# THE INFLUENCE OF COOKING AND STORAGE ON OXIDATIVE CHANGES IN FREEZE-DRIED BEEF AS EVALUATED BY THE 2-THIOBARBITURIC ACID TEST

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By

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# ABSTRACT

## THE INFLUENCE OF COOKING AND STORAGE ON OXIDATIVE CHANGES IN FREEZE-DRIED BEEF AS EVALUATED BY THE 2-THIOBARBITURIC ACID TEST

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This study was undertaken to measure changes in the deterioration of freeze-dried cooked beef by the 2-thiobarbituric acid (TBA) test. Uncooked beef, and beef cooked in an ordinary oven, an electronic oven, and in boiling water were freeze-dried for 20 hours before storage in aluminum foil and in stoppered glass bottles.

Thiobarbituric acid (TBA) tests, moisture determinations, and odor evaluations were conducted throughout an eight week period on samples from the various methods of cooking preparation and storage to find possible correlations. Interrelationships were found to exist between keeping quality as expressed by TBA numbers and odor evaluations and between moisture contents and method of storage. Freeze-dried cooked beef, when measured by four different TBA methods, was observed to reach its maximum TBA value early in the storage study.

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# INTRODUCTION

Freeze-drying, as a method of food preservation, has generated considerable interest in the last 10-15 years. Among the dehydration methods applicable to foods, freeze-drying is an ideal method for the maintenance of desirable functional and palatability characteristics. It has been considered the best means of dehydrating meat without losses in quality. Initially, freeze-dried meat possesses a number of ideal quality characteristics, but the deterioration can be quite excessive under adverse conditions or extended storage. Unsaturated lipids are the only well-known oxygen-labile reactants present in meat which could readily account for the rapid and large oxygen absorption noted.

Detection of lipid oxidation in foods organoleptically is considered the most sensitive and reliable method, though it does not lend itself well to quantitative measurements. In recent years the 2-thiobarbituric acid (TBA) test has been widely used for measuring oxidative rancidity changes in foods containing unsaturated fatty acids. It can be performed directly on the food without the necessity of extracting the fat. It has been reported that the TBA method is a sensitive test for the decomposition products of highly unsaturated fatty acids and the extent of the reaction has been correlated with off odors and flavor losses.

A search of the literature failed to uncover any mention of a successful application of the TBA test to freeze-dried meat. This study was designed to determine the applicability of the TBA test to beef that was uncooked, broiled in an ordinary oven, boiled in water, and cooked by microwaves in an electronic oven prior to freeze-drying and storage in aluminum foil and in stoppered glass bottles. Moisture analyses and odor evaluations were conducted to discover if they had any correlation with oxidative rancidity as measured by the TBA test.

# REVIEW OF LITERATURE

## Measurement of Oxidative Rancidity

Detection of lipid oxidation in foods organoleptically is considered the most sensitive and reliable method, though it does not lend itself well to quantitative measurements (19). Methods are now available for an objective determination of rancidity and of stability. The methods for measuring oxidative rancidity in fats have been discussed in reviews by Watts (49) and Dugan (7). √

The most commonly used methods for assessing stability or rancidity of a fat or fatty foods are the: active oxygen method, Schaal oven test, thiobarbituric acid test, carbonyl test, and peroxide value (7). Of these, the most widely used has been the peroxide value. √

Organoleptic changes which develop during storage in muscle tissues can be correlated with rancidity when assays are performed on the whole tissue (52), but not when fat alone, extracted with the usual solvents, is used (47, 48). It appears, therefore, that bound lipids are contributing markedly to the reactions responsible for rancid odors and flavors in cooked meats (53). Protein bound phospholipids are known to be important food constituents involved in the deteriorative reactions which take place during processing and storing (24). These substances are among the more labile components of foods but their role in the deterioration of only a few commodities, such as eggs and milk, have been studied (24). Little is known about their contributions to spoilage mechanisms in muscle tissues (53).

In recent years, the use of the 2-thiobarbituric acid test has become rather widespread. It can be performed directly on the meat tissue without the necessity of extracting the fat. It is a sensitive test for the decomposition products of highly unsaturated fatty acids (18).

