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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

by

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# 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'-sulfonalydianiline 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 singlet 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 investigations (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.

## 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 <u>trans</u> 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 susceptible 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

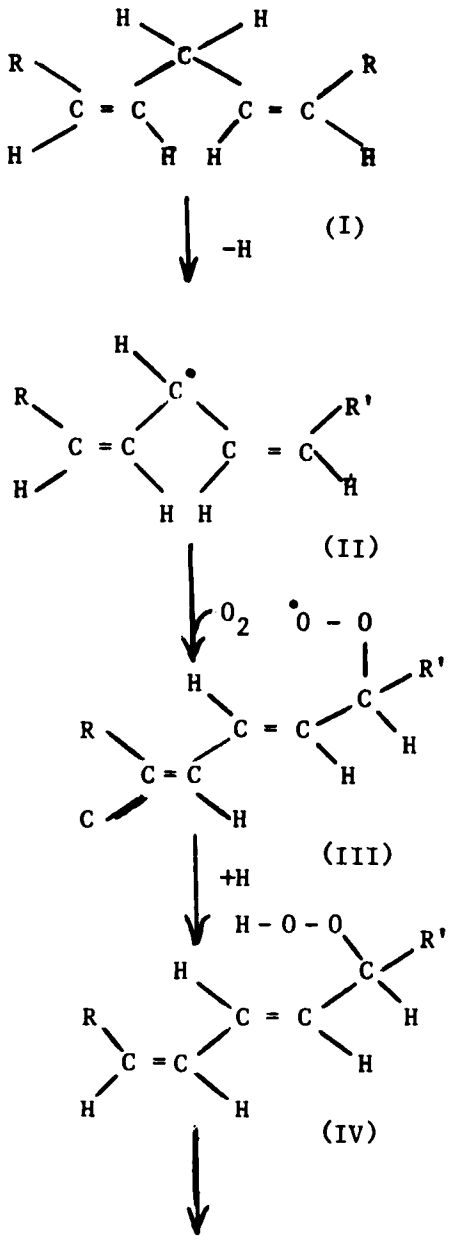
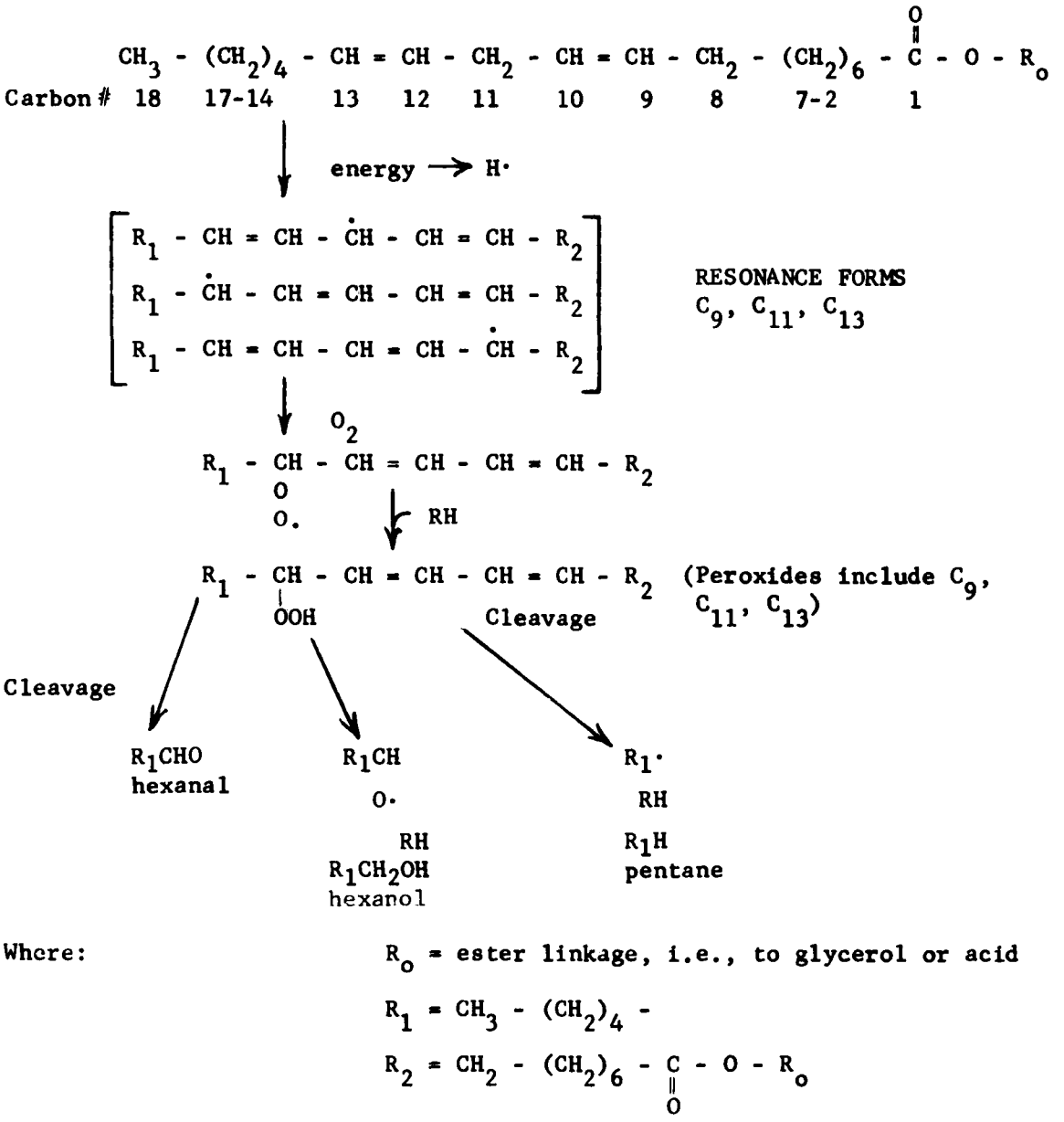



Figure 1. Reaction mechanisms for the autoxidation of an unsaturated fatty acid

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




Where:

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

$R_1 = CH_3 - (CH_2)_4 -$

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

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-enal |
| | $C_9$ | 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 $\beta$, $\gamma$ 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 $\beta$, $\gamma$ 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

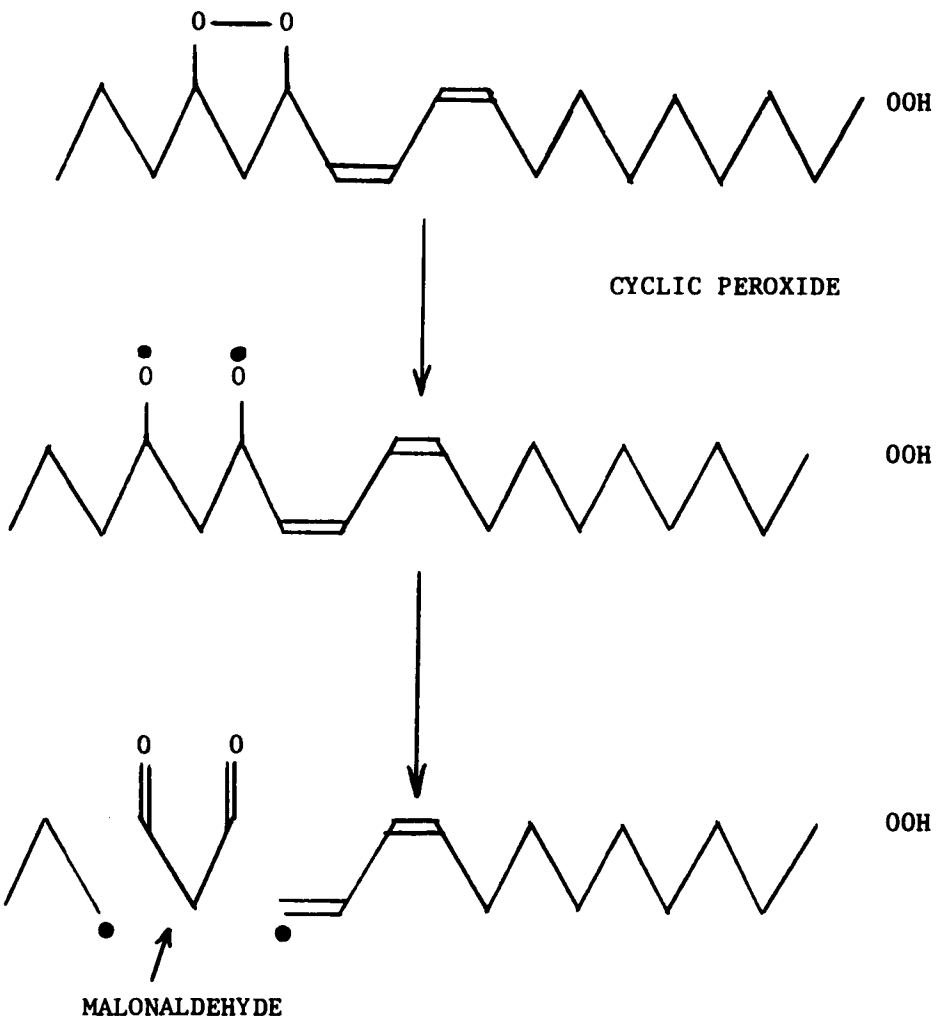



Figure 3. Mechanism for the formation of malonaldehyde (from Dahle et al., 1962)

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 monocarbonyls which are initial degradation products of lipid hydroperoxides.

