hv = UV light energy in photons.

Microwave energy could also activate oxygen (Rawls and van Santen,
1970). It has been found that singlet oxygen reacts about 10^3 to 10^4
faster than the normal oxygen with methyl linoleate (Rawls and van
Santen, 1971).

The acceptability of a fat-containing food depends on the extent
to which oxidative deterioration has occurred; hence, it is important
to have some criteria for assessing the extent of lipid oxidation.
A comprehensive review of the experimental techniques for the measure-
ment of lipid oxidation was written by Gray (1978). The spectrum of
tests ranges from sensory evaluation to chemical and physical
methods. Sensory evaluation is usually conducted with a taste panel
and the results are analyzed statistically. Even though consumers

use sensory characteristics to judge the quality of foods, the method has disadvantages because it is time-consuming and has poor reproducibility.

The chemical methods that have been developed include measurement of peroxide value, the TBA test, the Kreis test, determination of total and volatile carbonyl compounds and oxirane determination. These methods are more reproducible and sensitive than sensory measurements, but they do not correlate perfectly with sensory properties of oxidized lipids. Each method gives information about particular stages of the autoxidative process and some are more applicable to certain lipid systems than others. The method of choice depends on a number of factors including the nature and history of the oxidized sample, the type of information required, the time available and the test conditions. Gray (1978) discussed the need for a more thorough assessment of the available methods so that unreliable, cumbersome methods may be discarded and modifications made in the remaining methods to maximize the information obtained. Physical methods include conjugated diene methods, fluorescence, infrared spectroscopy, polarography, gas chromatography and refractometry.

B. Lipid Oxidation in Meat and Meat Products

The composition of meat lipids, the nature of the oxidative reaction, the catalysts involved and some methods of controlling oxidation have been adequately reviewed by Love (1971). Meat lipids can be classified

as tissue (intramuscular) or depot (intermuscular) lipids. The tissue lipids exist in close association with proteins and contain a large percentage of the total phospholipids (Watts, 1962). The phospholipids contribute about 1% of the tissue weight and the triglyceride fraction is about five times as large (Hornstein et al., 1961). The high unsaturated fatty acid content of these phospholipids make them susceptible to oxidation. High levels of linoleic and arachidonic acids are found in the phospholipids. Hornstein et al. (1961) found that the phospholipid fractions from pork and beef developed off-flavor more readily than the neutral fat and, hence, contribute more to off-flavor. The unsaturated fatty acid composition of phospholipids from different muscles and species may be quite variable (Kuchmak and Dugan, 1965) and may explain why different muscles and species have varying susceptibilities to oxidative rancidity.

Lipid oxidation develops more rapidly in cooked meat than raw (Timms and Watts, 1958). Frozen raw meat is quite stable for periods of several months, depending on the species from which it originated and storage conditions. Adverse changes in color and flavor have been reported to result from lipid oxidation in raw meat (Greene, 1969; Kesinkel et al., 1964). Frozen stored meat is generally fairly resistant to oxidation but rancidity can still develop during freezing and thawing (Watts, 1961). Cooked cured meat has lower levels of lipid oxidation as compared with the uncured samples. Cooked meat irradiated at pasteurizing levels and refrigerated has also been reported (Watts, 1961) to deteriorate as a result of lipid oxidation. Chang and Watts

(1950) showed that cooked, irradiation-sterilized beef did not develop oxidative rancidity when stored in air tight containers, and postulated that the pigments were converted to a catalytically inactive form during radiation treatment. Greene and Watts (1966) later indicated that the low TBA values observed for stored cooked, irradiated meat were due to a combination of antioxidant development and further reactions undergone by lipid oxidation products.

Catalysis of lipid oxidation in meat was well-covered in the review by Love (1971). The accelerating effect of hemoglobin and other iron-porphyrins on the oxidation of lipids is a generally accepted phenomenon and hemoproteins have been implicated as the major pro-oxidant in meat and meat products (Tappel, 1952; Younathan and Watts, 1959; Tappel, 1953). Ferric hemochromogen is postulated to be the active catalytic form of the muscle pigments (Younathan and Watts, 1959; Tappel, 1953). In cooked meat, the pigment is in the active denatured ferric hemochromogen form, accounting for the rapid initiation of lipid oxidation. The lower levels of oxidation in cured, stored meat results from the conversion of the pigments to the catalytically inactive ferrous nitric oxide hemochromogen (Zipser and Watts, 1967).

In fresh meat, the pigments exist in three forms: purple reduced myoglobin, red oxymyoglobin and brown metmyoglobin. Metmyoglobin is undesirable from the standpoint of meat color and also because of the catalytic effect of ferric hemes on the oxidation of unsaturated fatty acids. Free radical intermediates from lipid oxidation can decompose hemes, resulting in loss of color. Thus, pigment and lipid oxidation

are interrelated in fresh meat and of crucial importance from the standpoint of consumer acceptability (Greene, 1969). A more recent review (Pearson et al., 1977) covers the catalysis of lipid oxidation in greater detail. In this review, the involvement of heme compounds, metal ions and a comparison of heme and nonheme iron as prooxidant in muscle tissues during lipid oxidation were discussed. Liu and Watts (1970) found that both heme and nonheme iron function as catalysts of lipid oxidation in cooked meat. In contrast, Sato and Hegarty (1971) presented evidence that nonheme iron and ascorbic acid catalyzed lipid oxidation in cooked meat but found heme compounds to have little effect on the development of warmed-over flavor (WOF). Love (1972) presented findings that confirmed the observations of Sato and Hegarty (1971).

C. Significance of Malonaldehyde in Foods

Malonaldehyde occurs in foods and biological preparations as a product of lipid oxidation. High levels of malonaldehyde are generally found in rancid foods (Sinnhuber and Yu, 1958). Malonaldehyde has been detected in cooked meats (Tarladgis et al., 1960), cured meats (Zipser and Watts, 1962), raw ground beef (Hutchins et al., 1967), fish meat (Kurkhanova and Onekhova, 1971), fish oil (Sinnhuber and Yu, 1958; Koning and Silk, 1963), rancid salmon oil (Sinnhuber and Yu, 1958), rancid nuts (Holland, 1971), vegetable oils (Arya and Nirmala, 1971), fresh frozen green beans (Chow and Watts, 1969), fats (Sedlocek, 1964), milk (Downey, 1969), milk fat (Patton and Kurtz, 1951), rye

bread (Pein, 1964), and orange juice essence (Braddock and Petrus, 1971). Significant amounts of malonaldehyde have also been found in foods that do not have detectable rancid off-odors, therefore, the occurrence of malonaldehyde in foods seems not to be associated only with rancidity. Shamberger et al. (1977) found a range of 1-14 μg/g and 0.3-39 μg/g malonaldehyde in raw and cooked meats, respectively. Siu and Draper (1978) obtained a range of 0.35-2.9 μg/g in raw meats and 0.7-5.3 μg/g in cooked meats. In some meat products, the cooking process itself increases the malonaldehyde content (Newburg and Concon, 1980). It has been suggested that the malonaldehyde content of meats may have epidemiological significance (Shamberger, 1977), possibly accounting for a part of the strong correlation between per capita meat consumption and cancer incidence in various countries (Cairns, 1975). Malonaldehyde has been reported to be produced during the biosynthesis of prostaglandins and thromboxanes (Lands et al., 1977).

Malonaldehyde has been shown to be a potential carcinogen. It is mutagenic to __Salmonella__ __typhimurium__ (Mukai and Goldstein, 1976; Marnett and Tuttle, 1980) and initiates skin tumors in mice (Shamberger et al., 1974). Several reports have indicated that harmful effects resulted when experimental animals consumed peroxidized foods. Cutler and Hayward (1974) have summarized the effects of ingested oxidized unsaturated fatty acids in rats. These include damage to the intestinal mucosa with necrosis, edema, increased ctyoplasmic 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. Cutler and Schneider (1973) have reported an increase in the incidence of mammary tumors induced by 7,12-dimethylbenzanthracene among rats and mice receiving oxidized linoleic acid in their diet.

A disorder involving lipid peroxidation in humans occurs in Batten's disease (Siakotos et al., 1974). The brains of patients with this disease accumulate a ceroid pigment with some similarities to the lipofuscin "age" pigment. Chio and Tappel (1969a) synthesized and characterized fluorescent products derived from malonaldehyde and amino acids. The Schiff-base products of malonaldehyde crosslinked with the primary amino groups of amino acids, proteins, nucleic acids, and their bases, or phospholipids, have fluorescent properties similar to lipofuscin. Feeding oxidized corn to groups of 40 male Charles River rats resulted in an increased number of animals with focal myocarditis and focal fibrosis of the heart (Kaunitz and Johnson, 1973). Because of these harmful effects which are directly or indirectly related to malonaldehyde, levels of this aldehyde in foods should be measured and to achieve this, a very sensitive and selective method for malonaldehyde determination is necessary.

D. Detection of Malonaldehyde and Fluorescent Products

Malonaldehyde is not commercially available in pure form. Although crystalline malonaldehyde was prepared by Hüttel (1941), it is hygroscopic and volatile; and, thus, it can be kept only a very short time. Standards that have been recommended for use in the determina-

tion of malonaldehyde and its precursors are β-Ethoxyacrolein diethyl-acetal (1,3,3-triethoxyprop-1-ene); malonaldehyde bis sodium bisulphite; 1,1,3,3-Tetraethoxypropane; 1,1,3,3-Tetramethoxypropane; sodium malonal-dehyde; and N-prop-2-enal aminoacetic acid. These standards are readily hydrolyzed to malonaldehyde by acids.

Malonaldehyde can be determined in aqueous acidic (λ_{max} = 245 mμ, ε = 13,000) or alkaline (λ_{max} = 267 mμ, ε = 30,000) solutions. Obviously, the presence of organic compounds that absorb in the ultra-violet region would interfere seriously in this determination. Reagents that have been used in the analysis of malonaldehyde are shown in Table 2. Of these reagents, the most commonly used and perhaps most satisfactory is thiobarbituric acid (TBA). Measurement of malonaldehyde by the use of TBA derivatives has been employed for many years to measure rancidity of food or to follow peroxidative changes in animal tissues. Early investigations by Sinnhuber et al. (1958) helped to clarify the nature of the colorimetric reaction that occurs during the TBA test. They proposed that the chromagen was formed through the condensation of two molecules of TBA with one molecule of malonaldehyde (Figure 4).

The TBA test may be performed in several ways. A number of investigators have heated the macerated food directly with an acidified TBA reagent, extracting the pigment with an immiscible solvent (Turner et al., 1954; Yu and Sinnhuber, 1957). Others apply the test to a metaphosphoric or trichloroacetic acid extract of the food (Tappel and

Table 2. Reagents for the analysis of malonaldehyde and its standards (from Sawicki and Sawicki, 1975)

Reagent	λ_{max}(mϵ) or $F_{exc/em}$
Acid	250 (2·1)[a]
Alkali	267·5 (27·1)[b]
4'-Aminoacetophenone	504 (67)[c]
	F520/580
p-Aminobenzoic acid	F475/520[d]
p-Aminobenzoate, ethyl ester	F500/550[e]
4-Aminodiphenylamine	427 (29)
Aniline	387 (48·6)[f]
Anthrone	510 (7·9)
	F485/560
Azulene	702 (142)
Barbituric acid	485g
Benzenediazonium tetrafluoroborate	369 ($\sim$ 20)
10,9-Borazaronaphthalene	514
9,10-Diacetoxyanthracene	F495/560
N-Ethylcarbazole	590 (34)
4-Hexylresorcinol	603 (46)
Indole	550 (60)
3-Methyl-2-benzothiazolinone hydrazone (MBTH)	670 (17)
2-Methylindole	555

[a]At pH 1. However, at pH 2.2 or in 1 N H_2SO_4 λ_{max} 245 nm, mϵ 13.

[b]In 3% aq triethylamine. At pH 12 λ_{max} 267 nm, mϵ 30 and above pH 7 λ_{max} 267, mϵ 31.8.

[c]The pure neutral chromogen absorbs at λ_{max} 395 nm, mϵ 63·1 and its cationic salt at 409 nm.

[d]The chromogen cation is said to absorb at 400 nm.

[e]The pure neutral chromogen absorbs at λ_{max} 386 nm, mϵ 56·2, while its cation absorbs at 403 nm.

[f]The pure chromogen cation absorbs at 388 nm, mϵ 55·5 in ethanol while the anion absorbs at 437 nm, mϵ 61·7 in dimethylformamide and the neutral chromogen at λ_{max} 360 nm, mϵ 42·5 in 96% ethanol.

[g]Pure chromogen absorbs at 495 nm.

Table 2. Continued

Reagent	λ_{max}(mϵ) or $F_{exc/em}$
N-Methylpyrrole	558 (85)
p-Nitroaniline	580 (74·6)
4-Phenylazoaniline	605 (46)
Phloroglucinol	543
Sulphanilamide	F475/520
4,4'-Sulphonyldianiline	F475/545
Thiobituric acid	530 (15·8)[h]

[h] mϵ values of 153, 154 and 156 have been reported.

Zalkin, 1959) or to a distillate from the acidified food (Sidwell

et al., 1955; Tarladgis et al., 1960). All modifications of the method

involve an acid treatment of the food. The test has been criticized

on several points. One serious disadvantage is that the TBA reaction

as applied to animal tissue gives color reactions with various compounds

in addition to malonaldehyde (Landucci et al., 1955). Other evidence

that TBA can react with compounds other than those found in oxidizing

systems to produce characteristic red pigment has been presented in

the literature. Dugan (1955) has reported that sucrose and some

compounds in woodsmoke react with TBA to give a red color so that cured

and smoked meats require corrections for the sugar content and for the

smoke in the outer layer. Baumgartner et al. (1975) also found that a

mixture of acetaldehyde and sucrose when subjected to the TBA test

produced a pigment absorbing at 532 nm which was identical to that

produced by malonaldehyde and TBA. The authors suggested that this

reaction may interfere with the assay of lipid peroxidation in organs
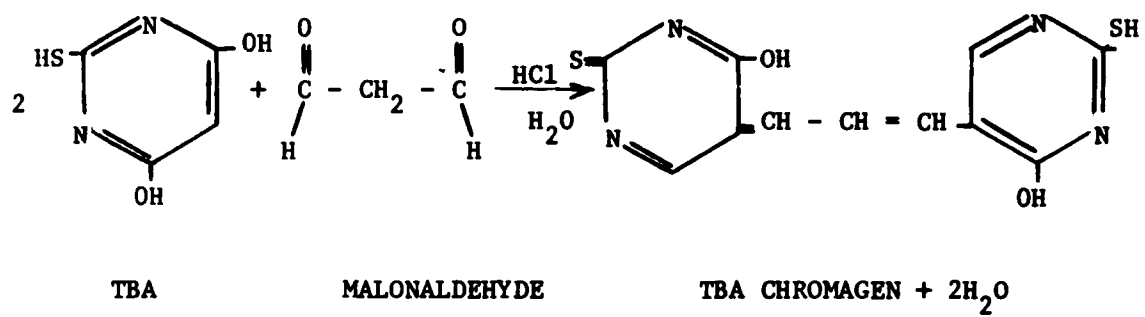
Figure 4. Proposed TBA reaction (Sinnhuber et al., 1958)

such as the liver where ethanol can be converted to acetaldehyde. Tarladgis and his co-workers (1962) considered the effect of acid, heat and oxidizing agents on the TBA reagent. They found that the structure of TBA was altered by acid and heat treatment as well as by the presence of peroxides and recommended that blank determinations be carried out in conjunction with the test.