## Application of the TBA Test

The TBA test has been widely used for oxidative changes in various foods. Many workers have applied this reaction to the estimation of rancidity in dairy products (3, 8, 9, 14, 17, 19, 27, 28, 34, 50), meats (37, 39, 45, 46, 52, 53, 58), fishery products (12, 15, 25, 31, 35, 54, 56, 57), cereal and baked products (4, 39), and fats and oils (6, 13, 14, 21, 30, 33, 36, 39, 40, 51).

## Evolution and Theory of the TBA Test

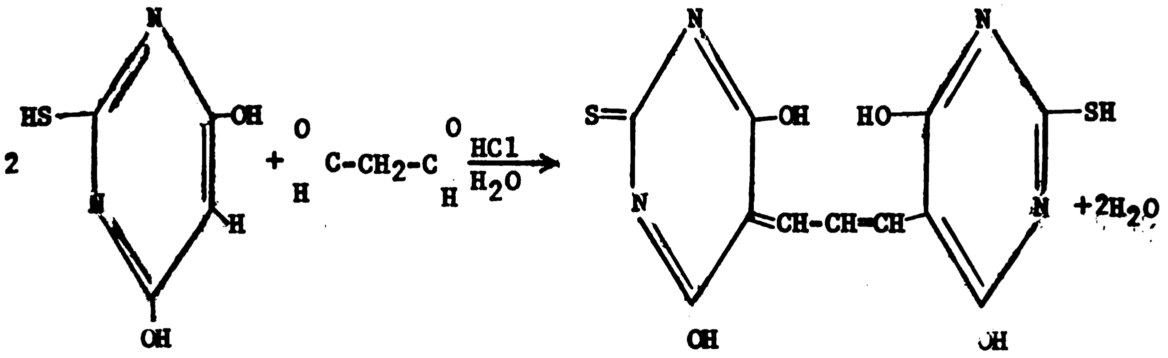
In 1944 Kohn and Liversedge (20) observed that animal tissues which had been incubated aerobically gave a color with 2-thiobarbituric acid (TBA). They suggested that the TBA reactive material was a carbonyl compound since the reaction was blocked by semicarbazide or phenylhydrazine. Bernheim, Bernheim, and Wilbur (2) concluded that the colors obtained by Kohn and Liversedge (20) were due to an oxidation product of unsaturated fatty acids, particularly linolenic acid. Wilbur, Bernheim, and Shapiro (51) explored the TBA color reaction with regard to certain sugars and aldehydes, as well as the oxidation products of linolenic and certain other unsaturated fatty acids. These studies revealed no specific compound which gives a color spectrum identical with those obtained from oxidized lipid materials or aerobically incubated animal tissues. Bernheim _et al_. (2) reported the isolation of an impure red TBA pigment, which contained some inorganic residue but no organic phosphate. On the basis of a combustion analysis, corrected for inorganic residue, it was suggested that a three carbon chain containing one oxygen atom may have added to the thiobarbituric acid molecule.

Patton and Kurtz (27) found malonic dialdehyde gave a red color when heated with TBA and spectral analysis of this color revealed it to be identical with the color obtained similarly from oxidized milk fat and to closely resemble the colors obtained with oxidized animal tissues. Kurtz, Price and Patton (22) tentatively identified the compound that is responsible for the results of the thiobarbituric acid test in oxidized milk fat to be malonaldehyde and postulated that the reaction occurred by attack of the monoenolic form of malonaldehyde on the active methylene group of thiobarbituric acid, followed by ring closure.

Patton, Keeney, and Kurtz (26) in a paper regarding the Kreis color reaction for rancid fats pointed out the similarity between epihydrin aldehyde and malonaldehyde. They reported that the TBA reactive material from oxidized milk fat was a water soluble, low molecular weight, Kreis positive, carbonyl compound similar to malonaldehyde. In addition, they postulated that malonaldehyde would be strongly acidic, enolic, and relatively stable on heating with dilute mineral acids. These properties are not expected of epihydrin aldehyde but would be expected of malonaldehyde. They were unable to demonstrate the actual existence of either compound as the free aldehyde in oxidized fat.