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 increases 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 hydroxytoluene (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:

$$^{1}S + h\nu \rightarrow {}^{1}S^{*} \rightarrow {}^{3}S^{*}$$

$${}^{3}S^{*} + {}^{3}O_{2} \rightarrow {}^{1}O_{2}^{*} + {}^{1}S$$

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

$$ROOH \rightarrow \text{Free radicals}$$

where $^{1}S$ = singlet state sensitizer

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

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

$^{3}O_{2}$ = normal triplet oxygen

$^{1}O_{2}^{*}$ = excited singlet state oxygen

$h\nu$ = 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 measurement 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 malonaldehyde; and N-prop-2-enal aminoacetic acid. These standards are readily hydrolyzed to malonaldehyde by acids.

Malonaldehyde can be determined in aqueous acidic ($\lambda_{max}$ = 245 mμ, ε = 13,000) or alkaline ($\lambda_{max}$ = 267 mμ, ε = 30,000) solutions. Obviously, the presence of organic compounds that absorb in the ultraviolet 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 | $\lambda_{max}$ (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 | 485[g] |
| Benzenediazonium tetrafluoroborate | 369 (~ 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 $H_2SO_4$ $\lambda_{max}$ 245 nm, mε 13.

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

[c] The pure neutral chromogen absorbs at $\lambda_{max}$ 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 $\lambda_{max}$ 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 $\lambda_{max}$ 360 nm, mε 42·5 in 96% ethanol.

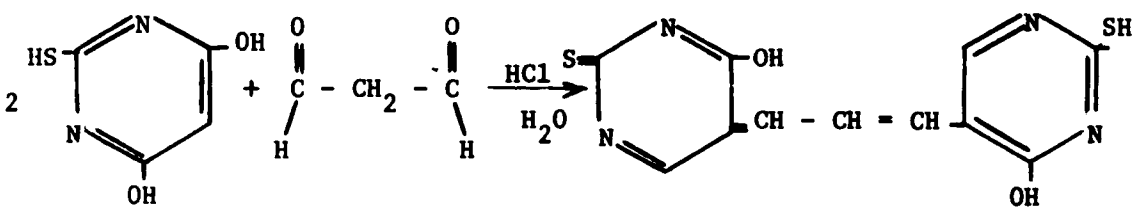
[g] Pure chromogen absorbs at 495 nm.

Table 2. Continued

| Reagent | $\lambda_{max}$(mμ) 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] mμ 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




TBA MALONALDEHYDE TBA CHROMAGEN + $2H_2O$

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 interference 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 peroxidizing lipids and the contaminant amines of a polyamide coating adsorbed on a glass or plastic plate as conventionally used in thin layer chromatographic separations. The fluorescence, which is measured at an excitation 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 spectrofluorometric 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.

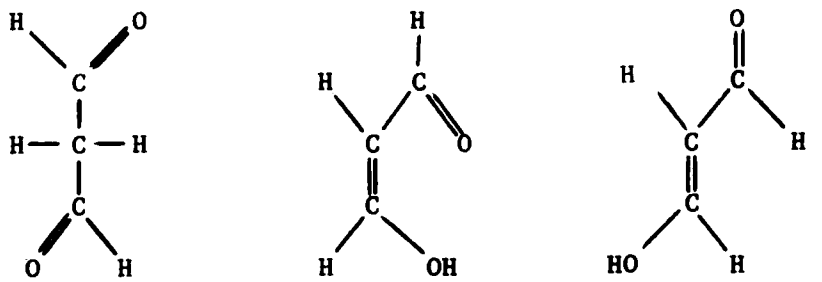
fluorescence intensity was stable for 30 minutes. 4,4'-Sulfonyldianiline was found to be the best reagent for this procedure.

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

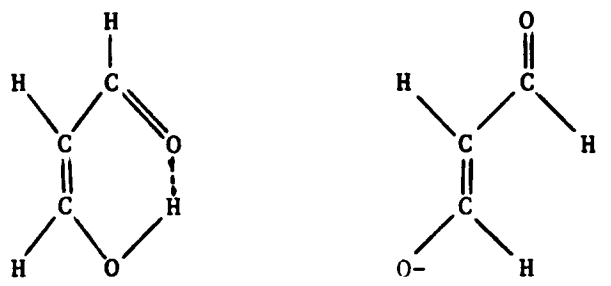
malonaldehyde in moist foods such as meat, which are commonly subjected to the TBA test.

## 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

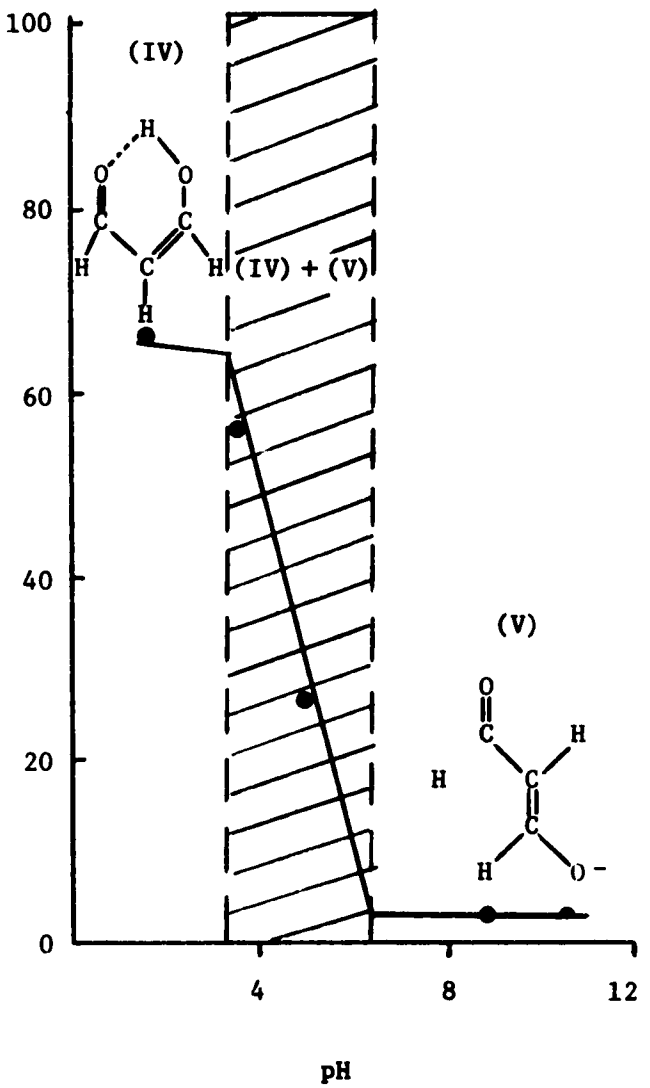



Figure 6. Effect of pH on recovery of malonaldehyde by distillation (from Kwon and Watts, 1964)

## 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 *Trans* 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% *trans* 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 *trans* fatty acids is that they are metabolized differently than their cis isomers. The interplay between the *trans* 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 ω6 series of PUFA esterify the β position of phosphatydylcholine and that in the absence of dietary sources of the ω6 series of fatty acids, the elongated ω9 series of fatty acids esterify this position. The trans fatty acids esterify the α position in the presence of dietary ω6 PUFA. However, in the absence of dietary ω6 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 ω6 PUFA preferentially esterifies cholesterol and is also preferentially hydrolyzed (Goller et al., 1970). However, in the absence of elongated ω6 PUFA, the ω9 series of fatty acids esterify cholesterol. In the presence of trans fatty acids, the ω6 PUFA preferentially esterifies cholesterol; however, the trans fatty acids also esterify cholesterol. Once esterified, ω6 cholesterol esters are preferentially hydrolyzed which can result in the accumulation of cholesterol esters of the elongated ω9 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<br>Metabolic products of hydrogenation |
|---|---|
| Butyrivibrio fibrisolvens | –[a] |
| B. fibrisolvens A38 | – |
| B. fibrisolvens A38 | 18:3 cis-9,trans-11,cis-15<br>18:2 $\Delta^{11},\Delta^{15}$ |
| Borrelia Treponema $B_25$ | Isomerized, then hydrogenated |
| Micrococcus sp. | Isomerized, then hydrogenated |
| Ruminococcus albus F2/6 | *18:3 cis-9,trans-11,cis-15<br>*18:2 trans-11,cis-15<br>18:1 trans (95%)<br>18:1 cis (5%) |
| Eubacterium F2/2 | 18:3 cis-9,trans-11,cis-15<br>18:2 trans-11,cis-15<br>18:1 trans-11 (5%) |
| Eubacterium W/461 | *18:3 cis-9,trans-11,cis-15<br>*18:2 trans-11,cis-15<br>18:1 trans (50%); trans-11 (65%)<br>18:1 cis (50%); cis-11 (95%) |
| Fusocillus T344 | *18:3 cis-9,trans-11,cis-15<br>*18:2 cis-9,trans-11<br>18:1 cis-15 (85%) |
| babrahamensis P2/2 | 18:2 trans-11,cis-15<br>18:1 cis-15 (85%) |
| R8/5 gram-negative rod | 18:2 trans-11,cis-15 (25%)<br>18:1 cis-15 (50%)<br>18:1 trans-15 (25%) |
| EC7/2 gram-negative rod | 18:2 trans-11,cis-15 (70%)<br>18:2 trans-11 (30%) |
| 2/9/1 gram-negative vibrio | 18:1 trans:cis ratio 2:1 (95%)<br>trans-11 (41%)<br>trans-12 (11%)<br>trans-10 (14%)<br>cis-11 (29%)<br>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<br>Metabolic products of hydrogenation |
|---|---|
| R7/5 gram-negative rod | 18:1 trans:cis ratio 1:1 (100%)<br>trans-11 (32%)<br>trans-12 (15%)<br>trans-10 (6%)<br>cis-11 (44%)<br>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%)<br>trans-11 (14%)<br>trans-10 (11%)<br>trans-12 (8%)<br>cis-11 (62%)<br>cis-12 (5%) | Not hydrogenated |
| 18:1 trans-11 (100%) | Not hydrogenated |
| 18:1 trans-11 (96%) | Not hydrogenated |
| 18:1 trans-11 (75%)<br>18:1 cis-9,cis-12 (25%) | – |