Many types of samples submitted to the TBA test have yielded an appreciable amount of absorption in the 440 to 460-mμ region (Kenney and Bassette, 1959; Dunkley and Jennings, 1951; Wertheim and Procter, 1956). Compounds such as glycidaldehyde and glyceraldehyde form a yellow chromagen at 456 mμ (Patton, 1960). A large amount of this background absorption would interfere seriously with the determination of malonaldehyde using thiobarbituric acid. Because of the non-specific nature of the TBA, malonaldehyde quantitation by this method will always be questioned.

Malonaldehyde is a very reactive compound which is known to react with amino groups of proteins, amino acids, and phosphatidyl-ethanolamine to form fluorescent compounds in small yields (Chio and Tappel, 1969a; Chio and Tappel, 1969b; Malshet and Tappel, 1973). Measurement of these compounds via fluorescence spectroscopy could provide sensitive and selective methods to determine the occurrence of in vivo and in vitro lipid peroxidation (Chio and Tappel, 1969a; Fletcher et al., 1973, Bidlack and Tappel, 1973). One problem with this technique is the presence in tissues of interfering fluorophores such as vitamin A. Fletcher et al. (1973) proposed that this problem could be removed

by irradiating samples with high intensity ultraviolet light for 30

seconds, but this method has been shown to give variable results (Csallany

and Ayaz, 1976). Csallany and Ayaz (1976) used Sephadex LH 20 to

separate the fluorescent (350 nm excitation, 435 nm emission) products

of rat and mouse tissues from interfering compounds. However, Purdy

and Tappel (1979), using Silylated glass beads in order to eliminate

the swelling problems of Sephadex LH 20, found quantitation impractical.

All of the fluorescent moeities of interest did not always co-

chromatograph. For this technique to be useful, the problem of inter-

ference and the chemistry of the formation of fluorescent products and

their specific characterization needs further exploration.

A rapid, fluorescence method for assessing the oxidative abuse

status of fats, oils and dry tissue lipids has been developed (Porter

et al., 1980). The method depends upon measurement of the fluorescence

of compounds produced by reaction of the volatiles arising from peroxi-

dizing lipids and the contaminant amines of a polyamide coating adsorbed

on a glass or plastic plate as conventionally used in thin layer chromato-

graphic separations. The fluorescence, which is measured at an excita-

tion maximum of 360 nm and an emission maximum of 425 nm, is presumed

to arise from polymer-bound amino-imino-propene compounds resulting from

the reaction of malonaldehyde and the known contaminant amines of the

polyamide (poly-epsilon-caprolactam). The same fluorescence was

generated from tetra-ethoxypropane (a malonaldehyde generator) in the

absence of peroxidizing lipids.

Procedures for the detection of malonaldehyde with aromatic amines
through spectrofluorometry have also been described (Sawicki et al.,
1963). Table 3 shows the aromatic amines that have been used. When
compared with other methods for determining malonaldehyde, this spectro-
fluorometric method was found to be highly selective and more sensitive.
Very good reproducibility was obtained, but the presence of water or
alcoholic solvent inhibited the formation of fluorescence. A linear
relationship between the concentration of malonaldehyde and the relative
fluorescent intensity was found from 5 to 170 ng of malonaldehyde. The

Table 3. Aromatic amine determination of malonaldehyde through
          spectrophotofluorimetry

Reagent	$F_{exc/em}$	Relative fluor. intensity
Quinine[a]	F350/450	1
4,4'-Sulphonyldianiline	F490/545	8
Ethyl p-aminobenzoate	F490/550	5
p-Aminobenzoic acid	F475/520	0·8
p-Aminoacetophenone	F490/580	0·5

[a]Standard.

fluorescence intensity was stable for 30 minutes. 4,4'-Sulfonyldianiline
was found to be the best reagent for this procedure.

Despite its advantages over the widely used TBA test, this
spectrofluorometric method has not been applied to the determination
of malonaldehyde in foods. This is probably due to the fact that water,
which inhibits the formation of fluorescence, is present with

malonaldehyde in moist foods such as meat, which are commonly subjected to the TBA test.

E.   Effects of pH on Malonaldehyde

The possible structures of malonaldehyde in neutral, acid and alkaline solutions are shown in Figure 5. The chelated form (IV) which occurs at very low pH is very volatile while the enolate anion (V) which occurs at high pH is nonvolatile. Therefore, the volatility of malonaldehyde during distillation depends on the pH of the solution being distilled. Kwon and Watts (1964) tested this assumption by distilling the same amounts of malonaldehyde at different pHs. The results are shown in Figure 6. The recovery of the malonaldehyde below pH 3 was about 65%, while above pH 6.5, the recovery was negligible. Between pH 3 and 6.5, which is the shaded area in Figure 6, the recoveries differ depending upon the proportions of the volatile chelated form (IV) and the nonvolatile enolate form (V). Where distillation is employed to separate malonaldehyde from other food constituents, maximum volatilization of malonaldehyde would not be expected at pH values above 3.

(I)   (II)   (III)

(IV)   (V)

Chelated Form   Enolate Anion
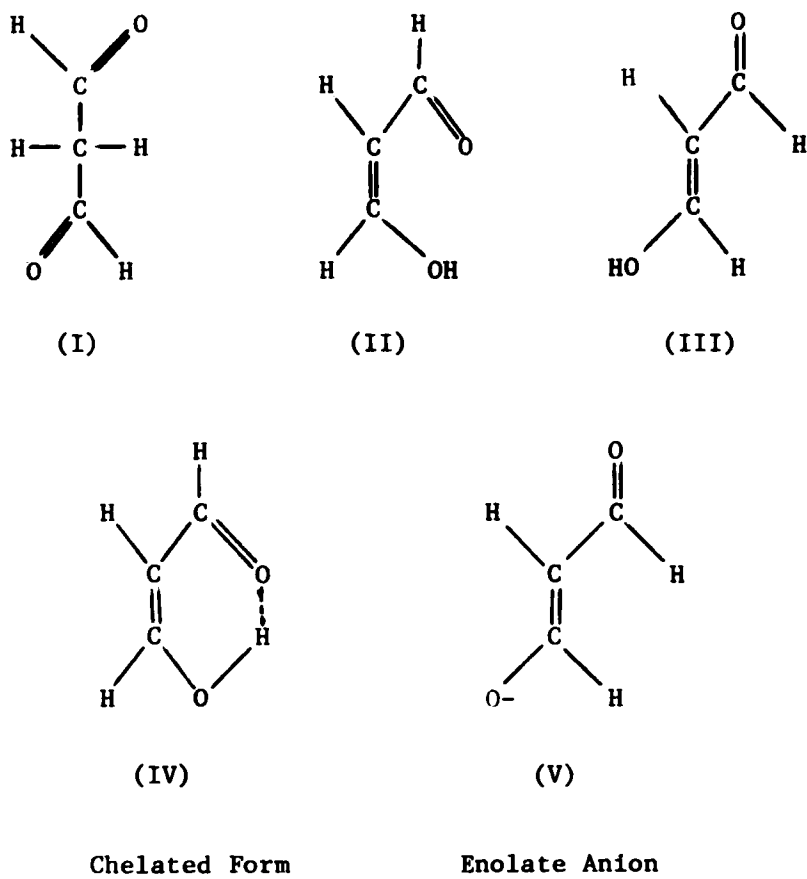
Figure 5. Structures of malonaldehyde in neutral, acid and alkaline solution
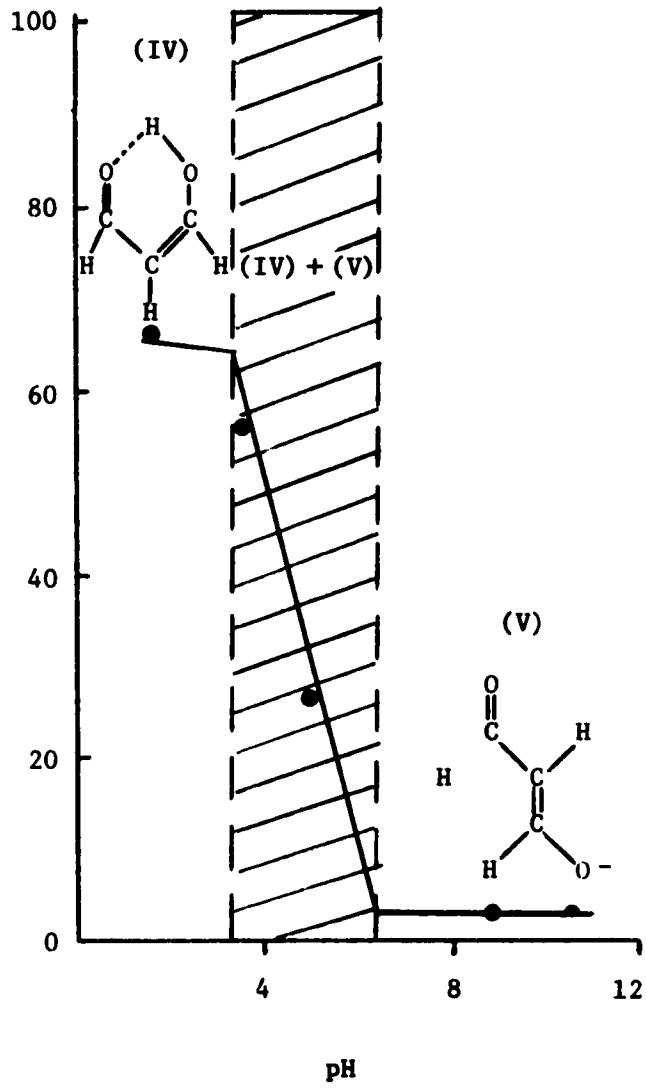
Figure 6. Effect of pH on recovery of malonaldehyde by distillation
(from Kwon and Watts, 1964)

F. Microwave Heating of Foods

The use of microwave energy for the preparation and processing of foods attracts much interest today. By 1990, it is projected that 25% of American households will have a microwave oven (Anonymous, 1979). Microwave ovens are also gaining in popularity in large-scale feeding operations to reheat conventionally prepared foods and to thaw and warm precooked frozen foods (Keefe and Goldblith, 1973). The many potential users such as elementary schools, colleges, hospitals, nursing homes, military feeding operations and welfare institutions, however, have a great concern for the nutritional quality of the foods they serve and data on the nutritional status and the stability of the microwave cooked foods are needed.

Foods which are cooked with microwave energy are heated as the result of the transformation of electrical energy into heat energy on absorption of the microwaves by the food material. Foods are heterogeneous mixtures containing molecules which are not electrically neutral. In an electric field, these molecules behave like magnets trying to line up with the field, which is changing millions of times each second. They are unable to do so because of other forces which restrict their movement (Decareau, 1972). The energy of the microwaves in trying to overcome these forces is converted to heat. Theoretically, the effect of microwaves upon various components of our foods could differ significantly from that of conventional cooking process.

The advantages of microwave cooking are convenience, quickness, and reduced loss of some nutrients via elution or chemical alterations.

Thermador (1978) found greater retention of ascorbic acid in fruits and vegetables when microwave cooked were compared to conventionally cooked. Tsen et al. (1977) found less destruction of lysine in microwave heated than in conventionally heated bread. Beef, pork and lamb roasts cooked by microwave retained less sodium chloride, phosphorous and iron compared to the same types of roast prepared by conventional methods (Baldwin et al., 1976). Some disadvantages of microwave cooking include the inadequate browning, lack of texture development, "crisping" in certain products and uneven heating.

G. Effects of Microwave Energy on Meat Lipids

1. Total lipid and lipid oxidation

Many factors are known to catalyze autoxidation of lipids. Among these are heat, light and high-energy radiation. Since microwaves are a low-energy form of radiation, they might also have a catalytic effect. Very little work has been done concerning the stability of microwave cooked meat products as compared to those cooked by conventional means. Ziprin and Carlin (1976) reported that sensory evaluation indicated that fat oxidation and post-treatment flavor in meats were affected by microwave heating. Ke et al. (1978) studied the effect of microwave heating pretreatment on lipid oxidation in frozen mackerel fillets. They reported an acceleration of lipid oxidation in microwave treated products during the 6 months frozen storage. These workers explained their observations in terms of the fact that the microwave energy at 2450 mHz used for pretreatment of the fish was great enough

to activate oxygen on either a direct attack basis or to promote a reaction to produce singlet oxygen using tissue pigments as sensitizers (Rawls and van Santen, 1970). In order to be sure that the observed increase in lipid oxidation is due to the microwave energy per se, microwave-heated samples should be compared to samples subjected to other forms of heat treatment.

Penner and Bowers (1973) compared freshly cooked boneless loins of pork with conventionally reheated and microwave reheated samples, which were precooked to 65°C and reheated to 55°C. TBA values were significantly different among the three heating treatments. TBA values were highest for pork exposed to the longest total heating time (precooked and conventionally heated) and lowest for pork exposed to only one heating (freshly cooked). The microwave reheated pork had an intermediate TBA value. Differences were not attributed to microwave effects but to the catalytic effect of heat in increasing the oxidation reaction rates.

2. Fatty acid composition

Significant changes occurred in the composition of palmitic, oleic and linoleic acids in beef patties cooked by conventional and microwave methods (Janicki and Appledorf, 1974). The palmitic acid underwent the greatest percent loss during cooking. The percent oleic and linoleic fatty acids increased following all cooking treatments. Compared to the raw meat, these differences were significant but comparing the different heat treatments, no significant differences could be established. Myers and Harris (1975) studied the

to activate oxygen on either a direct attack basis or to promote a reaction to produce singlet oxygen using tissue pigments as sensitizers (Rawls and van Santen, 1970). In order to be sure that the observed increase in lipid oxidation is due to the microwave energy per se, microwave-heated samples should be compared to samples subjected to other forms of heat treatment.

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2. Fatty acid composition

Significant changes occurred in the composition of C-16:0, C-18:1 and C-18:2 fatty acids in beef patties cooked by conventional and microwave methods (Janicki and Appledorf, 1974). The C-16:0 fatty acid underwent the greatest percent loss during cooking. The percent C-18:1 and C-18:2 fatty acids increased following all cooking treatments. Compared to the raw meat, these differences were significant but comparing the different heat treatments, no significant differences could be established. Myers and Harris (1975) studied the

effect of microwave energy and conventional heating on the fatty acid composition of meats and poultry. Gas-liquid chromatographic analysis of the methyl esters of myristic, palmitic, stearic, oleic, linoleic and linolenic acids revealed no significant difference between samples as a result of heat treatment. Berry and Cunningham (1970) reported only a slight difference between the fatty acid composition of chicken meat cooked by different heating methods including microwave cooking. Mai et al. (1980) found similar fatty acid composition in all raw and microwave cooked beef tallow and chicken fat.

Clearly, the effect of microwave energy on food lipids is still not well-understood. The several findings that lipid changes in food products subjected to microwave radiation are negligible are in accord with the conclusions drawn by Rosen (1972), who had pointed out that microwave energy is too low to cause major chemical changes due to direct interaction with molecules and chemical bonds. However, the statement by Rawls and van Santen (1970), that the microwave energy at 2450 mHz was great enough to activate oxygen on either a direct attack basis or to promote a reaction to produce singlet oxygen, using tissue pigments as sensitizers, seems logical based on the relatively few reports that microwave energy accelerates lipid oxidation. More studies are needed on this accelerating effect on lipid oxidation in order to be able to evaluate the different conclusions reached by Rosen (1972) and Rawls and van Santen (1970).