As shown in Table 4, some micrococcus 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 obtained from Land O'Lakes, Ellsworth, Ia. Cooking and storage treatments 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 obtained 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 recorder (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 approximately 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°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°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. Extraction of lipid from tissues, margarine and fermented soy products

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 chloroform. 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 extract 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 amount 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:

$$K(\text{distillation}) = \frac{\text{conc. in moles/5 ml of distillate}}{\text{optical density}} \times \frac{\text{molecular wt. of malonaldehyde}}{} \times \frac{10^7}{\text{wt. of sample}} \times \frac{100}{\% \text{ recovery}}$$

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 refrigerator 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 transferred 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 spectrophotofluorometer: Slit arrangement was 3, 1, 3 for the emission monochromator 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 cyclohexane-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. Attempts to follow lipid oxidation by quantitating the fluorescent compounds extracted from tissues

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

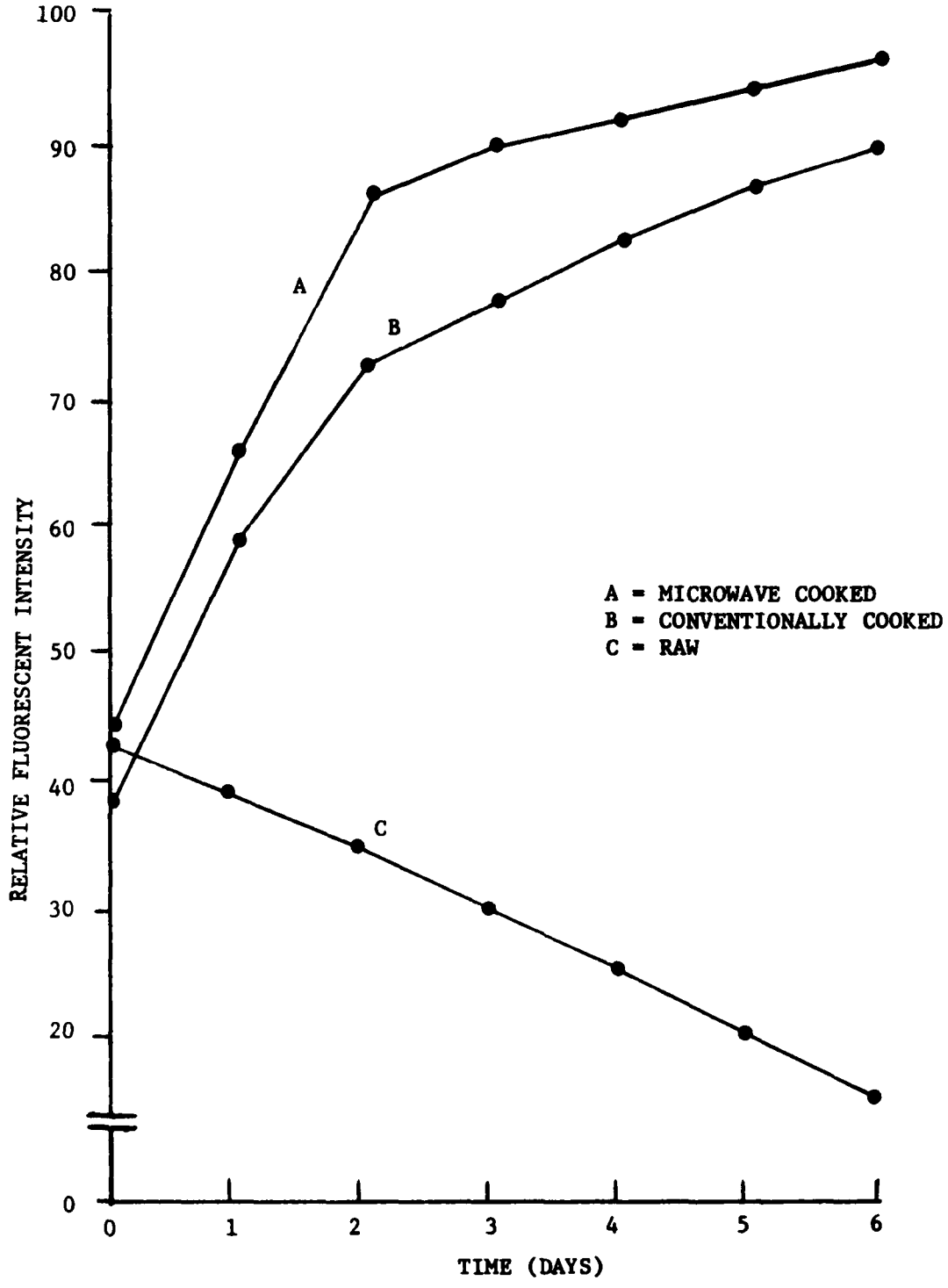


Figure 7. Assay of fluorescent compounds extracted from raw and cooked turkey meat

in the cooked samples the rate of destruction of the fluorescent compounds was slower than the rate of formation and, hence, increased spectrofluorometric 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 ultraviolet 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 dimethylformamide 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

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 malonaldehyde bis acetal was determined and compared with values obtained when the TBA test was performed on the same solution. The values for malonaldehyde, 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 malonaldehyde 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