H.   Sources and Physiological Effects of <u>Trans</u> Fatty Acids

Kummerow (1975) reported that Americans consumed approximately ten billion pounds of "visible" fats per year and listed the major source as 6.3 billion pounds of soybean oil which was converted to baking or frying fats, salad oils or margarines. The stabilization of soybean oil towards autoxidation by means of hydrogenation has made it possible to supply an abundance of economical calories to the American consumers. In commercial practice, the undesirable linolenic acid in the soybean oil is converted to the more stable monoenoic (oleic) and saturated fatty acids through hydrogenation of the double bonds. In this process, the double bonds are also isomerized and up to 50-60% of these bonds are converted from the natural cis to trans forms. Stick margarine contains from 25-35%, tub margarines 15-25%, shortenings 20-30% and salad oils from 0-15% <u>trans</u> fatty acids. Household consumption data (USDA, 1971) indicate that margarine represents 7%, shortenings 13.2% and cooking and salad oils 12.4% of the visible fat intake. On this basis, Kummerow (1975) estimated the total fatty acid intake from visible fat as approximately 8%.

The primary concern about the ingestion of <u>trans</u> fatty acids is that they are metabolized differently than their cis isomers. The interplay between the <u>trans</u> fatty acids and the essential ω6 series of PUFA has been unraveled by the research of several workers (Klenk, 1965; Stoffel and Ahrens, 1959; Lands et al., 1966, Privett and Blank, 1964, De Tomas et al., 1963; Mohrhauer and Holman, 1963; Sgoutas, 1968, 1970; and Sgoutas and Kummerow, 1969, 1970). These workers have shown

that in the absence of dietary _trans_ fatty acids, the $\omega6$ series of

PUFA esterify the β position of phosphatydylcholine and that in the

absence of dietary sources of the $\omega6$ series of fatty acids, the

elongated $\omega9$ series of fatty acids esterify this position. The _trans_

fatty acids esterify the α position in the presence of dietary $\omega6$

PUFA. However, in the absence of dietary $\omega6$ PUFA, the elongated _trans_

fatty acid esterifies the β position. The PUFA in the β position of

phosphotidylcholine are used to esterify cholesterol.

The interplay between the esterification and hydrolysis of the

cholesterol esters is very precise. The elongated $\omega6$ PUFA preferentially

esterifies cholesterol and is also preferentially hydrolyzed (Goller

et al., 1970). However, in the absence of elongated $\omega6$ PUFA, the $\omega9$

series of fatty acids esterify cholesterol. In the presence of _trans_

fatty acids, the $\omega6$ PUFA preferentially esterifies cholesterol; however,

the _trans_ fatty acids also esterify cholesterol. Once esterified,

$\omega6$ cholesterol esters are preferentially hydrolyzed which can result

in the accumulation of cholesterol esters of the elongated $\omega9$ fatty

acids in the tissue (Kummerow, 1975).

The levels of dietary linoleic acid in a fat which contains

"elaidinized" fat, that is, _trans_ fatty acids may be important to

atherogenesis. It was shown by McMillan et al. (1963) that a higher

serum cholesterol level and more atherosclerosis occurred in rabbits fed

elaidinized olive oil than in those fed olive oil. Other physiological

effects of _trans_ fatty acids have been discussed by Kummerow (1974) and

Hwang and Kinsella (1979). Feeding _trans_ fatty acids has been shown

to affect the fatty acid composition of vital organs (Anderson et al.,

1975; Takatori et al., 1976; Privett et al., 1977, Yu et al., 1980a).

In a recent study, Yu et al. (1980b) found that dietary trans-trans-linoleate, when fed to rats in increasing amounts, caused a reduction in lung weights, particularly at very high dietary concentrations of trans acids. The fatty acid composition of the lung phospholipids and triglycerides was altered. The percentage of oleic and arachidonic acids in lipids decreased as dietary trans 18:2 was increased. Eicosatrienoic acid (20:3) appeared in the phospholipids of lungs from rats receiving 100% dietary trans 18:2, but its concentration was much lower than in lungs from rats on an essential fatty acid deficient diet, indicating that trans 18:2 inhibited the enzymes synthesizing 20:3. Because of these potential physiological effects, dietary advice or food tables should take into consideration the amount of trans fatty acids from all dietary sources.

The trans fatty acid composition of milk fat has been determined (Woodrow and Deman, 1968). Microorganisms in the rumen are known to be responsible for the formation of these trans fatty acids. Reiser (1951) was the first to demonstrate this during experiments in which linseed oil was incubated with sheep rumen contents in vitro. Later, analyses by Shorland et al. (1955) showed that the linolenic acid present in pasture grasses was readily hydrogenated in the rumen to yield a cis-trans-dienoic acid, cis- and trans-monoenoic acids and stearic acid. Confirmation of this was obtained in vivo by Wood et al. (1963) using sheep given intraruminal injections of $(1-{}^{14}C)$-linolenic acid and in vitro by Ward et al. (1964) using $(U-{}^{14}C)$-linoleic and oleic acids. Ward et al. (1964) observed that the products of biohydrogenation in

the rumen were absorbed in the ileum and that any unsaturated fatty acids that escaped biohydrogenation in the rumen were biohydrogenated in the caecum and colon with the result that nearly all the fatty acids excreted in the feces were saturated. These workers also showed that a major intermediate in the biohydrogenation of unsaturated fatty acids in the rumen, caecum and colon was a trans C_{18} monoenoic acid.

Wright (1959, 1960) attempted to determine which microbial population of the rumen was responsible for the biohydrogenation of unsaturated fatty acids. The bacteria and protozoa isolated from the rumen of cows were incubated with unsaturated fatty acids. The results of these experiments led Wright to suggest that biohydrogenation activity was present in both bacteria and protozoa and also showed that biohydrogenation by rumen bacteria was greatly stimulated by the presence of cell-free supernatant fractions from centrifuged rumen contents. However, Viviani (1970) has shown that it is bacteria that are largely responsible for biohydrogenation in the rumen and that protozoa are of only secondary importance.

There have been a number of attempts to isolate in pure culture the microorganisms responsible for biohydrogenation in the rumen. Table 4 shows the isolates obtained to date, along with the substrates used by the organisms and the major end-products of biohydrogenation. The techniques used by the workers listed in Table 4 have generally involved the isolation in pure culture of large numbers of different strains of rumen bacteria and the subsequent screening of each isolate for the ability to carry out biohydrogenation. Such methods are time-consuming and as can be seen from Table 4, only a limited number of strains have been identified.

Table 4. Ability of bacteria isolated from the rumen to hydrogenate
linolenic, linoleic and oleic acids (from Harfoot, 1978)

Organism	Linolenic acid Metabolic products of hydrogenation
Butyrivibrio fibrisolvens	$-^a$
B. fibrisolvens A38	$-$
B. fibrisolvens A38	18:3 cis-9,trans-11,cis-15 18:2 Δ^{11},Δ^{15}
Borrelia Treponema B₂5	Isomerized, then hydrogenated
Micrococcus sp.	Isomerized, then hydrogenated
Ruminococcus albus F2/6	*18:3 cis-9,trans-11,cis-15 *18:2 trans-11,cis-15 18:1 trans (95%) 18:1 cis (5%)
Eubacterium F2/2	18:3 cis-9,trans-11,cis-15 18:2 trans-11,cis-15 18:1 trans-11 (5%)
Eubacterium W/461	*18:3 cis-9,trans-11,cis-15 *18:2 trans-11,cis-15 18:1 trans (50%); trans-11 (65%) 18:1 cis (50%); cis-11 (95%)
Fusocillus T344	*18:3 cis-9,trans-11,cis-15 *18:2 cis-9,trans-11 18:1 cis-15 (85%)
babrahamensis P2/2	18:2 trans-11,cis-15 18:1 cis-15 (85%)
R8/5 gram-negative rod	18:2 trans-11,cis-15 (25%) 18:1 cis-15 (50%) 18:1 trans-15 (25%)
EC7/2 gram-negative rod	18:2 trans-11,cis-15 (70%) 18:2 trans-11 (30%)
2/9/1 gram-negative vibrio	18:1 trans:cis ratio 2:1 (95%) trans-11 (41%) trans-12 (11%) trans-10 (14%) cis-11 (29%) cis-12 (4%)

^{a}Not determined.
* = transient appearance as intermediate.
^{b}Percentages indicate proportion of original (1-^{14}C)-labeled
substrate appearing in products of isomerization and hydrogenation.

Substrate hydrogenated linoleic acid	Oleic acid
Metabolic products of hydrogenation	

18:1 (46%)[b]	—
18:2 cis-9,trans-11 (68%)	Not hydrogenated
18:1 trans-9 and trans-11 (23%)	
18:2 cis-9,trans-11	—
18:1 trans-11	
18:2 cis-9,trans-11 (5%)	—
18:1 trans-11 (95))	
18:1 trans-11 major intermediate	
*18:2 cis-9,trans-11	Not hydrogenated
18:1 trans (95%)	
18:1 cis (5%)	
18:2 cis-9,trans-11	Not hydrogenated
18:1 trans-11 (95%)	
*18:2 cis-9,trans-11	
18:1 trans (50%); trans-11 (65%)	Not hydrogenated
*18:2 cis-9,trans-11	18:1 trans-11 (5%)
18:1 trans-11 (65%)	18:1 cis-9 (5%)
18:0	18:0 (90%)
18:1 trans-11 (70%)	18:0 hydroxy (20%)
18:0 (30%)	
18:1 trans-11 (50%)	18:1 cis-9 (60%)
18:0 (40%)	18:0 hydroxy (40%)
18:1 trans-11 (100%)	Not hydrogenated
18:1 trans:cis ratio 2.5:1 (100%)	Not hydrogenated
trans-11 (46%)	
trans-12 (12%)	
trans-10 (15%)	
cis-11 (25%)	
cis-12 (5%)	

Table 4. Continued

| Organism | Linolenic acid |
	Metabolic products of hydrogenation
R7/5 gram-negative rod	18:1 trans:cis ratio 1:1 (100%)
	trans-11 (32%)
	trans-12 (15%)
	trans-10 (6%)
	cis-11 (44%)
	cis-12 (5%)
LM8/1A, LM8/1B gram-negative rods	18:2 trans-11,cis-15 (100%)
R8/3 gram-negative rod	18:2 trans-11,cis-15 (100%)
2/7/2	18:2 trans-11,cis-15 (100%)

Substrate hydrogenated linoleic acid	Oleic acid
Metabolic products of hydrogenation	

18:1 trans:cis ratio 1:2 (95%)
    trans-11 (14%)
    trans-10 (11%)
    trans-12 (8%)
    cis-11 (62%)
    cis-12 (5%)
	Not hydrogenated

18:1 trans-11 (100%)	Not hydrogenated
18:1 trans-11 (96%)	Not hydrogenated
18:1 trans-11 (75%)	—
18:1 cis-9,cis-12 (25%)	

As shown in Table 4, some microccus species are capable of causing the formation of trans fatty acids. Mills et al. (1970) found that pure cultures of a gram-negative micrococcus isolated from the rumen contents of sheep are able to partially but not completely hydrogenate linolenic and linoleic acids. The initial product formed during the hydrogenation of linolenic acid is cis-9, trans-11, cis-15 octadecadienoic acid which is presumably hydrogenated to the trans-11, cis-15-octadecadienoic acid. This diene is further reduced to form a trans-11-monoene. The bacteria used as starters in fermented sausages are selected strains belonging to the genera Micrococcus, Lactobacillus, and Pediococcus. The latter two function as biological acidulating agents and the micrococci are added primarily because of their nitrate-reducing activity which is so important in maintaining the desirable pink to red color of the sausage. Micrococci also play a part in desirable flavor development.

The possible formation of trans fatty acids by micrococci during the fermentation of sausages has not been investigated. In fact, no study has been made to see if microorganisms used in fermented foods do cause hydrogenation and formation of trans fatty acids during the fermentation process.

Formation of trans fatty acids during the microwave cooking of potatoes was reported by Maga et al. (1977). Very recently, Mai et al. (1980) studied the effects of microwave treatments on the fatty acid composition of several foods including chicken fat, beef tallow, bacon fat, rainbow trout, peanut oil and potato lipids. They did not find any evidence for chemical alteration or isomerization of the

fatty acids as a result of microwave cooking. Mai et al. (1980) worked only with the adipose fat trimmed from both raw and microwave cooked chicken and beef. The effect on tissue lipid was not reported. Since other food components such as proteins, prooxidants and antioxidants play an important role in governing the rate of lipid alteration during heating, the effects of microwave heating on tissue lipids should be investigated. Other species should also be examined.

III. MATERIALS AND METHODS

A. Materials

Thigh and breast muscles from freshly slaughtered turkeys were ob-
tained from Land O'Lakes, Ellsworth, Ia. Cooking and storage treat-
ments commenced within a few hours after sample collection. Frozen
turkey thighs, fresh pork loin, ground beef, beef round steak, and
margarine were purchased from local grocery stores. Fifty/fifty pork
trim and 80/20 beef trim were obtained from the Iowa State University
Meat Laboratory.

Malonaldehyde bis(dimethyl acetal), which was used as a standard
in the determination of malonaldehyde, was purchased from Aldrich Chemical
Co., Milwaukee, Wis. 4,4'-Sulfonyldianiline, thiobabituric acid, boron
tri-fluoride-methanol and fatty acid methyl ester standards were all
purchased from Sigma Chemical Co., St. Louis, Mo. Ten percent aqueous
tetra-n-propyl ammonium hydroxide was purchased from Fisher Scientific
Co., Pittsburg, Pa. Formula LTI (Pediococcus) and formula LTII
(Pediococcus + Micrococcus) lactic acid starter cultures were ob-
tained from Trumark, Inc., Roselle, NJ. Other materials including
reagent grade chemicals and solvents were obtained from the Iowa
State University Chemistry Stores. Solvents were purified before
use. Distilled water was used in all analyses involving water.

An Aminco Ratio Spectrophotofluorometer linked to an X-Y re-
corder (American Instrument Co., Silver Spring, Md.) was used for
fluorometric measurements and for spectrophotometric work, a Beckman
DU Spectrophotometer was employed. The Amana Touchmatic II Radarange

microwave oven (model RR-10) and a conventional General Electric
oven were used for cooking meat samples. Processing facilities at
the Iowa State University Meat Laboratory, including a grinder with
3/8" and 1/8" plates, Vemag stuffer, casing slipper, mixer and 2"
fibrous summer sausage casings, were used for the preparation of semi-dry
summer sausages. The Tekmar tissumizer (model SDT) with high torque
was used for homogenizing meat samples while the Büchi Rotavapor-R was
used for evaporating solvents under vacuum. Fatty acid methyl esters were
analyzed with the Beckman gas chromatograph, model GC 72-5, coupled
with an automatic digital integrator. Moisture analysis was done with
the Brabender moisture tester, and meat grinding with the Oster food
grinder. The Vitris freeze dryer (Vitris, Gardner, NY) was used to dry
fermented soy products. The Goldfish apparatus (Laboratory Construction
Co., Kansas City, Mo.) was used for extracting fat from the soy
products.