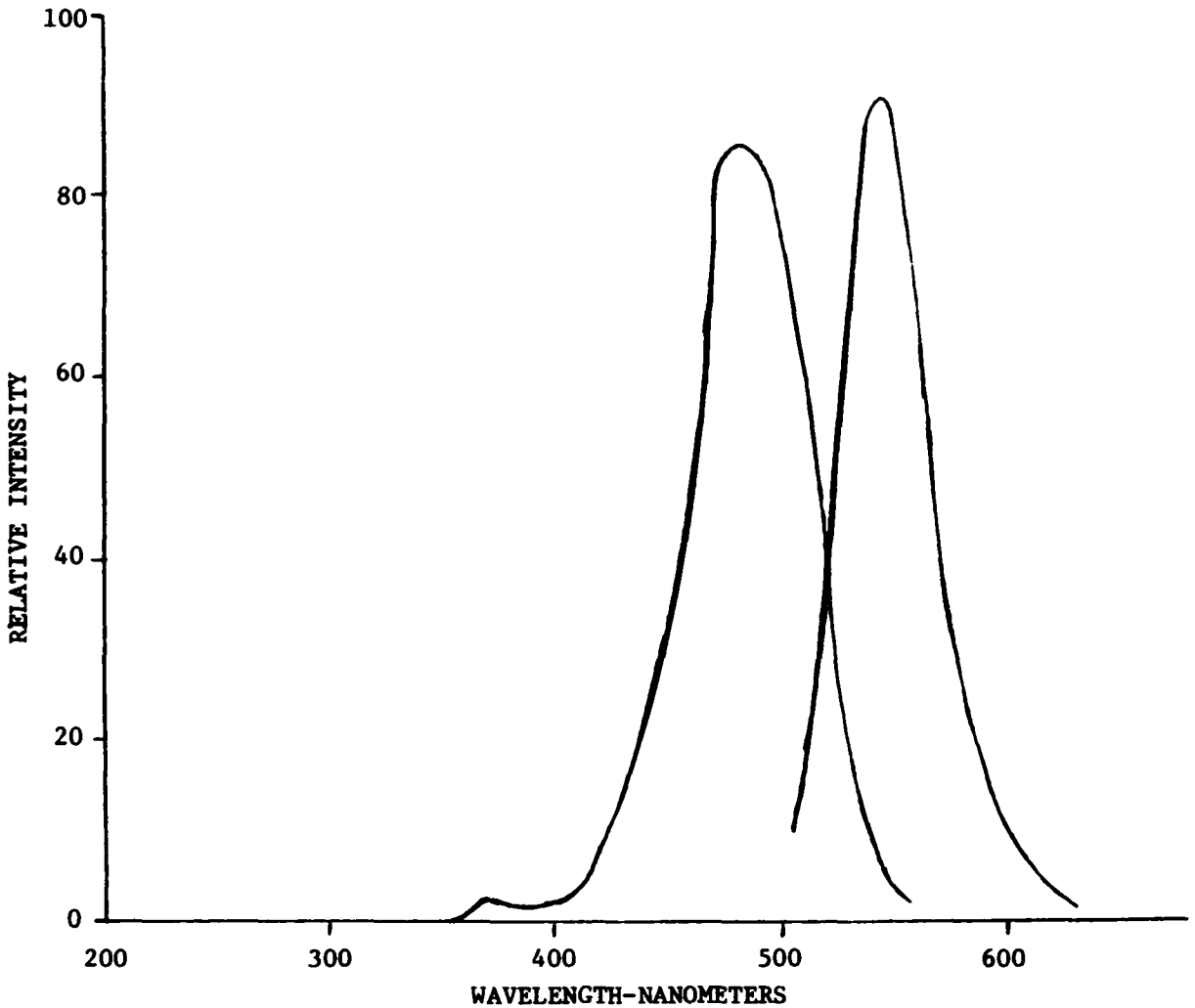


Figure 8. Spectrofluorometric curve obtained in determination of malonaldehyde

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 dimethylformamide, 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-dihydroxyacetone, 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 malonaldehyde 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 (± 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 $p < 0.05$.