B. Methods

1. Meat processing

a. Cooking   All meat samples, except the ground beef, were
chopped into small pieces which were ground through the fine grinding
plate of the Oster food grinder. Each batch of ground meat was then
thoroughly mixed and formed into 300-g patties, 3 cm thick and ap-
proximately 10 cm in diameter, using a metal ring to shape the patties.
A sample of the raw ground meat was saved for moisture and fat
analyses. Some of the 300-g patties were cooked with the Amana micro-

wave oven at 2450 mHz and 1450 watts cooking power. This microwave oven had an automatic temperature sensing capability to monitor the internal temperature of the cooked meat. Each patty was placed in the center of a 6" x 9" x 2" pyrex pan with a temperature probe placed in the center of the patty, and cooked to an internal temperature of 60°C (140°F). The patty was then turned and cooked to an internal temperature of 82.2°C (180°F). The final internal temperature was confirmed by a thermometer placed in the center of the patty immediately after cooking.

For the conventionally cooked patties, the electric oven was set at 176.7°C (350°F) and samples were cooked to an internal temperature of 82.2°C (180°F). Weight loss during cooking was determined by weighing the samples before and after cooking.

Fresh ground beef was mixed thoroughly, made into 300-g patties and treated just as described above for the other meat samples. In all turkey samples, the skin was removed before grinding.

b. Preparation of summer sausage   The following ingredients were used to prepare the summer sausage: 171.1 lbs. pork trim (50/50), 14.5 lbs. beef trim (80/20), 12 oz. salt, 8 oz. water, 6 oz. dextrose, 2 oz. BC 1520 Zanzibar brand seasoning, 1.8 gm sodium nitrate, 0.9 gm sodium nitrite and LTI or LTII lactic acid starter culture.

The pork and beef trim was ground through the 3/8" plate of the meat grinder. All ingredients were then mixed thoroughly into the meat except for the lactic acid starter culture. Then the starter culture was suspended in water, added to the meat and mixed with the meat for about one minute. The mixture was then reground through a 1/8" plate, stuffed into 2" fibrous casing and placed on smoke

trucks. The trucks were moved into the smokehouse, where the necessary controls were set for automatic smoking and fermentation.

Two batches of sausages were prepared, one batch with the LTI (Pediococcus) starter culture and the other with the LTII (Pediococcus + Micrococcus) starter culture. Prior to the addition of ingredients and starter culture, a sample of the ground meat was removed and saved for analysis. Samples from the two types of sausages as well as the raw meat were analyzed for their fatty acid composition.

2. Sample storage following processing

All stored samples were held at refrigerator temperature, approximately $5^{\circ}C$. After cooking, patties were broken into fine particles by hand. Samples of the thoroughly mixed meat weighing 50g were put in plastic bags, which were sealed and stored at $5^{\circ}C$. Five 50g samples were prepared for each cooked patty and each type of cooked meat and for the raw meat. One bag from each set of samples was used each day for TBA, spectrofluorometric and fatty acid analyses.

3. Moisture content analysis

The procedure described in the Brabender Instrument, Inc. manual for using the Brabender moisture tester was followed to determine moisture content. Ten grams of sample was weighed into moisture tester dishes and dried to a constant weight. The water loss was read directly in percent. Moisture content analyses were made for raw, microwave and conventionally cooked beef, turkey breast, turkey thigh and pork.

4. <u>Extraction of lipid from tissues, margarine and fermented soy products</u>

Lipids from all meat samples were extracted by the method of

Bligh and Dyer (1959) with slight modifications. Representative

samples of meat (20g) were homogenized with a Tekmar tissumizer for

2 minutes with a mixture of methanol (100 ml) and chloroform (50 ml).

Fifty ml of chloroform was then added to the mixture which was

blended for 30 seconds. Next, 50 ml of distilled water was added and

blending was continued for another 30 seconds. The homogenate was

filtered through Whatman No. 1 filter paper on a Buchner funnel with

slight suction. The filtrate was transferred into a 500 ml separatory

funnel. The residue and filter paper were transferred into a flask

and blended with 50 ml chloroform for 30 seconds. The mixture was

then filtered through the Buchner funnel used in the first filtration

and the flask and residue were rinsed with a total of 25 ml of chloro-

form. This second filtrate was mixed with the first filtrate in

the separatory funnel. After complete separation and clarification of

the chloroform layer, the chloroform layer was transferred into a 250 ml

rb flask, dried over anhydrous sodium sulfate and concentrated to

dryness on a rotary evaporator at 40°C. The concentrated lipid ex-

tract was quantitatively transferred to a 25 ml volumetric flask and

made up to volume with chloroform. Aliquots from this extract were

used to determine the total lipid content. Butylated hydroxytoluene

(BHT) at a concentration of 0.05% (of the lipid) was added to the

remaining lipid extract and the extract stored at - 40°C until further

analysis.

Lipids were extracted in this way from raw, microwave cooked and conventionally cooked turkey and beef as well as from fermented sausages. The procedure was also used with slight modifications, to extract lipids from margarine. All extractions and further analyses were done in triplicate.

Lipids were also extracted from natto (a soy fermented product) by the Soxhlet method (AOAC, 1975). The natto was freeze dried with the Virtis freeze dryer before extraction. Two-gram samples were extracted with anhydrous diethyl ether for about 5 hours at a condensation rate of 5-6 drops/sec.

5. Determination of total lipid

A 5 ml aliquot from the lipid extract was transferred into a pre-heated, cooled and weighed aluminum dish which was resting on a hot plate with low heat under a hood. A stream of nitrogen was directed onto the dish to evaporate the sample aliquot to dryness (verified by the absence of chloroform odor). The sample was then dried in an 80°C oven for 20 minutes. After this, the sample was cooled to room temperature in a desiccator and weighed within 4 hours. Calculations were made as follows:

$$\text{Total lipids (mg)} = \frac{\text{wt, mg x volume of sample}}{\text{volume of the aliquot}}$$

6. Fatty acid analyses

a. **Methylation**   The method of Morrison and Smith (1964) was used for the preparation of fatty acid methyl esters. An aliquot of lipid solution (containing 15 mg of lipid material) was evaporated to

dryness under nitrogen in a centrifuge tube provided with a teflon-lined screw cap. Boron fluoride-methanol reagent (25% Boron fluoride-methanol, 20% benzene, 55% methanol) was added under nitrogen. The tube was then heated in a boiling water bath for 30 minutes, cooled to room temperature and the methyl esters were extracted by adding 2 volumes of pentane, then 1 volume of water, shaking briefly, and centrifuging until both layers were clear. The pentane extract was used for gas chromatographic analysis.

b. _Gas chromatography_   A Beckman GC 72-5 gas chromatograph was used for the analysis of fatty acid methyl esters (FAME). A 12 ft x 1/8 in. stainless steel column packed with 10% Silar 10C on 100/120 mesh Gas-Chrom Q (Applied Science Lab., State College, Pa.) was used. The column temperature was programmed from 180° to 212°C at 1°C/min. The injection port temperature was 250°C and the detector temperature was also 250°C. The carrier gas (nitrogen) flow rate was 15 ml/min. A second column (20 ft x 1/8 in., packed with Silar 10c on 100/120 mesh Gas-Chrom Q) was also used to improve the resolution of cis-trans 18:1 methyl esters. This run was isothermal at 200°C with a carrier gas flow rate of 10 ml/min. Detector and injection temperatures were also 250°C. Routine analyses were done with the 12 ft column and the 20 ft column was only used to quantitate _trans_ 18:1 methyl esters. Peaks on the chromatogram were identified by comparing to retention times of known fatty acid methyl ester standards. The areas of the peaks were integrated electronically by an Infotronics automatic digital integrator (model CRS-208). All samples were run in triplicate.

7. TBA value analyses

The distillation procedure for the quantitative determination of malonaldehyde in rancid foods, described by Tarladgis et al. (1960), was used. A 10g sample of meat was blended with 50 ml of distilled water with a Tekmar tissumizer for 2 minutes. The mixture was then quantitatively transferred into a macro Kjeldahl flask. An additional 47.5 ml of distilled water was added, using a portion of it to rinse the flask in which the meat was blended. To bring the pH to 1.5, 2.5 ml of 4N hydrochloric acid was added to the mixture. A small amoun. of Dow antifoam A was put into the lower neck of the flask and a few boiling beads added to prevent bumping. The mixture in the flask was then distilled at the highest setting of a macro Kjeldahl apparatus (Precision Scientific Co., Chicago, Ill.) until 50 ml of distillate was collected.

The distillate was mixed thoroughly and 5 ml transferred into a 50 ml test tube. Five ml TBA reagent (0.02 m TBA solution in 90% glacial acetic acid) was added to the 5 ml distillate in the test tube. The tube was stoppered, the contents were mixed, then it was immersed in a boiling water bath for 35 minutes. During the 35-minute boiling, the tubes were stoppered with marbles for condensers. A distilled water-TBA reagent blank was prepared and treated like the samples. After heating, the tubes and contents were cooled in tap water for about 10 minutes and portions were transferred into cuvettes and the optical density of the samples read against the blank at 538 mμ using a Beckman DU spectrophotometer. The average recovery of malonaldehyde obtained in 50 ml of distillate was found to be about 68%;

hence, the K (distillation) value used to convert optical density

measurements to "TBA number," was 7.8 as calculated by Tarladgis et al.

(1960). TBA number is the mg of malonaldehyde per 1,000g of sample

and it is calculated by multiplying the absorbancy by a constant K,

the value of which may be obtained from the standard curves and the

known dilutions as follows:

$$K(distillation) = \frac{conc. \ in \ moles/5 \ ml \ of \ distillate}{optical \ density}$$

$$x \ \frac{molecular \ wt.}{of \ malonaldehyde} \ x \ \frac{10^7}{wt. \ of \ sample} \ x \ \frac{100}{\% \ recovery}$$

Malonaldehyde bis(dimethyl acetal) was used as a standard in all

TBA tests. TBA analyses were done every 24 hours, in six replicates on

raw, microwave and conventionally cooked meat samples, stored at re-

frigerator temperature for a period of 3 days.

8. Spectrofluorometric analyses

Portions of the 50 ml distillate obtained during the initial

steps of TBA analyses of beef and turkey samples were used for the

spectrofluorometric assay. Five ml of the distillate was transferred

into a 250 ml round-bottom flask and 1 ml of 0.1N sodium hydroxide

was added. To this mixture, 45 ml of N,N-dimethylformamide was added

and the flask contents mixed by shaking. Next, about 35 ml of solvent

was removed from the mixture by distillation under reduced pressure

using the rotary evaporator with the water bath containing the sample

at 60°C. The remaining solution in the flask was quantitatively trans-

ferred into a 50 ml volumetric flask and made up to volume with

dimethylformamide.

Two ml portions of this dimethylformamide test solution were treated essentially as described by Sawicki et al. (1963). To 2 ml of the dimethylformamide test solution, 1 ml of the 4,4'-sulfonyldianiline reagent was added. The 4,4'-sulfonyldianiline reagent was made up by dissolving 1% 4,4'-sulfonyldianiline and 1% (vol/vol) of concentrated hydrochloric acid (38%) in dimethylformamide. The mixture of reagent plus test solution was heated for 10 minutes on a boiling water bath and then cooled to room temperature. One-half milliliter of 10% aqueous tetra-n-propylammonium hydroxide was added. Readings were taken at the emission wavelength maximum of 545 nm while exciting at the excitation wavelength maximum of 475 nm. The fluorescence intensity was stable for 30 minutes.

The following settings were used with Aminco-Bowman ratio spectro-photofluorometer: Slit arrangement was 3, 1, 3 for the emission mono-chromator entrance slit 3, excitation monochromator exit slit 2 and the rotary exit slit 4, respectively. The excitation light source was the Hanovia xenon lamp. The instrument was daily calibrated to read 80 relative fluorescence units against a quinine sulfate solution (1 μg/ml 0.1N H_2SO_4) at excitation wavelength of 350 nm and emission wavelength of 450 nm. Duplicate runs were made on each of the six replicates of the distillate obtained from the initial steps of the TBA test. This gave twelve fluorometric readings for each sample. For all procedures, the blank had the same composition as the analyzed solution, except that the malonaldehyde aqueous solution was replaced with distilled water. The experimental justification for the spectro-

fluorometric procedure, just described, will be clear from the observations in the section for results and discussion.

In preliminary studies, fluorecent compounds were extracted from meat tissues with a mixture of chloroform:methanol, 2:1, and analyzed spectrofluorometrically, as described by Fletcher et al. (1973). The extracted fluorescent compounds were fractionated using silicic acid column chromatography. Chloroform-methanol mixtures were used to elute the column, starting with pure chloroform and ending with pure methanol. Fractions from the silicic acid column chromatography were further fractionated using thin-layer chromatography (TLC). Solvents used for development of the TLC plates, which were coated with silica gel Gr, included cyclo-hexane-chloroform-methanol, 70:30:3; chloroform-methanol-acetic acid-water, 60:40:2:1. Fluorescent components on the TLC plates were identified using an ultraviolet lamp. Spots which fluoresced were removed and extracted with chloroform. The fluorometric spectra of the chloroform extracts were obtained.

9. Statistical analyses

For all statistical analyses, an analysis of variance was carried out and the "F" test performed to test treatment means.

IV. RESULTS AND DISCUSSIONS

A. Spectrofluorometric Procedures

1. <u>Attempts to follow lipid oxidation by quantitating the fluorescent compounds extracted from tissues</u>

Initial efforts in this study were directed toward using the fluorometric assay described by Fletcher et al. (1973) to follow lipid oxidation in raw and cooked meat. The method consists of a chloroform-methanol extraction of tissue followed by measurement of fluorescent intensity due to the products resulting from the reaction of malonaldehyde with amino acids. The fluorescent system, N-C=C-C=N, has an excitation maximum of 360 nm and an emission maximum of 460 nm. The fluorometric technique has been used successfully for measurements of membrane damage during aging (Tappel, 1972), and for measurement of damage to tissues of animals placed under stress conditions, for example, animals fed a diet high in unsaturated fatty acids and low in antioxidants (Fletcher et al., 1972).