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

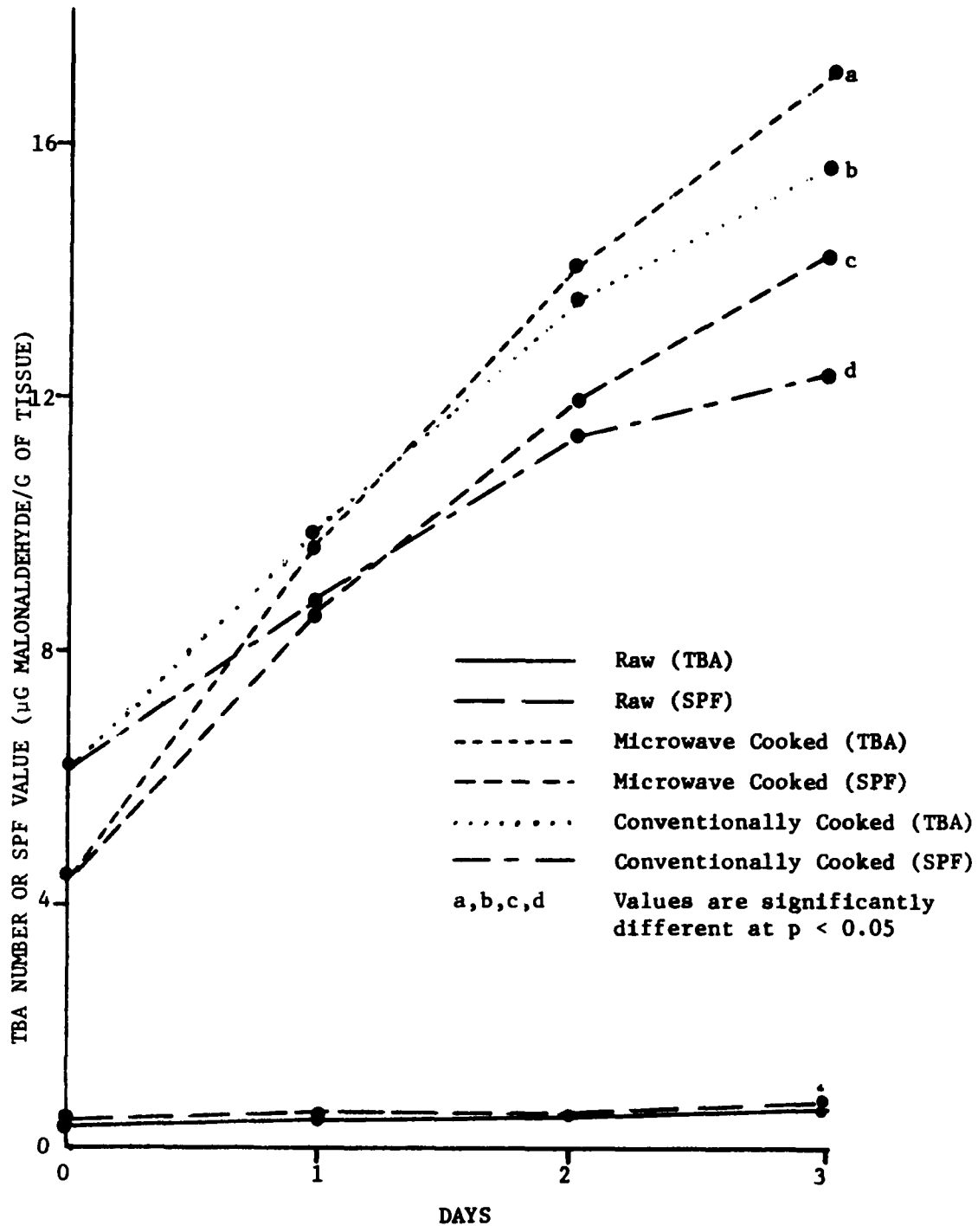


Figure 9. Stability of raw and cooked turkey as measured by TBA and SPF tests

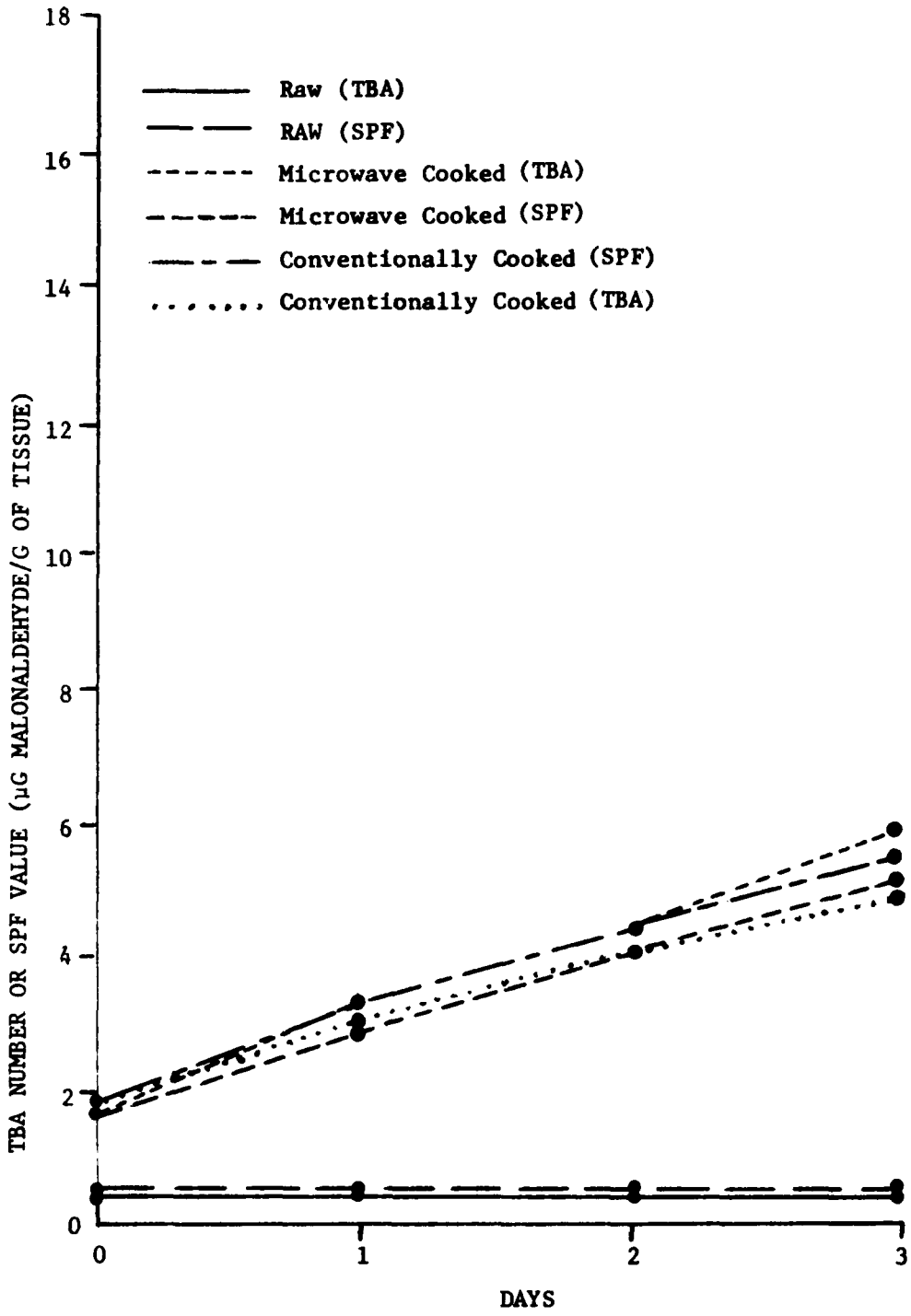


Figure 10. Stability of raw and cooked beef as measured by TBA and SPF. tests

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

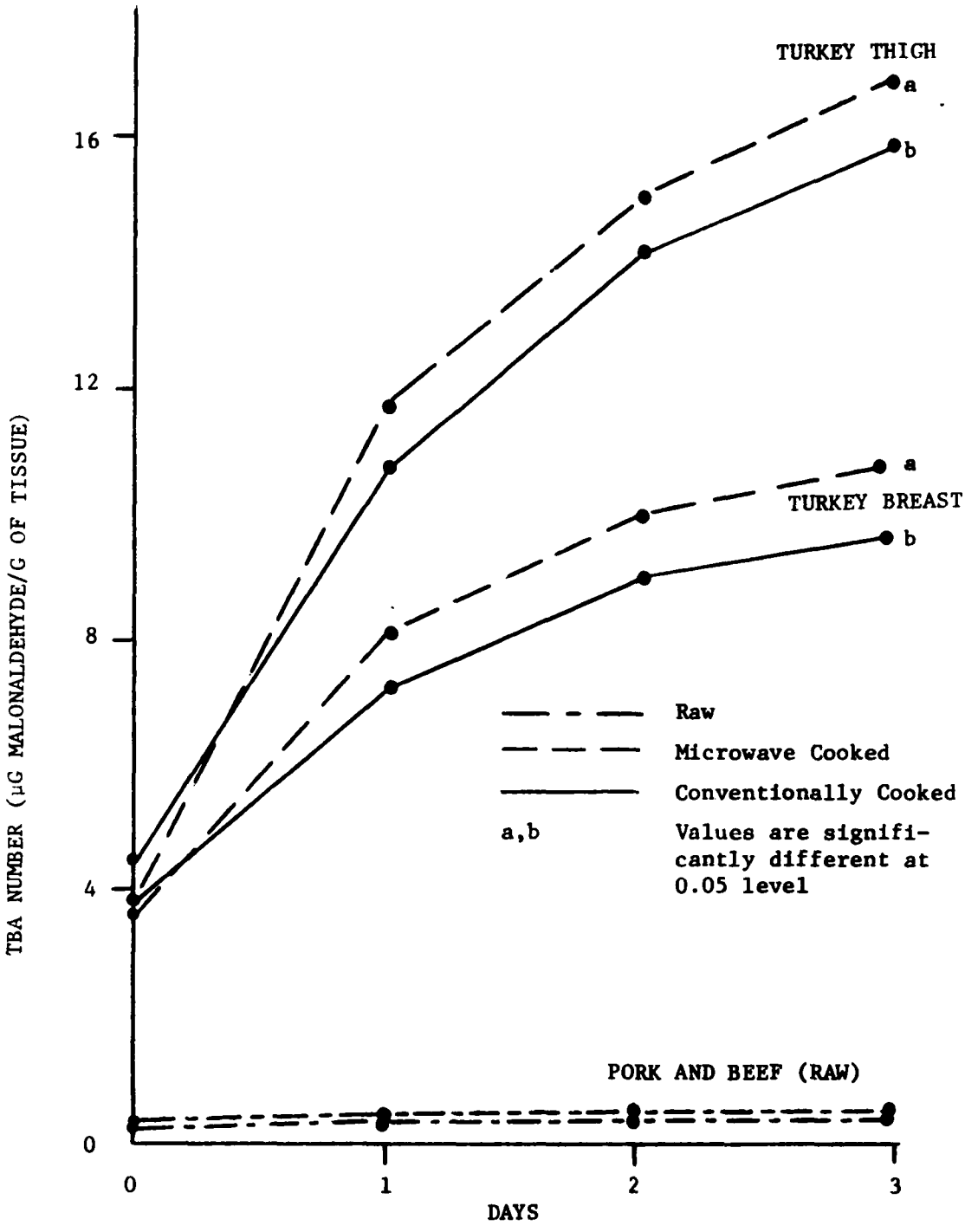


Figure 11. Stability of raw and cooked turkey breast and thigh as measured by TBA

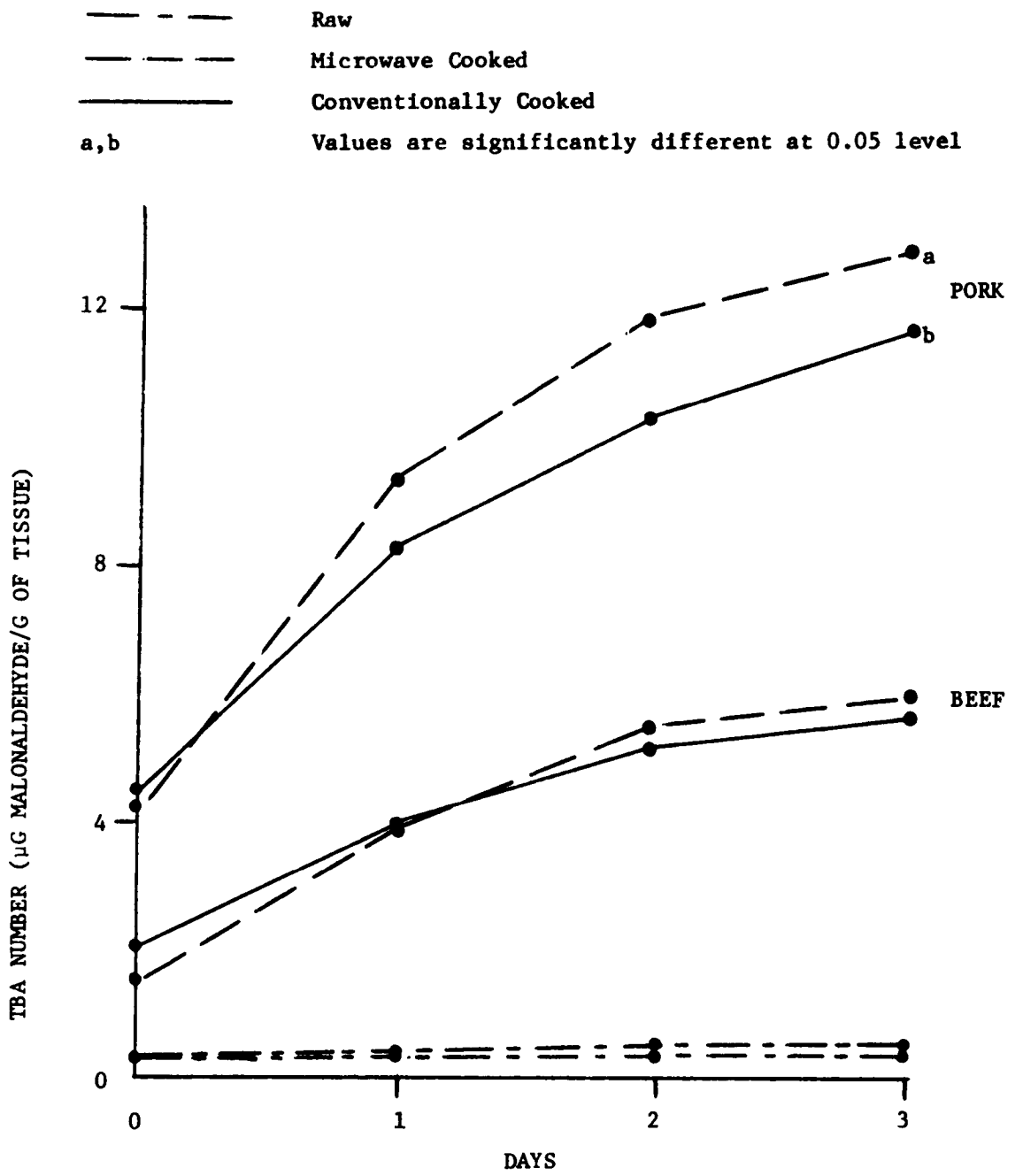


Figure 12. Stability of raw and cooked pork and beef as measured by TBA

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, microwave 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, $\Delta$ = 18%; CC - 1.75-1.55, $\Delta$ = 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]

| | Days | | | |
|---|---|---|---|---|
| Fatty Acid | 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]

| | Days | | | |
|---|---|---|---|---|
| Fatty Acid | 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]

| | Days | | | |
|---|---|---|---|---|
| Fatty Acid | 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.

# VI. BIBLIOGRAPHY

Anderson, R. L., C. S. Fullmer, Jr., and E. J. Hollenbach. 1975. Effects of trans isomers of linoleic acid on the matabolism of linoleic acid in rats. J. Nutr. 105:393-400.