Raw and cooked beef were stored at about 5°C and the spectrofluorometric assay performed every 24 hours for 6 days. Figure 7 shows the results obtained. Fluorescent intensity increased in extracts from the cooked samples during storage but decreasing values were obtained for the raw samples. The increasing values for the cooked meats presumably reflect the lipid oxidation which would be expected to occur in stored cooked meat. However, the decreasing values for the raw samples are not easily explained since slight increases in lipid oxidation should occur with storage. It is possible that the fluorescent compounds produced in the meat samples from lipid oxidation products are not stable. Perhaps
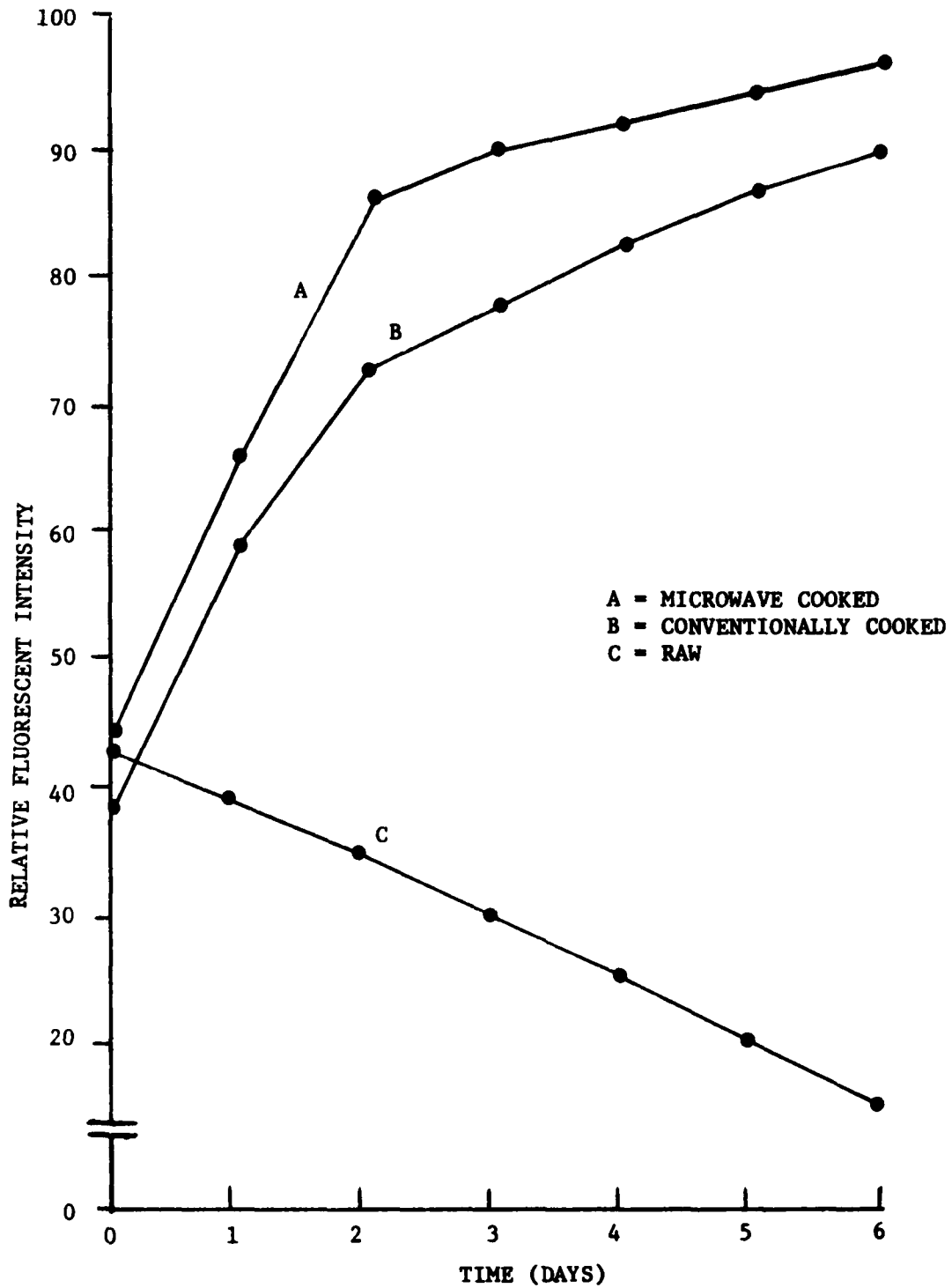
Figure 7. Assay of fluorescent compounds extracted from raw and cooked turkey meat

in the cooked samples the rate of destruction of the fluorescent compounds
was slower than the rate of formation and, hence, increased spectrofluoro-
metric values were obtained. But in the raw samples, relatively small
quantities of fluorescent compounds may have been formed during storage
because of the slow rate of oxidation and the rate of destruction may
have been higher than the rate of formation; hence the observed decrease
with storage. It is not known why the values obtained for the cooked
meats are not higher than for the raw meat at zero time.

Efforts were then made to isolate the fluorescent compounds that
were being measured with the hope of studying their stabilities. It
would also be desirable to isolate the fluorescent compounds to eliminate
the possible interference in the assay due to retinol. Silicic acid
column and thin-layer chromatographic techniques were employed to
fractionate the fluorescent products extracted from the tissues, as
described in the methods section. Fractions collected during the silicic
acid chromatography contained families of compounds whose fluorescent
characteristics were the same as those of the Schiff base fluorophores
obtained from the reaction of malonaldehyde with amino acids. The
fluorescent components recovered from the silicic acid column were further
fractionated with thin-layer chromatography. Fluorescent components on
the thin-layer chromatographic plates were identified using an ultra-
violet lamp. Spots which fluoresced were removed and extracted with
chloroform. The fluorometric spectra of the chloroform extracts were
obtained. Several peaks were noted in the spectrum obtained from each
fluorescent spot, indicating that more than one fluorescent species was

present. Complete fractionation of the various compounds in each spot was not possible with further TLC work when different combinations of chloroform, methanol, acetic acid and water were used for development of the plates.

Since the fluorescent moieties of interest did not always co-chromatograph, quantitation of lipid oxidation via isolation of fluorescent materials from the tissue was not practical. Fractions containing fluorescent compounds decreased in fluorescent intensity when they were allowed to stand for one, two and three days, indicating that the compounds were not stable, at least after extraction from the tissue. For the spectrofluorometric assay described by Fletcher et al. (1973) to be useful for the measurement of the extent of lipid oxidation (rancidity), the fluorescent compounds formed when the oxidation products react with amino acids should remain stable and accumulate in the tissue. Since these compounds do not seem to be stable, it is not practical to use the procedure to follow lipid oxidation.

2. A spectrofluorometric procedure using 4,4'-sulfonyldianiline

a. Transfer of malonaldehyde from aqueous solution into dimethyl-formamide   Malonaldehyde is easily and efficiently removed from meat and biological tissues by acidic aqueous extraction or by collecting the distillate from an acidified aqueous extract of tissue. The 4,4'-sulfonyldianiline reagent, known to be selective for malonaldehyde, cannot be used to assay for this compound when these procedures are used, since the presence of water has been shown to inhibit the reaction leading to formation of a fluorescent compound (Sawicki et al., 1963).

In preliminary experiemnts, extremely low fluorescence intensity readings were obtained on an aqueous solution of malonaldehyde which had been reacted with 4,4'-sulfonyldianiline, confirming the observation made by Sawicki et al. (1963). It is therefore necessary to transfer malonaldehyde from the aqueous solution into a solvent such as dimethylformamide which is a suitable medium for fluorophore development.

To transfer malonaldehyde from water into dimethylformamide, advantage was taken of the fact that malonaldehyde exists in a nonvolatile form at alkaline pH (Kwon and Watts, 1964). By changing the pH of a malonaldehyde solution to 6.5 or above, it was anticipated that a large amount of solvent could be removed by distillation with a negligible loss of malonaldehyde. To test this assumption, aqueous solutions of malonaldehyde were prepared by distillation of an acidified solution of malonaldehyde bis acetal and a cooked meat slurry. TBA tests were performed on these distillates, as well as on a malonaldehyde bis acetal solution. 50 ml of the aqueous solutions of malonaldehyde were then made alkaline (pH 11-12) and distilled until 30 ml of distillate was collected. The residues and distillates were diluted to 50 ml and aliquots from then used for the TBA test. Data on the recovery of malonaldehyde are presented in Table 5.

The malonaldehyde aqueous solutions obtained by distillation of an acidified malonaldehyde precursor or cooked meat showed negligible losses of malonaldehyde into the distillate during the alkaline distillation. But malonaldehyde solution C, which is a 10^{-5} M solution of malonaldehyde bis acetal, showed a 95% loss of malonaldehyde into the distillate during

58

Table 5. Recovery of malonaldehyde and malonaldehyde bis acetal from alkaline solutions during distillation[a]

Malonaldehyde Solution	% Malonaldehyde Found in	
	Residue	Distillate
A[b]	99.78	0.02
B[c]	99.61	0.39
C[d]	4.01	95.99

[a] Means are based on six observations.

[b] Malonaldehyde aqueous solution obtained by acid distillation of 10^{-5} M solution of malonaldehyde bis acetal.

[c] Malonaldehyde aqueous solution obtained by acid distillation of cooked meat slurry.

[d] 10^{-5} M standard solution of malonaldehyde bis acetal.

the alkaline distillation. Apparently at alkaline pH, the malonaldehyde bis acetal is volatile and most of it distilled over.

To achieve the transfer of malonaldehyde from aqueous solutions into dimethylformamide, advantage was also taken of the fact that a mixture of dimethylformamide and water could be separated by fractional distillation with water distilling off first. When 5 ml of aqueous malonaldehyde solution was mixed with 45 ml dimethylformamide and the solution made alkaline (pH 11-12) with 0.1 N sodium hydroxide, the water was removed by distilling off 30-40 ml of the mixture. A test employing anhydrous copper sulfate indicated that the residue did not

contain water. Under the alkaline conditions, malonaldehyde did not

distill off with the water, hence the malonaldehyde present in the

original aqueous solution was left in the dimethylformamide residue.

This residue was made up to 50 ml volume with dimethylformamide. Aliquots

from the dimethylformamide solution were used for the spectrofluorometric

test.

b. Standard curve   A standard curve for the spectrofluorometric

procedure was first prepared using malonaldehyde bis acetal dissolved in

dimethylformamide with the assumption that a theoretical yield of

malonaldehyde would be obtained from the acetal. Based on the standard

curve obtained, the malonaldehyde content of an aqueous solution of

malonaldehyde obtained by distillation of acidified solution of malon-

aldehyde bis acetal was determined and compared with values obtained when

the TBA test was performed on the same solution. The values for malon-

aldehyde, as measured by TBA, were 2.5 times larger than the values

obtained by the SPF method. These results indicated that the assumption

that a theoretical yield of malonaldehyde would be obtained from malon-

aldehyde bis acetal did not appear to be correct. Presumably, relatively

less malonaldehyde was released under the conditions of the SPF test

when compared to the conditions for the TBA test.

In another attempt to obtain a standard curve for the SPF assay,

malonaldehyde was first liberated from its precursor by distillation of

the acidified malonaldehyde bis acetal. 50 ml distillate was collected

from 100 ml of a 10^{-5} M aqueous solution of malonaldehyde bis acetal

that was acidified with 2 ml 4 N hydrochloric acid. The percentage

recovery of malonaldehyde during the distillation process was determined

by performing the TBA test on aliquots from the distillate and also from

the original 10^{-5} M solution of malonaldehyde bis acetal. Since the

percentage recovery was known, it was possible to calculate the amount of

malonaldehyde in the 50 ml distillate. Portions of the aqueous distillate

were transferred into dimethylformamide in the same way described above.

Aliquots from the dimethylformamide-malonaldehyde solution, representing

a concentration range from 19.44 to 388.8 ng malonaldehyde were used to

prepare the standard curve. A linear relationship that obeyed Beer's

law only up to about 150 ng of malonaldehyde concentration was obtained.

The slope of the line was 2.05.

Using this new standard curve, the SPF and TBA methods were again

compared using the same aqueous solution of malonaldehyde. Similar

values for malonaldehyde content were obtained when the two methods were

used to analyze the same sample. The standard curve for the SPF assay

must be prepared using malonaldehyde solutions. Malonaldehyde will not

be completely liberated from the precursor during the SPF assay. Aliquots

taken from aqueous malonaldehyde solution obtained from meat samples, for

the SPF procedure, were chosen in such a way that measurements were

within the range of malonaldehyde concentration, which obeyed Beer's

law. Figure 8 shows a typical spectrofluorometric curve obtained in the

determination of malonaldehyde.

3. Advantages and limitations of the spectrofluorometric method

Even though the distillation procedure for extracting malonaldehyde

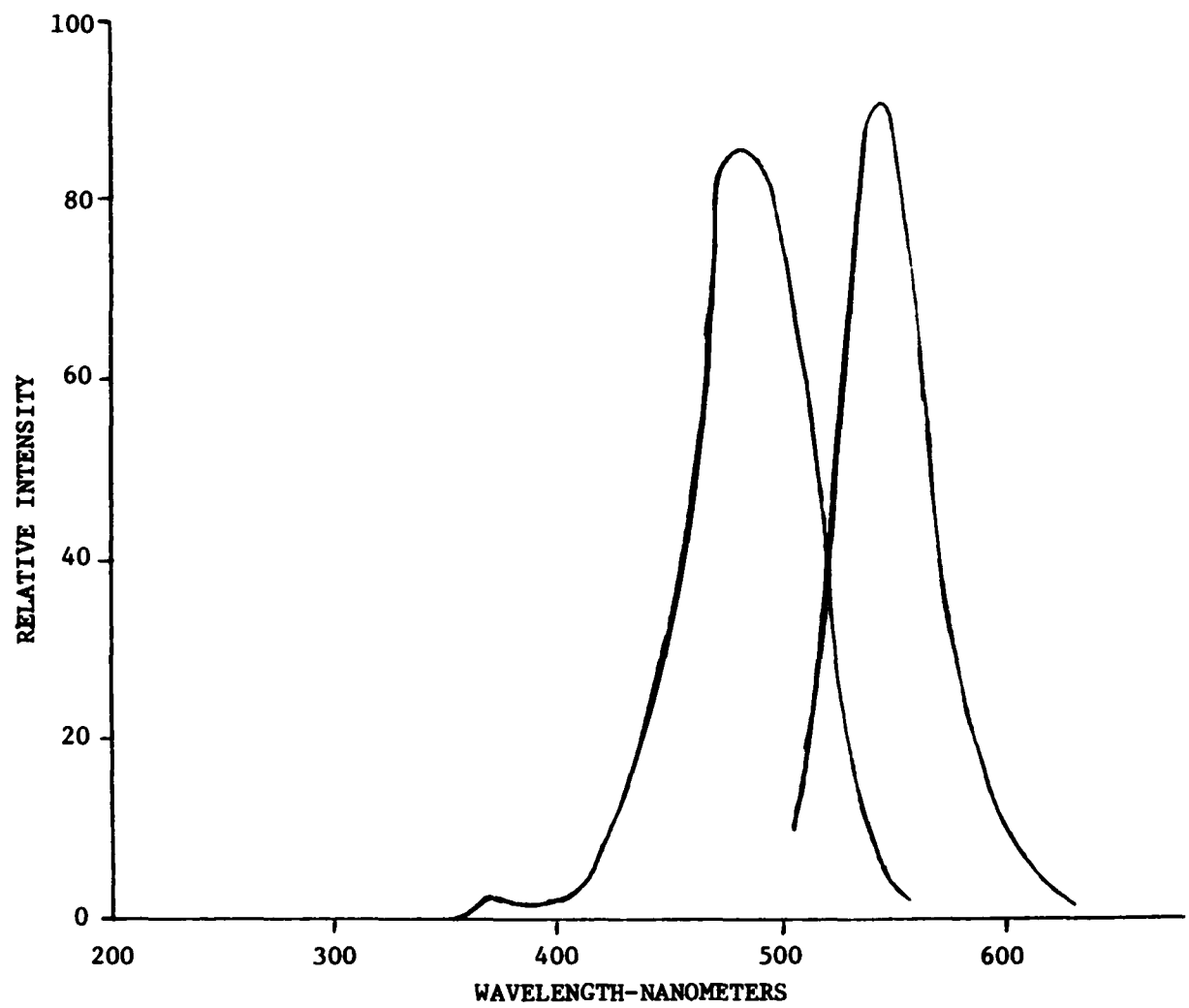from foods for the TBA test eliminates most interfering compounds, for

Figure 8. Spectrofluorometric curve obtained in determination of malonaldehyde

example, sucrose in cured meats, volatile interfering compounds would still give problems. Volatile compounds, for example, the 2-alkenals and the 2-4 alkadienals, interfered with the TBA test (Marcuse and Johansson, 1973). During the alkaline distillation in the SPF method, when malonaldehyde is transferred from aqueous solution into dimethyl-formamide, malonaldehyde is retained because of the structure it assumes at an alkaline pH. Other volatile interfering compounds which are not affected by pH changes in the same way as malonaldehyde might be expected to distill off during the alkaline distillation. This would contribute more to the selective nature of the procedure. It has been shown that the 4,4'-sulfonyldianiline reagent used in the spectrofluorometric procedure gave negative results with formaldehyde, acetaldehyde, glyoxal, propionaldehyde, acrolein, glyceraldehyde, pyruvaldehyde, 1,3-dihydroxy-acetone, glycidol, biacetyl, 2-aminopyrimidine, 4-pyridinecarboxaldehyde, benzaldehyde, and sulfadiazine (Sawicki et al., 1963).

In the current study, the SPF method was found to be more sensitive than the TBA test, at least by a factor of 25. The SPF procedure, as described in this paper, is for moist tissues like meat. However, the reagent, 4,4'-sulfonyldianiline could be used to determine the malonalde-hyde content of other materials as long as water and alcohol are not present. The spectrofluorometric procedure could be carried out in about the same period of time as the TBA test. Boiling of reagent with malonaldehyde solution took 35 minutes for the TBA test, but only 10 minutes for the SPF assay. However, the time required to transfer

malonaldehyde out of the aqueous solution compensates for the time gained during the boiling stage.

B. Assay for Malonaldehyde Using
the TBA and SPF Methods

The SPF and TBA procedures were used to determine the malonaldehyde content of raw and freshly cooked beef and turkey. Data obtained are presented in Table 6. As shown in the table, the malonaldehyde content of each sample as measured by the TBA and the malonaldehyde specific, SPF methods are not significantly different. This result seems to show that in raw or freshly cooked samples, interfering compounds do not contribute significantly to the malonaldehyde content as measured by the TBA test using the distillation method. Shamberger et al., 1977, reported similar results when the malonaldehyde content of beef extracts was measured by the TBA procedure and a gas chromatographic technique. These workers indicated that the TBA procedure did not measure interfering compounds to any significant level. However, as oxidation products accumulate in cooked meat during storage, interfering compounds might affect the malonaldehyde content measured by the TBA test.

There were significant differences among the TBA values for the meat type and also between cooking methods, as shown in Table 7. The cooked turkey samples had higher values than the corresponding beef samples. This is probably due to the fact that poultry meat contains more polyunsaturated fatty acids than beef (Chang and Watts, 1952).

Table 6. Malonaldehyde in raw and freshly cooked turkey and beef, as determined by TBA and spectrofluorometric assays[a]

Samples	Malonaldehyde Content (μg/g of Tissue)	
	TBA	SPF[b]
Turkey I[c]		
Raw	0.28 ± 0.01^g	0.27 ± 0.02^g
MC[d]	4.46 ± 0.23^h	4.47 ± 0.19^h
CC[e]	6.35 ± 0.18^i	6.37 ± 0.20^i
Turkey II[f]		
Raw	0.35 ± 0.02^g	0.39 ± 0.02^g
MC	5.68 ± 0.31^h	5.68 ± 0.12^h
CC	6.63 ± 0.33^i	6.34 ± 0.29^i
Beef		
Raw	0.29 ± 0.02^g	0.30 ± 0.02^g
MC	0.50 ± 0.01^h	0.49 ± 0.02^h
CC	0.74 ± 0.05^i	0.76 ± 0.03^i

[a]Values for each treatment are means ($\pm$ S.D.) based on six observations.

[b]Spectrofluorometric assay.

[c]Obtained from Land O' Lakes.

[d]Microwave cooked.

[e]Conventionally cooked.

[f]Obtained from a local retail outlet.

[g,h,i]Values for each type of meat followed by different letters are significantly different at $p < 0.05$.

65

Table 7. Analysis of variance table showing the significant
differences between various treatments

Source	DF	ANOVA SS	F Value	PR > F
Meat[a]	1	281.37518935	1954.95	0.0001
Cook[b]	2	394.32921296	1213.54	0.0001
Cooked*Meat	2	143.43019537	498.27	0.0001
Sample (Cooked*Meat)	30	0.14167917	0.03	1.0000
Test[c]	1	0.02370370	0.16	0.6862
Cooked*Test	2	0.06207963	0.22	0.8066
Meat*Test	1	0.01516713	0.11	0.7465
Cooked*Meat*Test	2	0.03527870	0.12	0.8849

[a]Turkey versus beef.

[b]Raw versus microwave and conventionally cooked.

[c]TBA versus SPF.

Microwave cooked samples had significantly lower malonaldehyde contents than the conventionally cooked samples. This can be explained in terms of the time of cooking. Conventional cooking took a longer time, thereby allowing more time for the promotion of lipid oxidation by heat.

C. Stability of Meat Products as Influenced

by Method of Cooking

An experiment was designed to compare the ability of the TBA and SPF procedures to follow increases in malonaldehyde during storage. The malonaldehyde contents of raw, microwave and conventionally cooked turkey and beef were measured during a three-day refrigerator storage period.

TBA values and SPF readings are shown in Table A.1. (Appendix A) and
are presented graphically in Figures 9 and 10. As can be seen from
these figures, both the TBA and the SPF assays show that malonaldehyde
increases more rapidly in the cooked samples than in the raw meats. The
increased development of oxidative rancidity as a result of cooking has
been noted previously by many authors (Younathan and Watts, 1959;
Chang et al., 1961, Kesinkel et al., 1964; Sato and Hegarty, 1971;
Keller and Kinsella, 1973). In this study, significantly higher
$(p < 0.05)$ TBA and SPF values were observed for the microwave cooked
turkey than the conventionally cooked turkey samples at three days of
storage (Figure 9).

The malonaldehyde contents measured by the TBA procedure were
similar to those measured by the SPF method for all raw and freshly
cooked samples. But with storage, significantly different values for
malonaldehyde in cooked turkey were obtained with the two methods. The
TBA method gave progressively higher values than the SPF assay with
time of storage in these samples. The reason for this is probably
because interfering compounds were formed as oxidation progressed.
Since the SPF method is more selective for malonaldehyde, it gave lower
values than the TBA test, which measures some of the interfering
compounds in addition to malonaldehyde. The TBA test gave only slightly
higher values for malonaldehyde content than did the SPF method at all
storage times for beef samples (Figure 10).

Another experiment was designed to compare the effects of microwave
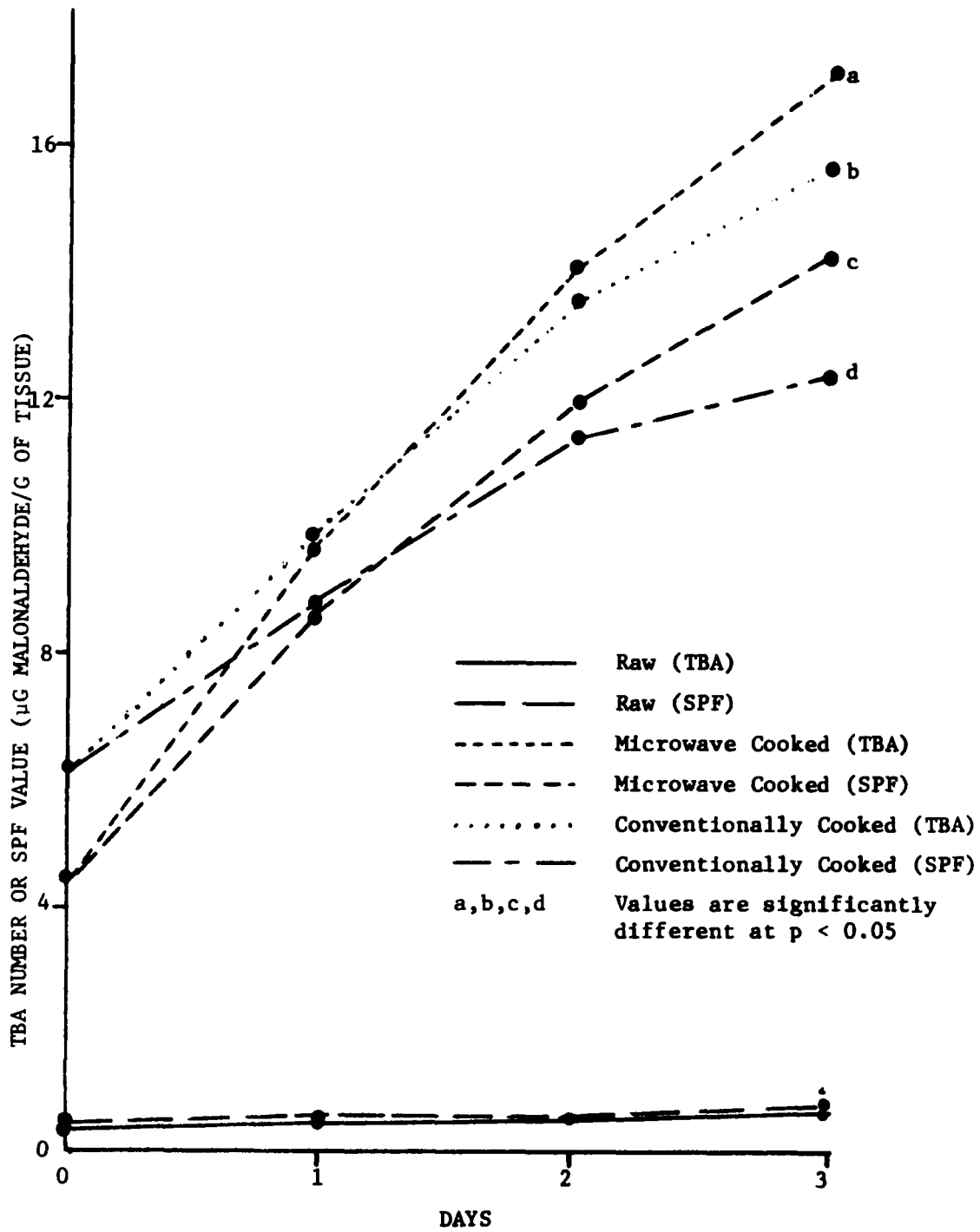and conventional cooking on the stability of lipds in pork, beef, turkey

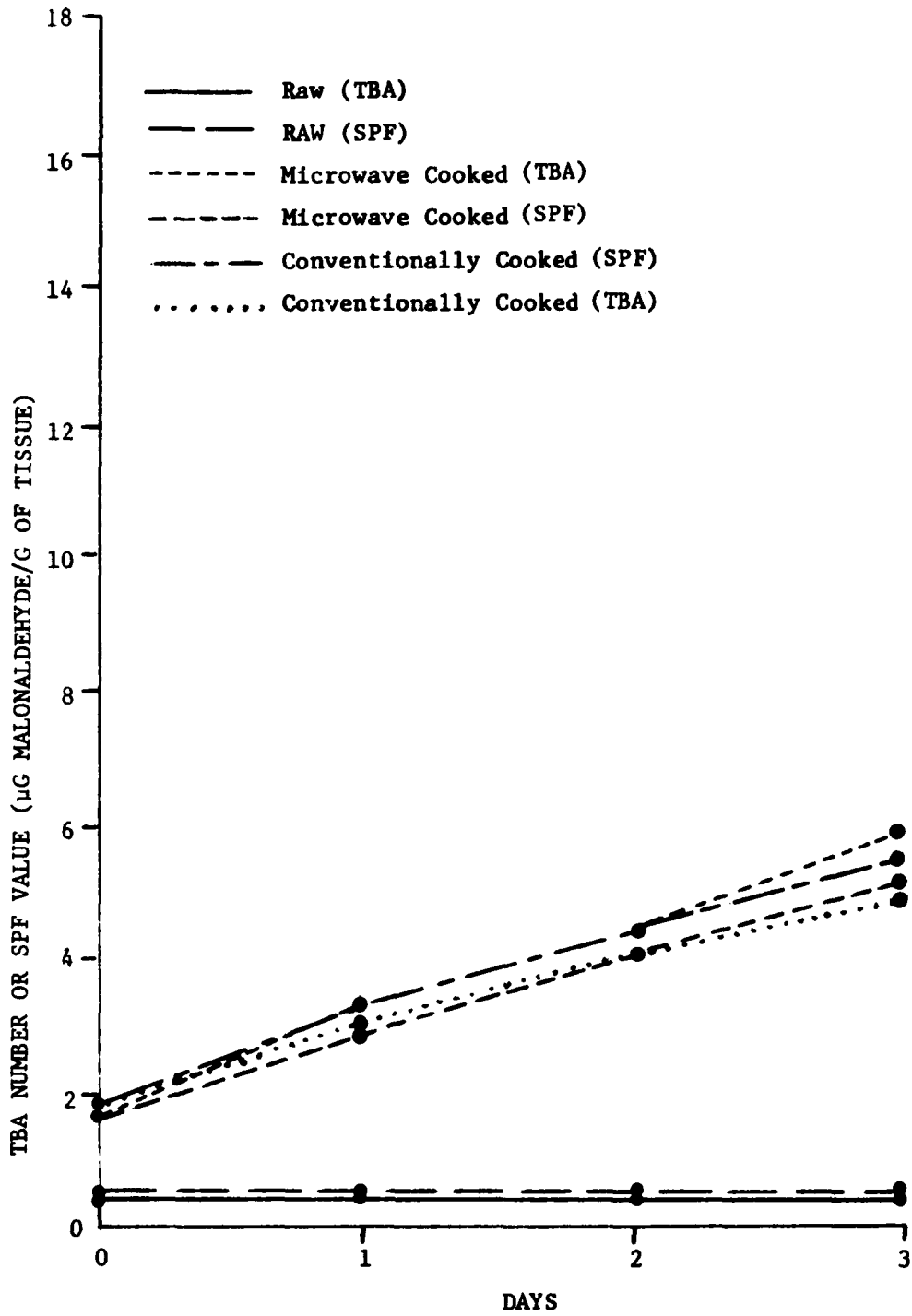Figure 9. Stability of raw and cooked turkey as measured by TBA and SPF tests

Figure 10. Stability of raw and cooked beef as measured by TBA and SPF.
tests

thigh and breast muscles. Cooking losses were measured, as were moisture and fat contents in the cooked products. The results are presented in Table 8. The time necessary to obtain an internal temperature of 180°F (82.2°C) was longer for conventional cooking (40-45 minutes) than for microwave cooking (4-5 minutes). This observation agrees with the findings of other research workers (Berger, 1958; Marshall, 1960, Meyers and Harris, 1975; Korschegen et al., 1974). Slightly higher or similar total cooking losses were observed for all microwave cooked samples compared to conventionally cooked samples. A number of research workers have also made the same observation (Marshall, 1960; Kylen et al., 1964; Bowers and Heier, 1970; Moody et al., 1978; Moore et al., 1980). Some of these workers who cooked meat samples to an internal temperature of approximately 180°F also showed losses of a magnitude similar to those reported in Table 8. Meyers and Harris (1975) did not find any significant differences in weight loss as a result of microwave versus conventional cooking for beef and pork. Comparing the percentage cooking losses among the various meats in Table 8, the greatest losses occurred in turkey, followed by pork and then beef.