Anonymous. 1979. Watching appliances sales trends indicates product opportunities. Food Prod. Dev. 13:41, 44.

AOAC. 1975. Official methods of analysis. 12th ed. Association of Official Analytical Chemists, Washington, D.C.

Apgar, J., N. Cox, J. Downey, and F. Fenton. 1959. Cooking pork electronically. J. Am. Diet. Assoc. 35:1260-1269.

Arya, S. S., and N. Nirmala. 1971. Determination of free malonaldehyde in vegetable oils. J. Food Sci. Technol. 8:144-180.

Baldwin, R., B. M. Korschgen, M. S. Russell, and L. Mabes. 1976. Proximate analysis, free amino acid, vitamin and mineral content of microwave cooked meat. J. Food Sci. 41:762-765.

Baumgartner, W. A., N. Baker, V. A. Hill, and E. T. Wrights. 1975. Novel interference in TBA assay for lipid peroxidation. Lipids 10:309-311.

Berger, L. R. 1958. An experiment in the electronic cookery of meat. Food News and Views No. 147. Swift and Co., Chicago, IL.

Berry, J. G., F. E. Cunningham. 1970. Factors affecting the flavor of frozen fried chicken. Poult. Sci. 49:1236-1242.

Bidlack, W. R., and A. L. Tappel. 1973. Damage to microsomal membrane by lipid peroxidation. Lipids 8:177-182.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Boatman, C., and E. G. Hammond. 1964. Effect of manufacturing conditions and storage on centain polyunsaturated fatty acids of dairy products. J. Dairy Sci. 47:194-195.

Bowers, J. A., and M. Heier. 1970. Microwave cooked turkey: heating patterns, eating quality and histological appearance. Microwave Application Newsletters 3(6):3-7.

Braddock, R. J., and D. R. Petrus. 1971. Malonaldehyde in aqueous orange juice essences. J. Food Sci. 36:1095-1097.

Buttery, R., G. C. Hendel, and M. M. Boggs. 1961. Autoxidation of potato granules. Part 1. Changes in fatty acids. J. Agr. Food Chem. 9:245-252.

Cairns, J. 1975. The cancer problem. Sci. Am. 233-264.

Chang, I., and B. M. Watts. 1950. Some effects of salt and moisture on rancidity in fats. Food Res. 15:313-321.

Chang, I. C. L., and B. M. Watts. 1952. The fatty acid content of meat and poultry before and after cooking. J. Am. Oil Chem. Soc. 29:334-338.

Chang, Pi-Yu, M. T. Younathan, and B. M. Watts. 1961. Lipid oxidation of precooked beef preserved by refrigeration, freezing and irradiation. Food Technol. 15:168-170.

Chio, K. S., and A. L. Tappel. 1969a. Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. Biochemistry 8:2821-2827.

Chio, K. S., and A. L. Tappel. 1969b. Inactivation of ribonuclease and other enzymes by peroxidising lipids and by malonaldehyde. Biochemistry 8:2827-2832.

Chow, L., and B. M. Watts. 1969. Origin of off-odors in frozen green beans. Food Technol. 23:973-974.

Csallany, A. S., and K. L. Ayaz. 1976. Quantitative determination of organic solvent soluble lipofuscin pigments in tissues. Lipids 11: 412-417.

Cutler, M. C., and M. A. Hayward. 1974. Effect of lipid peroxides on fat absorption and folic acid status in the rat. Nutr. Metabol. 16: 87-93.

Cutler, M. G., and R. Schneider. 1973. Malformations produced in mice and rats by oxidized linoleate. Fd. Cosmet. Toxicol. 11:935-942.

Dahle, L. K., E. G. Hill, and R. T. Holman. 1962. The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methyl esters. Arch. Biochem. Biophys. 98:253-261.

Decareau, R. V. 1972. The ABC's of microwave cooking. J. Microwave Power 7(4):397-409.

De Tomas, M. E., R. R. Brenner, and R. O. Peluffo. 1963. Position of eicosatrienoic acid in the phosphatidyl-ethanolamine from rats deficient in essential fatty acids. Biochim. Biophys. Acta 70: 472-473.

Downey, W. K. 1969. Lipid oxidation as a source of off-flavor development during the storage of dairy products. J. Soc. Dairy Technol. 22: 154-162.

Dugan, Jr., L. R. 1955. Stability and rancidity. J. Am. Oil Chem. Soc. 32:605-609.

Dunkley, W. L., W. G. Jennings. 1951. A procedure for application of the thiobarbituric acid test to milk. J. Dairy Sci. 34:1064-1069.

Fletcher, B. L., C. J. Dillard, and A. L. Tappel. 1973. Measurement of fluorescent lipid peroxidation products in biological systems and tissues. Anal. Biochem. 52:1-9.

Fletcher, B. L., A. K. M. Reddy, C. J. Dillard, C. K. Chow, and A. L. Tappel. 1972. Fluorescence measurement of lipid peroxidation and the effect of dietary vitamine E on biochemical damage in rats exposed to $O_3$ and $NO_2$. Fed. Proc. 31:713.

Goller, H. J., D. S. Sgoutas, I. A. Ismail, and F. D. Gunstane. 1970. Dependence of sterol ester hydrolase activity on the position of ethylenic bond in cholesteryl cis-octadecenoates. Biochemistry 9: 3072-3076.

Gray, J. I. 1978. Measurement of lipid oxidation: A review. J. Am. Oil Chem. Cos. 55:539-546.

Greene, B. E., and B. M. Watts. 1966. Lipid oxidation in irradiated beef. Food Technol. 20:1087-1090.

Greene, B. E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food. Sc. 34:110-113.

Harfoot, C. G. 1978. Lipid metabolism in the rumen. Prog. Lipid Res. 17:25-54.

Holland, D. C. 1971. Determination of malonaldehyde as an index of rancidity in nut meats. J. Assoc. Off. Anal. Chem. 54:1024-1026.

Holman, R. T. 1954. Autoxidation of fats and related substances. In Holman, W. O. Lundberg, and T. Mallicin, eds. Progress in the chemistry of fats and other lipids. Academic Press, New York, NY.

Hornstein, I., P. F. Crowe, and M. J. Heimberg. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci. 26:581-586.

Hutchins, B. K., T. H. P. Liu, and B. W. Watts. 1967. Effects of additives and refrigeration on reducing activity, metmyoglobin and malonaldehyde of raw ground beef. J. Food Sci. 32:214-217.

Hüttel, R. 1941. Über Malonaldehyd. Berichte 74:1825.

Hwang, D. H., and J. E. Kinsella. 1979. The effects of trans-trans methyl linoleate on the concentration of prostaglandins and their precursors in rats. Prostaglandins 17:543-559.

Ingold, K. U. 1961. Inhibition of the autoxidation of organic substances in the lipid phase. Chem. Rev. 61:563-589.

Janicki, L. J., and H. Appledorf. 1974. Effect of broiling, grill frying and microwave cooking on moisture, some lipid components and total fatty acids of ground beef. J. Food Sci. 39:715-717.

Karel, M., and T. P. Labuza. 1967. Mechanisms of deterioration and formulation of space diets. AF Contract 49:609.

Kaunitz, H., and R. E. Johnson. 1973. Exacerbation of heart and liver lesions in rats by feeding of various mildly oxidized fats. Lipids 8:329-336.

Ke, P. J., B. A. Linke, and R. G. Ackman. 1978. Acceleration of lipid oxidation in frozen mackerel fillet by pretreatment with microwave heating. J. Food Sci. 43:38-40.

Keefe, R. T., and S. A. Goldblith. 1973. Hospital feeding via microwave kitchenettes -- a cost analysis. Food Technol. 27(8):16-23.

Keller, J. D., and J. E. Kinsella. 1973. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. J. Food Sci. 38:1200-1204.

Kenney, M., and R. Bassette. 1959. Detection of intermediate compounds in early stages of browning reaction in milk products. J. Dairy Sci. 42:945-960.

Kesinkel, A., J. C. Ayres, and H. E. Snyder. 1964. Determination of oxidative changes in raw meats by the 2-thiobarbituric acid method. Food Technol. 18:223-226.

Klenk, B. 1965. The metabolism of polyenoic fatty acids. J. Am. Oil Chem. Soc. 42:580-582.

Koch, R. B. 1956. Mechamisms of fat oxidation. Baker's Dig. 30(2): 48-53, 68, 71.

Koning, A. M., and M. H. Silk. 1963. The 2-thiobarbituric acid reagent for determination of osidative rancidity in fish oils. J. Am. Oil Chem. Soc. 40:167-169.

Korschgen, B. M., R. E. Baldwin, and S. Snider. 1976. Quality factors in beef, pork and lamb cooked by microwaves. J. Am. Diet. Assoc. 69:635-640.

Kuchmak, M., and L. R. Dugan, Jr. 1965. Composition and positional distribution of fatty acids in phospholipids isolated from pork tissues. J. Am. Oil Chem. Soc. 42:45-48.

Kummerow, F. A. 1974. Current studies on relation of fat to health. J. Am. Oil Chem. Soc. 51:255-259.

Kummerow, F. A. 1975. Lipids in atherosclerosis. J. Food Sci. 40:12-17.

Kurkhanova, V. M., and N. V. Onekhova. 1971. Determination of thiobarbituric acid number in fish meat. Issled. Technol. Ryb:73-78.

Kwon, T. W., and B. M. Watts. 1964. Malonaldehyde in aqueous solution and its role as a measure of lipid oxidation in foods. J. Food Sci. 29:294-304.

Kylen, A. M., B. H. McGrath, E. L. Hallmark, and F. O. Van Duyne. 1964. Microwave and conventional cooking of meat. J. Am. Diet. Assoc. 45:139-145.

Labuza, T. P. 1971. Kinetics of lipid oxidation in foods. C. R. C. Crit. Rev. in Food Technol. 355-405.

Lands, W. E. M., M. L. Blank, L. J. Nutter, and O. S. Privett. 1966. A composition of acyltransferase activities in vitro with the distribution of fatty acids in lecithins and triglycerides in vivo. Lipids 1:224-229.

Lands, W. E. M., M. E. Hemler, and C. G. Crawford. 1977. Functions of polyunsaturated fatty acids. Pages 193-228 in Wolf-H. Kunau, ed. Polyunsaturated fatty acids. Am. Oil Chem. Soc., Champaign, IL.

Landucci, J. M., J. Pouradier, and M. Durante. 1955. À propos de la réaction de l'acide 2-thiobarbiturique sur les tissus. Compt. Rend. Soc. Biol. 149:919-921

Lillard, D. A., and E. A. Day. 1964. Degradation of monocarbonyls from oxidizing lipids. J. Am. Oil Chem. Soc. 41:549-552.

Liu, H. P, and B. M. Watts. 1970. Catalysis of lipid peroxidation in meats. 3. Catalysts of oxidative rancidity in meats. J. Food Sci. 35:590-592.

Love, J. D. 1971. Lipid oxidation in meat and meat products -- a review. J. Am. Oil Chem. Soc. 48:547-549.

Love, J. D. 1972. A comparison of myoglobin and noniron as prooxidants in cooked meat and dispersions of phospholipid. Unpublished Ph.D. Dissertation. Michigan State University, East Lansing, MI.

McMillan, G. C., M. D. Silver, and B. I. Weigensberg. 1963. Elaidinized olive oil and cholesterol atherosclerosis. Arch. Pathol. 76:106-112.

Maga, J. A., J. A. Twomey, and M. Cohen. 1977. Effect of baking methods on the fatty acid composition of potatoes. J. Food Sci. 42:1669-1670.

Mai, J., C. H. Tsai, G. Armbruster, P. Chu, and J. E. Kinsella. 1980. Effects of microwave cooking on food fatty acids: No evidence of chemical alterations or isomerization. J. Food Sci. 45:1753-1744.

Malshet, V. G., and A. L. Tappel. 1973. Fluorescent products of lipid peroxidation: 1. Structural requirement for fluorescence in conjugated Schiff bases. Lipids 8:194-198.

Marcuse, R., and L. Johansson. 1973. Studies on the TBA test for rancidity grading: 2. thiobarbituric acid reactivity of different aldehyde classes. J. Am. Oil Chem. Soc. 50(10):387-391.

Marnett, L. J., and M. A. Tuttle. 1980. Comparison of the mutagenicities of malonaldehyde and the side products formed during its chemical synthesis. Cancer Res. 40:276-282.

Marshall, N. 1960. Electronic cookery of top round of beef. J. Home Econ. 52:31-34.

Meyers, S. J., and N. D. Harris. 1975. Effect of electronic cooking on fatty acids in meats. J. Am. Diet. Assoc. 67:232-234.

Mills, S. C., T. W. Scott, G. R. Russell, and R. M. Smith. 1970. Hydrogenation of $C_{18}$ unsaturated fatty acids by pure cultures of a rumen micrococcus. Aust. J. Biol. Sci. 23:1109-1113.

Mohrhauer, H., and R. T. Holman. 1963. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. Lipid Res. 4:151-159.

Moody, W. G., C. A. Bedau, and B. E. Langlois. 1978. Beef thawing and cookery methods: Effect of thawing and cookery methods, time in storage and bread on the microbiology and palatability of beef cuts. J. Food. Sci. 43:834-838.

Moore, L. J., D. L. Harrison, and A. D. Dayton. 1980. Differences among top round steaks cooked by dry or moist heat in a conventional or a microwave oven. J. Food Sci. 45:777-781.

Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600-608.

Mukai, F. H., and B. D. Goldstein. 1976. Mutagenicity of malonaldehyde, a decomposition product of polyunsaturated fatty acids. Science 191:868-869.

Newburg, D. S., and J. M. Concon. 1980. Malonaldehyde concentrations are affected by cooking conditions. J. Food Sci. 45:1681-1683.

Patton, S., and G. W. Kurtz. 1951. 2-thiobarbituric acid as a reagent for detecting milk-fat oxidation. J. Dairy Sci. 34:669-674.

Patton, S., 1960. Response of epihydrin aldehyde and glyceraldehyde in the TBA test for fat oxidation. Food Res. 25:554-556.

Pearson, A. M., J. D. Love, and F. B. Shorland. 1977. "Warmed-over" flavor in meat, poultry and fish. Adv. in Food Res. 23:1-74.

Pein, G. 1964. Distillation method for the determination of the shelf-life of whole grain products. Brot Bebaeck 18:12-14.

Penner, K. K., and J. A. Bowers. 1973. Flavor and chemical characteristics of conventionally and microwave reheated pork. J. Food Sci. 38:553 -555.

Porter, W. L., A. M. Wetherby, and J. G. Kapsalis. 1980. Fluorescence test of oxidative abuse status of fats, oils and dry whole tissue. J. Am. Oil Chem. Soc. 57(2):497.

Privett, O., and M. L. Blank. 1964. Studies on the metabolism of linoelaidic acid in the essential fatty acid deficient rat. J. Am. Oil Chem. Soc. 41:292-300.

Privett, O. S., F. Phillips, H. Shimasaki, T. Nozawa, and E. Nickell. 1977. Studies of effects of trans fatty acids in the diet on lipid metabolism in essential fatty acid deficient rats. Am. J. Clin. Nutr. 30:1009-1017.

Purdy, R. E., and A. L. Tappel. 1979. Permeation chromatography of fluorescent products from tissues and peroxidized lipids. J. Chrom. 170:217-220.

Rawls, H. R., and P. J. Van Saten. 1971. A possible role for singlet oxygen in the initiation of fatty acid antoxidation. J. Amer. Oil Chem. Soc. 47:121-125.

Rawls, H. R., and P. J. Van Saten. 1970. Singlet oxygen: A possible source of original hyperoxides in fatty acids. Ann. N.Y. Acad. Sci. 171:135-137.

Reiser, R. 1951. Hydrogenation of polyunsaturated fatty acids by the ruminant. Fed. Proc. 10:236.

Rosen, C. G. 1972. Effects of microwave on food and related material. Food Technol. 26(7):36-40, 55.

Sawicki, E., T. J. Stanley, and H. Johnson. 1963. Comparison of spectrophotometric and spectorfluorometric methods for the determination of malonaldehyde. Anal. Chem. 35:199-204.

Sawicki, E., and C. R. Sawicki. 1975. Malonaldehyde. Pages 115-137 in E. Sawicki, and C. R. Sawicki, eds. Aldehyde-photometric analysis. Vol. 2. Academic Press, London.

Sato, K., and G. R. Hegarty. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36:1098-1102.

Schultz, H. W., E. A. Day, R. O. Sinuhuber. 1962. Symposium on foods: Lipids and their oxidation. The AVI Publishing Co., Inc., Westport, CT.

Sedlocek, B. 1964. Rancidity of fats by ultraviolet spectrophotometry and other methods. Nagrung 8:176-187.

Sgoutas, D. S. 1968. Hydrolysis of synthetic cholesterol esters containing trans fatty acids. Biochim. Biophys. Acta 164:317-326.

Sgoutas, D. S. 1970. Effect of geometry and position of ethylenic bond upon acyl coenzyme A-cholesterol-O-acryltransferase. Biochemistry 9:1826-1833.

Sgoutas, D. S., and F. A. Kummerow. 1969. Cis-trans isomerization of unsaturated fatty acid methyl esters without double bond migration. Lipids 4:283-287.

Sgoutas, D. S., and F. A. Kummerow. 1970. Incorporation of trans fatty acids into tissue lipids. Am. J. Clin. Nutr. 23:111.

Shamberger, R. J., T. L. Andreone, and C. E. Willis. 1974. Antioxidants and cancer. IV. Malonaldehyde has initiating activity as a carcinogen. J. Nat. Cancer Inst. 53:1771-1773.

Shamberger, R. J. 1977. Antioxidants and cancer. Presence of malonaldehyde in beef and othermeats and its epidemiological significance. In Trace Substances in Environmental Health. Vol. 27. University of Missouri Press, Columbia, MO.