In all meats studied, slightly greater moisture losses occurred with microwave cooking than conventional cooking. A similar result has been shown by other workers. Significantly more moisture was lost in microwave heated beef patties compared with conventionally cooked patties in a study by Janicki and Appledorf (1974). Likewise, Apgar et al., (1959) showed higher moisture loss in microwave cooked pork patties than in those cooked conventionally. Other workers (Wing and Alexander, 1972;

Table 8. Effect of cooking on the composition of various meats[a]

Meat Type	Treatment[b]	Total Cooking Loss (%)	Moisture (%)	Total Lipid[c]	
				A	B
Turkey Thigh	R	---	74.0 ± 2.5	4.0 ± 0.4	15.2
	MC	33.7 ± 0.9	63.0 ± 3.1	5.0 ± 0.5	13.4
	CC	33.3 ± 1.1	66.9 ± 2.7	3.5 ± 0.4	10.5
Turkey Breast	R	---	72.0 ± 3.0	2.0 ± 0.4	7.2
	MC	31.0 ± 1.3	63.7 ± 2.4	2.4 ± 0.4	6.7
	CC	30.2 ± 1.0	64.0 ± 2.1	1.1 ± 0.3	2.9
Beef	R	---	66.6 ± 1.9	11.8 ± 1.0	35.3
	MC	26.8 ± 0.8	42.5 ± 3.4	14.2 ± 1.2	24.6
	CC	25.1 ± 0.9	43.0 ± 2.3	14.0 ± 1.0	24.5
Pork	R	---	58.0 ± 2.0	25.1 ± 2.4	59.8
	MC	28.2 ± 1.5	48.8 ± 2.9	27.4 ± 1.9	53.42
	CC	27.9 ± 0.9	49.1 ± 2.6	26.2 ± 1.7	51.53

[a]Mean (±S.D.) is based on six observations. All meat cooked to internal temperature of 180°F (82.2°C).

[b]R = raw; MC = microwave cooked; CC = conventionally cooked.

[c]A, gm/100 gm on wet weight basis; B, gm/100 gm on dry weight basis.

Penner and Bowers, 1973) have also shown greater moisture losses in microwave cooked than conventionally cooked meat products. In this study, the method of cooking affected the fat content in the cooked meats. Cooking decreased the fat content (calculated on dry weight basis) of the samples. Slightly greater fat losses occurred during the conventional cooking of turkey and pork meats when compared to microwave

cooking. The longer time needed to cook in the conventional oven allowed more fat to be rendered resulting in samples with a lower fat content.

Samples were stored for a period of three days at refrigerator temperatures. TBA values for the stored samples are presented in Tables A.2 (Appendix A) and graphically in Figures 11 and 12. As shown in these figures, the microwave cooked samples initially appeared to oxidize faster than the conventionally cooked samples of pork and turkey. A similar result was obtained in the previous experiment. Unlike the earlier study, there are no differences in the TBA values obtained for microwave and conventionally cooked turkey immediately after cooking. Additional samples of turkey thighs from two different sources were obtained and the TBA values measured immediately after microwave and conventional cooking. Slightly higher TBA values (average of 5.92) were obtained for the conventionally cooked than for the microwave cooked (average of 4.81).

Acceleration of lipid oxidation in frozen mackerel fillets, pre-treated with microwave energy, has been reported by Ke et al. (1978). These workers did not compare the microwave treatment to any other form of heating. As a result, one cannot be sure that the acceleration of lipid oxidation observed was due to microwave energy. However, the results presented in Figures 11 and 12 indicate that stored microwave heated turkey and pork had significantly higher TBA values than did conventionally cooked samples. Rawls and Van Saten (1970) stated that microwave energy can activate oxygen on either a direct basis or to
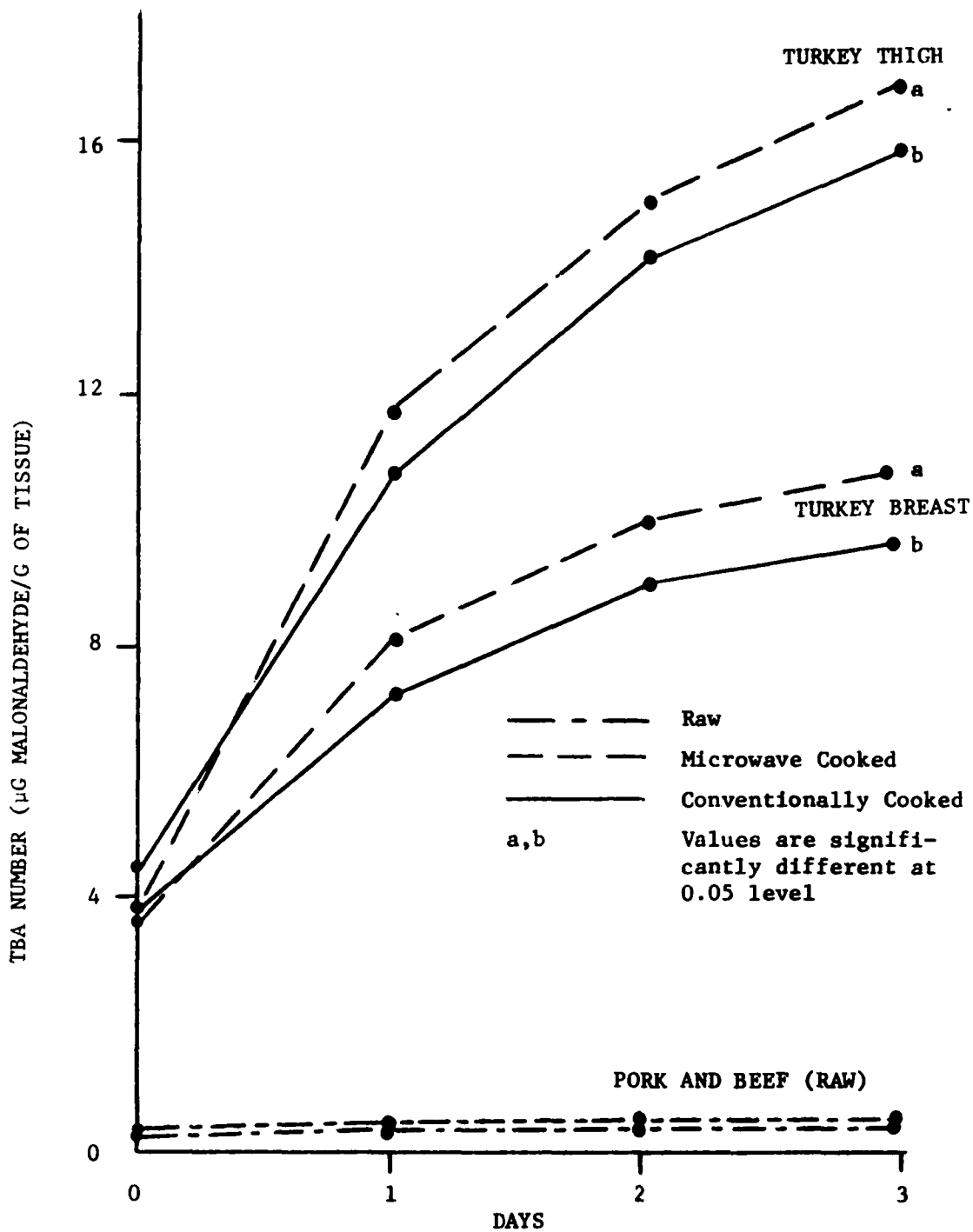
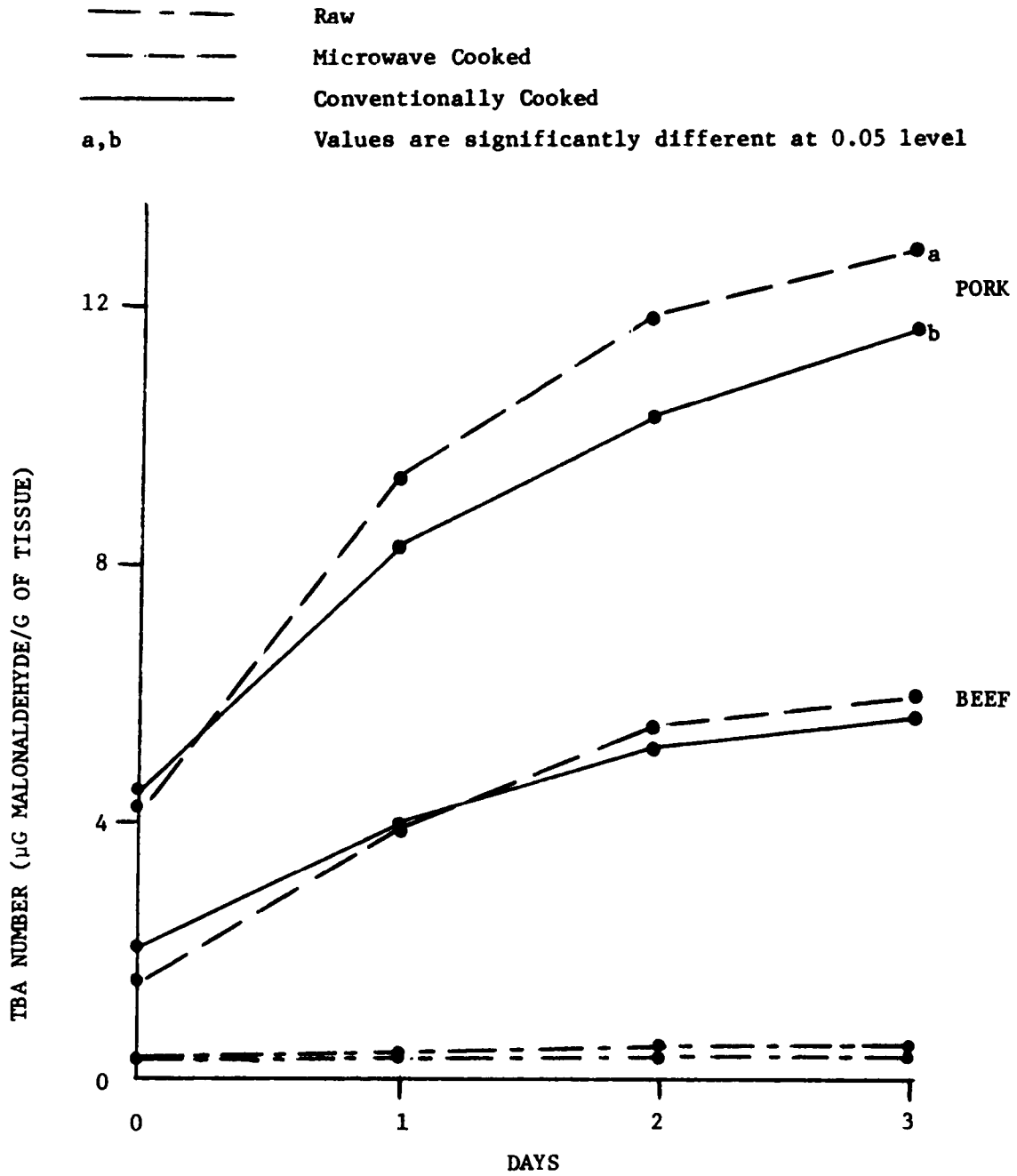Figure 11. Stability of raw and cooked turkey breast and thigh as measured by TBA

Figure 12. Stability of raw and cooked pork and beef as measured by TBA

promote a reaction to produce singlet oxygen using tissue pigments as sensitizers. Since singlet oxygen reacts $10^3 - 10^4$ faster than normal oxygen in initiating lipid oxidation (Rawls and Van Saten, 1970, 1971), it seems logical to attribute the acceleration of oxidation to the production of singlet oxygen by microwave energy. However, the results obtained for beef seem not to support this explanation. Since beef has an appreciable amount of pigment which could act as a sensitizer, singlet oxygen should be produced during microwave treatment and, as a result, a significantly different rate of lipid oxidation should be observed for the microwave and conventionally cooked samples. Cooking methods did not affect the TBA values of beef in the present study.

Other observations made in this study seem to shed some light on what might be responsible for the influence of microwave energy on the stability of the meat products. These observations will be discussed in the next section, after presenting the results of the fatty acid analysis on the raw and cooked meat products.

D. Effect of Cooking and Storage on the Fatty Acid Composition of Meat

Since Maga et al. (1977) had reported that microwave cooking resulted in the production of trans fatty acids in potatoes, it was of interest to see if microwave cooking would cause the formation of trans fatty acids in meats. A 20-foot Silar 10C column was used to improve the separation of cis, trans isomers of oleic acid (18:1). The cis, trans isomers of linoleic acid (18:2) were more resolved than the isomers of oleic acid. A commercial margarine was also analyzed because it was

presumed to contain readily measurable quantities of trans 18:1. Table

9 shows the results of the margarine analysis.

Table 9. Percent relative fatty acid composition of a commercial margarine[a]

Fatty Acid	Relative % Contribution
14:0	0.78
16:0	12.09
16:1	0.11
18:0	7.84
trans 18:1	13.00
cis 18:1	21.64
trans, trans 18:2	0.84
cis, cis 18:2	41.69
18:3	1.61
20:0	0.40

[a]Means based on three observations.

The fatty acid composition of raw and cooked samples is shown in

Table 10-13. As is evident in the tables, no trans fatty acids were

detected in any turkey or pork samples. Very small amounts of trans,

trans 18:2 (linoleic acid) were detected in all beef samples and cooking

did not increase the content of this component. Mai et al. (1980)

Table 10. Effect of cooking method on relative percent fatty acid
composition of turkey thigh muscle[a]

Fatty Acid[b]	Raw	Microwave Cooked	Conventionally Cooked
14:0	3.70	3.01	3.26
14:1	2.10	1.89	2.01
16:0	25.60	25.20	25.52
16:1	8.31	7.41	7.39
18:0	7.24	6.91	7.10
trans 18:1	---	---	---
cis 18:1	29.81	31.56	30.63
trans, trans 18:2	---	---	---
cis, cis 18:2	21.02	21.83	21.94
18:3	0.68	0.69	0.71
20:4	1.51	1.46	1.41
22:6	0.03	0.03	0.03
U/S ratio	1.74	1.85	1.79

[a]Means are based on three observations.

[b]Number identifies chain length and number after colon signifies
the number of double bonds.

Table 11. Effect of cooking method on relative percent fatty acid
composition of turkey breast muscle[a]

Fatty Acid[b]	Raw	Microwave Cooked	Conventionally Cooked
14:0	3.81	3.25	3.24
14:1	2.07	1.99	2.02
16:0	25.50	24.81	25.30
16:1	7.51	7.41	7.06
18:0	7.62	7.31	7.49
trans 18:1	---	---	---
cis 18:1	30.02	30.59	30.81
trans, trans 18:2	---	---	---
cis, cis 18:2	21.32	22.50	21.98
18:3	0.59	0.62	0.60
20:4	1.43	1.51	1.49
22:6	0.02	0.01	0.01
U/S ratio	1.71	1.83	1.78

[a]Means are based on three observations.

[b]Number identifies chain length and the number after colon
signifies the number of double bonds.

Table 12. Effect of cooking method on relative percent fatty acid
composition of pork meat[a]

Fatty Acid[b]	Raw	Microwave Cooked	Conventionally Cooked
14:0	2.64	1.97	2.12
14:1	1.00	0.74	0.76
16:0	24.43	23.73	24.13
16:1	4.51	3.87	3.75
18:0	11.20	11.17	11.22
trans 18:1	---	---	---
cis 18:1	45.60	46.89	46.40
trans, trans 18:2	---	---	---
cis, cis 18:2	9.17	10.00	9.99
18:3	0.80	0.89	0.90
20:4	0.65	0.74	0.73
U/S ratio	1.61	1.71	1.67

[a]Means are based on three observations.

[b]Number identifies chain length and the number after colon
signifies the number of double bonds.

Table 13.   Effect of cooking method on relative percent fatty acid
composition of beef[a]

Fatty Acid[b]	Raw	Microwave Cooked	Conventionally Cooked
14:0	3.28	3.31	3.36
14:1	1.16	1.43	1.44
16:0	29.26	28.80	28.88
16:1	4.90	4.91	4.89
18:0	14.14	12.40	12.37
trans 18:1	---	---	---
cis 18:1	43.65	44.97	44.87
trans, trans 18:2	0.02	0.02	0.01
cis, cis 18:2	2.24	2.76	2.82
18:3	1.30	1.32	1.29
20:4	0.05	0.08	0.07
U/S ratio	1.14	1.25	1.24

[a]Means are based on three observations.