Shamberger, R. J., B. A. Shamberger, and C. E. Willis. 1977. Malonaldehyde content of food. J. Nutr. 107:1404-1409.

Shorland, F. B., R. O. Weenink, and A. T. Johns. 1955. Effect of the rumen on dietary fat. Nature, London 175:1129-1130.

Siakotos, A. N., N. Koppang, B. S. Youmans, and C. Bucana. 1974. Blood levels of α-tocopherol in a disorder of lipid peroxidation: Batten's disease. Am. J. Clin. Nutr. 27:1152-1157.

Sidwell, C. G., H. Salwin, and J. H. Mitchel, Jr. 1955. Measurement of oxidation in dired milk products with thiobarbituric acid. J. Am. Oil Chem. Soc. 32:13-16.

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

Sinnhuber, R. O., T. C. Yu, and Y. T. Chang. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23:626-633.

Siu, G. M., and H. H. Draper. 1978. A survey of the malonaldehyde content of retail meats and fish. J. Food Sci. 43:1147-1149.

Stoffel, W., and E. G. Ahrens. 1959. The unsaturated fatty acids in menhaden body oil. The $C_{18}$, $C_{20}$ and $C_{22}$ series. J. Lipid Res. 1: 139-146.

Swern, D. 1961. Primary products of olefinic autoxidations. Pages 1-54 in W. O. Lundberg, ed. Autoxidation and antioxidants. Vol. 1. Interscience Publishers, Inc., New York, NY.

Tappel, A. L. 1952. Linoleate oxidation catalysed by hog muscle and adipose tissue extracts. Food Res. 17:550-559.

Tappel, A. L. 1972. In B. F. Trump and A. Arstila, eds. Pathological aspects of cell membranes. Academic Press, New York, NY.

Tappel, A. L. 1953. Linoleate oxidation catalyst occurring in animal tissues. Food Res. 18:104-108.

Tappel, A. L., and H. Zalkin. 1959. Lipid peroxidation in isolated mitochondria. Arch. Biochem. Biophys. 80:326-332.

Tarladgis, B. G., B. M. Watts, M. T. Younathan, and L. Dugan, Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44-48.

Tarladgis, B. G., A. M. Pearson, and L. R. Dugan, Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39:34-39.

Takatori, T., F. Phillips, and O. S. Privett. 1976. Effects of dietary saturated and trans fatty acids on cholesteryl ester synthesis and hydrolysis in the testes of rats. Lipids 11:357-363.

Terao, J., and S. Matsushita. 1977. Products formed by photosensitized oxidation of unsaturated fatty acid esters. J. Am. Oil Chem. Soc. 54(6):234-238.

Thermador/Waste King Division of Norris Industries. 1978. Comparison of reduced ascorbic acid content of microwave and conventional cooked fruits and vegetables. Final Report. Author, 5119 District Blvd., Los Angeles, CA.

Thomas, M. J., and W. A. Pryor. 1980. Singlet oxygen oxidation of methyl linoleate: Isolation and characterization of the $NaBH_4$-reduced products. Lipids 15(7):544-548.

Timms, M. J., and B. W. Watts. 1958. Protection of cooked meats with phosphates. Food Technol. 12:24-243.

Tsen, C. C., P. R. K. Reddy, and C. W. Gehrke. 1977. Effects of conventional baking, microwave baking and steaming on the nutritive value of regular and fortified breads. J. Food Sci. 42:402.

Turner, E. W., W. D. Paynter, E. T. Montie, M. W. Bessert, G. M. Struck, and F. C. Olson. 1954. Use of the 2-thiobarbituric acid reagent to measure rancidity in frozen pork. Food Technol. 8:326.

U.S.D.A. 1971. Civilian consumption of visible and invisible food fats per person. U. S. Fats and Oils Statistics, 1950-1971. 1959-71-ERS/USDA Statistical Bulletin 489.

Viviani, R. 1970. Metabolism of long-chain fatty acids in the rumen. Adv. Lipid Res. 8:278-346.

Ward, P. F. V., T. W. Scott, and R. M. C. Dawson. 1964. The hydrogenation of unsaturated fatty acid in the ovine digestive tract. Biochem. J. 92:60-68.

Watts, B. M. 1961. Pages 83-96 in Campbell flavor chemistry symposium. Campbell Soup Co., Camden, NJ.

Watts, B. M. 1962. Meat products. Pages 202-214 in H. W. Schultz, E. A. Day, and R. C. Sinnhuber, eds. Lipids and their oxidations. The AVI Publishing Co., Westport, CT.

Wertheim, J. H., and B. E. Proctor. 1956. Radiation preservation of milk and milk products. III. The TBA test as a means of evaluating radiation induced changes in milk. J. Dairy Sci. 39:391-401.

Wing, R. W., and J. C. Alexander. 1972. Effect of microwave heating on Vitamin $B_6$ retention in chicken. J. Am. Diet. Assoc. 61:661-664.

Wood, R. D., M. C. Bell, R. B. Grainger, and R. A. Teekel. 1963. Metabolism of labelled linoleic-1-$C^{14}$ acid in the sheep rumen. J. Nutr. 79:62-68.

Woodrow, I. L., and J. M. Deman. 1968. Distribution of trans fatty acids in milk fat. Biochim. Biophys. Acta 152:472-478.

Wright, D. E. 1959. Hydrogenation of lipids by rumen protozoa. Nature, London 184:875-876.

Wright, D. E. 1960. Hydrogenation of chloroplast lipids by rumen bacteria. Nature, London 185: 546-547.

Younathan, M. T., and B. M. Watts. 1959. Relationship of meat pigments to lipid oxidation. Food Res. 24:728-734.

Yu, T. C., J. Mai, and J. E. Kinsella. 1980a. Effects of dietary trans trans methyl octadecadienoate acid on composition and fatty acids of rat-heart. Am. J. Clin. Nutr. 33:598-605.

Yu, P., J. Mai, and J. E. Kinsella. 1980b. The effects of dietary 9-trans, 12-trans-octadecadienoate on composition and fatty acids of rat lungs. Lipids 15:975-979.

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

Ziprin, Y. A., and A. F. Carlin. 1976. Microwave and conventional cooking in relation to quality and nutritive value of beef and beef-soy loaves. J. Food Sci. 41:4-8.

Zipser, M. W., and B. M. Watts. 1962. A modified 2-thiobarbituric acid method for the determination of malonaldehyde in cured meats. Food Technol 16:102-104.

Zipser, W. W., and B. M. Watts. 1967. Ascorbate and tripolyphosphate in cured, cooked, frozen pork. J. Agric. Food Chem. 15:80-82.

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# 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 ± .02[c] | | | .44 ± .02[c] |
| Microwave Cooked[b] | 3.79 ± | | | .51[d] |
| Conventionally Cooked[b] | 4.21 | | | 60[e] |
| Turkey Breast | | | | |
| Raw | | | | |
| Microwave Cooked[b] | | | | |
| Conventionally Cook | | | | |
| Beef | | | | |
| Raw | | | | |
| Microwave Cooked[b] | | | | |
| Conventionally Coo | | | | |
| 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 ± .02[c,d] | 0.41 ± .02[c,d] | 0.44 ± .03[c,d] | 0.44 ± .02[c,d] |
| Microwave Cooked[b] | 3.79 ± .11[d,e] | 11.81 ± .41[d,e] | 14.95 ± .44[d,e] | 16.89 ± .51[d,e] |
| Conventionally Cooked[b] | 4.21 ± .19[e] | 10.74 ± .40[e] | 14.10 ± .50[e] | 15.80 ± .60[e] |
| Turkey Breast | | | | |
| Raw | 0.31 ± .02[c] | 0.39 ± .01[c] | 0.39 ± .03[c] | 0.41 ± .03[c] |
| Microwave Cooked[b] | 3.79 ± .09[d] | 8.11 ± .39[d] | 9.95 ± .48[d] | 10.75 ± .42[d] |
| Conventionally Cooked[b] | 3.81 ± .23[e] | 7.20 ± .33[e] | 9.00 ± .39[e] | 9.60 ± .41[e] |
| Beef | | | | |
| Raw | 0.36 ± .04[c] | 0.39 ± .02[c] | 0.40 ± .03[c] | 0.40 ± .02[c] |
| Microwave Cooked[b] | 1.62 ± .08[d] | 4.01 ± .21[d] | 5.55 ± .25[d] | 6.10 ± .19[d] |
| Conventionally Cooked[b] | 2.01 ± .13[e] | 4.12 ± .20[e] | 5.20 ± .18[e] | 5.65 ± .21[e] |
| Pork | | | | |
| Raw | 0.35 ± .03[c,d] | 0.42 ± .01[c,d] | 0.41 ± .01[c,d] | 0.52 ± .02[c,d] |
| Microwave Cooked[b] | 4.21 ± .30[d,e] | 9.47 ± .57[d,e] | 12.04 ± .61[d,e] | 13.00 ± .59[d,e] |
| Conventionally Cooked[b] | 4.40 ± .27[e] | 8.37 ± .40[e] | 10.47 ± .51[e] | 11.78 ± .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.