[b]Number identifies chain length and the number after colon
signifies the number of double bonds.

studied the effect of microwave heating on beef tallow, chicken fat and bacon fat and also failed to find any evidence of chemical alteration or isomerization of fatty acids due to microwave energy. They reported traces of _trans_ fatty acids in the beef tallow samples they analyzed, and noted an apparent slight increase in the _trans_ fatty acid content following cooking. Lack of formation of _trans_ fatty acids in microwave cooked meat products, as opposed to the increase Maga et al. (1977) reported for potatoes, may be due to the differences in composition between the two types of food.

The fatty acid compositions of raw and cooked samples of meat were very similar. This finding agrees with reports from other workers (Berry and Cunningham, 1970; Janicki and Appledorf, 1974; Meyers and Harris, 1975; Mai et al., 1980). Table 14 shows the changes in the polyunsaturated:saturated (U/S) ratio of the various meat products following cooking. In general, cooking caused an increase in the U/S ratio. Microwave cooking caused a significantly higher U/S ratio than did conventional cooking for pork and turkey meats. There is not a significant difference in the U/S ratio for microwave and conventionally cooked beef. The samples with higher U/S ratios might be expected to undergo more lipid oxidation during storage than those with lower unsaturation. In previous experiments, it was noted that microwave cooked pork and turkey had higher TBA values after refrigerated storage than did conventionally cooked samples. The insignificant difference in the TBA values for microwave and conventionally cooked beef may be due to the similar U/S ratios for these samples.

Table 14. Changes in the U/S ratio of various meats as a result of cooking[a]

Meat Type	U/S Ratio		
	Raw	Microwave Cooked	Conventionally Cooked
Turkey Thigh	1.74^b	1.85^c	1.79^d
Turkey Breast	1.71^b	1.83^c	1.78^d
Pork	1.61^b	1.71^c	1.67^d
Beef	1.14^b	1.25^c	1.24^c

[a]Means are based on three observations.

[b,c,d]Values followed by these letters are significantly different at $p < 0.05$ for each meat type.

Changes with storage in the fatty acid composition of raw, micro-wave and conventionally cooked turkey lipids were also studied. The fatty acids composition of stored samples is shown in Tables 15-17. Only slight loss of unsaturated lipid occurred in the stored raw samples and the U/S values for raw samples were essentially unchanged. Greater losses were noted in the cooked samples. Microwave heated samples lost 5.5% more unsaturated fatty acids during the six days of storage than did the conventionally cooked samples (U/S: MC - 1.79-1.47, Δ = 18%; CC - 1.75-1.55, Δ = 12.5%).

The greater loss of unsaturation by the microwave cooked samples as compared to the conventionally cooked ones with storage is a further

Table 15. Changes in the relative percent fatty acid composition of
raw turkey thigh meat lipids during refrigerated storage[a]

Fatty Acid	Days			
	0	2	4	6
14:0	4.73	5.41	5.39	5.52
14:1	2.07	2.46	2.89	3.12
16:0	25.25	25.26	25.26	25.24
16:1	7.11	7.11	7.10	7.10
18:0	7.32	7.31	7.39	7.38
trans 18:1	---	---	---	---
cis 18:1	31.02	31.03	31.08	30.99
trans, trans 18:2	---	---	---	---
cis, cis 18:2	20.50	19.64	19.32	19.21
18:3	0.60	0.58	0.54	0.53
20:4	1.38	1.19	1.02	0.91
22:6	0.02	0.01	0.01	---
U/S ratio	1.68	1.63	1.62	1.62

[a]Figures represent averages of three replicates.

Table 16. Changes in the relative percent fatty acid composition of
conventionally cooked turkey thigh meat lipids during
refrigerated storage[a]

Fatty Acid	Days			
	0	2	4	6
14:0	4.03	4.86	5.14	5.93
14:1	2.11	2.42	2.70	2.96
16:0	24.96	25.26	25.30	25.43
16:1	7.02	7.00	6.94	6.84
18:0	7.26	7.48	7.79	7.79
trans 18:1	---	---	---	---
cis 18:1	31.55	30.86	30.78	30.17
trans, trans 18:2	---	---	---	---
cis, cis 18:2	21.19	20.70	20.05	19.63
18:3	0.62	0.64	0.64	0.63
20:4	1.24	0.77	0.65	0.62
22:6	0.02	0.01	0.01	---
U/S ratio	1.75	1.65	1.61	1.55

[a] Figures represent averages of three replicates.

Table 17. Changes in the relative percent fatty acid composition of
microwave cooked turkey thigh meat lipids during refrigerated
storage[a]

Fatty Acid	Days			
	0	2	4	6
14:0	3.20	4.58	5.67	6.99
14:1	2.09	2.33	2.11	3.19
16:0	25.14	25.81	25.88	25.61
16:1	7.29	7.19	7.16	7.11
18:0	7.17	7.80	7.75	7.85
trans 18:1	---	---	---	---
cis 18:1	31.47	31.24	30.54	30.09
trans, trans 18:2	---	---	---	---
cis, cis 18:2	21.14	19.87	19.87	18.22
18:3	0.61	0.55	0.51	0.47
20:4	1.55	0.63	0.51	0.47
22:6	0.02	---	---	---
U/S ratio	1.79	1.61	1.55	1.47

[a]Figures represent averages of three replicates.

indication that microwave cooking caused some changes that affected the stability of the lipids. The content of antioxidants or pro-oxidants in the tissue might also be affected by the method of cooking. Naturally occurring antioxidants, for example, tocopherol and ascorbic acid, might be destroyed at different rates by the two methods of cooking.

E. Lipid Composition of Fermented Food Products

The purpose of this study was to determine whether microorganisms hydrogenate food lipids during the fermentation process. Table 18 shows the results of the fatty acid analysis of sausages before and after fermentation. No trans fatty acids were detected in either the raw meat or the sausages. This result indicates that the Micrococcus species used as a starter culture did not hydrogenate the fatty acids in the meat. The reason for lack of hydrogenation by the organisms may be the lack of a suitable environment for the organisms to carry out the reaction. It is also probable that the Micrococcus species involved here are different from those isolated from the cow's rumen. Pediococcus species used in this study also did not cause isomerisation of fatty acids.

A fermented soy product, natto, was also investigated and the results of the fatty acid analysis are presented in Table 19. No trans fatty acids were detected in the product. Soybean lipids naturally contain only cis fatty acids and the fermented product also contained only cis fatty acids. This shows that the organism, Bacillus subtilis, used in the fermentation process did not cause hydrogenation of lipids.

Table 18. Relative percent fatty acid composition of summer sausage[a]

Fatty Acid	Raw	LT I[b]	LT II[c]
14:0	1.18	1.26	1.23
14:1	0.08	0.08	0.09
16:0	24.81	24.90	24.80
16:1	1.88	1.99	1.85
18:1	14.57	14.49	14.37
trans 18:1	---	---	---
cis 18:1	46.75	45.89	46.54
trans, trans 18:2	---	---	---
cis, cis 18:2	9.91	10.44	10.36
18:3	0.63	0.72	0.58
20:4	0.19	0.23	0.18
U/S ratio	1.47	1.46	1.48

[a]Means are based on three observations

[b]Pediococcus as starter culture.

[c]Pediococcus plus micrococcus as starter culture.

Table 19. Relative percent fatty acid composition of natto[a]

Fatty Acid[b]	Relative %
16:0	11.50
16:1	0.42
18:0	3.64
trans 18:1	---
cis 18:1	25.12
trans, trans 18:2	---
cis, cis 18:2	56.49
18:3	2.83

[a]Means are abased on three observations.

[b]Number identifies chain length and number after colon signifies the number of double bonds.

The results obtained in this study show that consumption of trans

fatty acids from the products studied is not likely. Studies by

Boatman and Hammond (1964) on the effect of manufacturing conditions and

storage on certain polyunsaturated fatty acids of dairy products also

suggest that the microorganisms involved in the manufacture of cheese

may not have the ability to hydrogenate the fatty aicds. These workers

did not see any changes in the polyunsaturated fatty acid content of the

products before and after fermentation. If hydrogentation of fatty

acids occurred, changes should be observed in the polyunsaturated fatty

acid levels of the products. It should be pointed out, however, that

the method used by Boatman and Hammond (1964) was not designed to measure

trans fatty acids.

V. CONCLUSIONS

A malonaldehyde specific reagent, 4,4'-sulfonyldianiline was used to determine spectrofluorometrically the malonaldehyde content of wet muscle tissues. In a comparison of this spectrofluorometric (SPF) method with the TBA test, similar values were obtained for raw and freshly cooked samples, but lower values were found by the SPF technique for cooked samples stored at refrigerator temperature for 72 hours. This gives further evidence that TBA assays measure more than malonaldehyde. Products other than malonaldehyde which react with TBA were probably formed in the stored cooked samples. In addition to the greater selectivity of the SPF method for malonaldehyde, it is also more sensitive than the TBA test. The time necessary to run the two methods is about the same.

Microwave energy affected the stability of lipids in turkey and pork meats. The higher TBA values in stored microwave cooked samples, when compared to the conventionally cooked, may be due to the quality and quantity of lipid left in the sample after cooking. No trans isomers of fatty acids could be detected in the meat samples as a result of microwave cooking.

Results obtained in this study indicate that the microorganisms used for the fermentation of meat and soybeans in the preparation of semi-dry summer sausages and Natto, respectively, did not cause the hydrogenation of fatty acids since no trans fatty acids were detected. However,

it would be interesting to study other microorganisms used in other food fermentation systems.

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VII. ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my adviser, Dr. Jane D. Love, for her assistance in planning and conducting these investigations, for her patient assistance in the preparation of this manuscript and for her guidance and encouragement during the pursuit of this Ph.D. degree.

I would also like to express my sincere appreciation to Dr. Mark Love, Department of Food and Nutrition, Dr. Joseph Sebranek, Department of Animal Science, and Dr. Earl Hammond and Dr. Allen Kraft, Department of Food Technology, for their guidance during the pursuit of this project. Sincere appreciation is extended to Dr. Jacqueline Dupont, head of my department, for her understanding and concern about my future. I wish to thank Diane Elas for her help and Gretchen Triplett for her assistance in typing this manuscript.

Finally, I wish to extend my heartfelt thanks to my wife, Mercy, without whose assistance and patient understanding this study would not have been possible. This dissertation is dedicated to my children, Moyo and Tosin.

VIII. APPENDIX

Table A.1. Stability of turkey and beef as measured by TBA and SPF tests[a]

| Samples | TBA Numbers (μg Malonaldehyde/g of Tissue) | | | |
	Day 0	Day 1	Day 2	Day 3
Turkey				
Raw	0.28 ± .02	0.42 ± .03	0.63 ± .01	0.68 ± .03
MC[b]	4.46 ± .20	9.89 ± .41	14.11 ± .40	17.16 ± .60
CC[c]	6.35 ± .21	10.11 ± .39	13.57 ± .41	15.68 ± .51
Beef				
Raw	0.42 ± .01	0.42 ± .03	0.43 ± .02	0.43 ± .02
MC[b]	1.41 ± .05	3.18 ± .18	4.32 ± .11	5.41 ± .22
CC[c]	1.89 ± .05	3.16 ± .12	4.26 ± .17	5.32 ± .24

[a]Means (±S.D.) are based on six observations.

[b]Microwave cooked.

[c]Conventionally cooked.

SPF Values (µg Malonaldehyde/g of Tissue)			
Day 0	Day 1	Day 2	Day 3
0.29 ± .03	0.48 ± .01	0.61 ± .02	0.68 ± .03
4.48 ± .19	8.90 ± .34	11.95 ± .32	14.27 ± .42
6.38 ± .24	9.02 ± .28	11.46 ± .34	12.49 ± .42
0.43 ± .02	0.42 ± .03	0.42 ± .02	0.43 ± .02
1.46 ± .04	2.85 ± .07	4.05 ± .09	5.10 ± .11
1.82 ± .03	3.03 ± .11	4.02 ± .12	5.00 ± .19

Table A.2. Stability of raw, microwave and conventionally cooked beef, pork and turkey meats[a]

Sample	TBA Number μg/g Tissue			
	Day 0	Day 1	Day 2	Day 3
Turkey Thigh				
Raw	0.37 ± .0??			?4 ± .02[c]
Microwave Cooked[b]	3.79 ±			± .51[d]
Conventionally Cooked[b]	4.21			60[e]
Turkey Breast				
Raw				
Microwave Cooked[b]				
Conventionally Cooked				
Beef				
Raw				
Microwave Cooked[b]				
Conventionally Cooked				
Pork				
Raw				
Microwave Cooked[b]				
Conventionally Cooked				

[a] Means are based on six

[b] Values are significantly di?? horizontal row.

[c,d,e] Within vertical rows for each meat type, values followed by different letters are significantly different at 0.05 level.

Table A.2. Stability of raw, microwave and conventionally cooked beef, pork and turkey meats[a]

Sample	TBA Number µg/g Tissue			
	Day 0	Day 1	Day 2	Day 3
Turkey Thigh				
Raw	$0.37 \pm .02^c$	$0.41 \pm .02^c$	$0.44 \pm .03^c$	$0.44 \pm .02^c$
Microwave Cooked[b]	$3.79 \pm .11^d$	$11.81 \pm .41^d$	$14.95 \pm .44^d$	$16.89 \pm .51^d$
Conventionally Cooked[b]	$4.21 \pm .19^e$	$10.74 \pm .40^e$	$14.10 \pm .50^e$	$15.80 \pm .60^e$
Turkey Breast				
Raw	$0.31 \pm .02^c$	$0.39 \pm .01^c$	$0.39 \pm .03^c$	$0.41 \pm .03^c$
Microwave Cooked[b]	$3.79 \pm .09^d$	$8.11 \pm .39^d$	$9.95 \pm .48^d$	$10.75 \pm .42^d$
Conventionally Cooked[b]	$3.81 \pm .23^e$	$7.20 \pm .33^e$	$9.00 \pm .39^e$	$9.60 \pm .41^e$
Beef				
Raw	$0.36 \pm .04^c$	$0.39 \pm .02^c$	$0.40 \pm .03^c$	$0.40 \pm .02^c$
Microwave Cooked[b]	$1.62 \pm .08^d$	$4.01 \pm .21^d$	$5.55 \pm .25^d$	$6.10 \pm .19^d$
Conventionally Cooked[b]	$2.01 \pm .13^e$	$4.12 \pm .20^e$	$5.20 \pm .18^e$	$5.65 \pm .21^e$
Pork				
Raw	$0.35 \pm .03^c$	$0.42 \pm .01^c$	$0.41 \pm .01^c$	$0.52 \pm .02^c$
Microwave Cooked[b]	$4.21 \pm .30^d$	$9.47 \pm .57^d$	$12.04 \pm .61^d$	$13.00 \pm .59^d$
Conventionally Cooked[b]	$4.40 \pm .27^e$	$8.37 \pm .40^e$	$10.47 \pm .51^e$	$11.78 \pm .56^e$

[a]Means are based on six observations.

[b]Values are significantly different at 0.05 level across the horizontal row.

[c,d,e]Within vertical rows for each meat type, values followed by different letters are significantly different at 0.05 level.