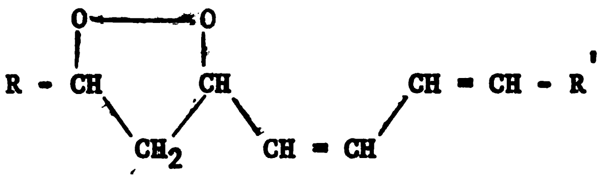
Schmidt (30), Shepherd (32), and Sinnhuber, Yu and Yu (36) have shown that certain pyrimidines are capable of forming the same pigment as malonaldehyde on reaction with TBA. The mechanism is thought to involve hydrolysis of the pyrimidine ring to produce an oxyacrolein intermediate (30), which is tautomeric with malonaldehyde and therefore can undergo condensation to produce the TBA pigment. Although TBA itself has a pyrimidine structure, it is substituted in the 4 and 6 positions and would therefore be unlikely to give rise to malonaldehyde on hydrolysis (30).

Sinnhuber and Yu (35) used 1,1,3,3-tetra-ethoxypropane (TEP) as a standard for the quantitative determination of malonaldehyde with TBA. Acid hydrolysis of this acetal yields malonaldehyde. It was therefore possible to express their results in terms of the "TBA number", defined as mg. of malonaldehyde per 1,000 g. of sample. Sinnhuber et al. (36) prepared a crystalline TBA pigment from rancid salmon oil and the acid hydrolysis product of TEP. Results obtained by elemental analyses, absorption spectrophotometry, and paper chromatography suggested that the pigments were identical. Only 2% of the malonaldehyde measured in the rancid salmon oil could be extracted in the free form, the rest apparently being bound in some form that was released upon heating in acid medium. Their data indicated that the crystalline TBA pigment was a condensation product of one molecule of malonaldehyde with two molecules of TBA with the probable elimination of two molecules of water.



TBA malonaldehyde TBA pigment

Dahle, Hill, and Holman (6) measured the TBA reaction of autoxidized single methylene - interrupted diene, triene, tetraene, pentaene, and hexaene fatty acid esters at various stages of autoxidation. They found that as the degree of unsaturation increased, the molar yield of TBA color (malonaldehyde) increased. They proposed a mechanism for TBA reactant formation from methylene-interrupted polyunsaturated compounds with a cyclic five-membered ring peroxide



as an intermediate in the formation of malonaldehyde.

The cyclic peroxide would appear only in the oxidation of methylene-interrupted polyunsaturated systems with three or more double bonds as the result of the cyclization of β,γ unsaturated peroxide radicals. Dahle et al. (6) observed that linolenate gave a TBA color but that oxidized linoleate, which they suggested could form only α, β and not β,γ unsaturated peroxide radicals, gave a slight color and only then at peroxide values over 3,000. They explained this slight color as occurring from autoxidation of α,β-unsaturated aldehydes, breakdown products of hydroperoxides (27). Kenaston, Wilbur, Ottolenghi, and Bernheim (18) and Wilbur *et al.* (51) obtained a low TBA color for oxidized linoleate but attributed it to linolenate impurity in the samples used.

Tarladgis and Watts (40) reported TBA color was developed during the autoxidation of oleate, linoleate, linolenate, and arachidonate. However, the system they employed for autoxidation included phosphate buffer, Tween 20 emulsifier and Cu++ as catalyst; and in their study of oleate, linoleate was added to the sample. Patton and Kurtz (27) found α,β-unsaturated aldehydes gave a TBA color in the presence of copper, but not in the absence of Cu++ until after a long period of autoxidation. This phenomenon could account for most of the TBA color from oxidized oleate and linoleate in the experiments of Tarladgis and Watts, for the α,β-unsaturated aldehydes would appear as breakdown products in the experiments of the latter (17, 18).

## Limitations of the TBA Test

Almost all methods employing the TBA test, utilize additional acid besides TBA. Tarladgis, Pearson, and Dugan (42) ascertained by UV, visible, and IR spectra as well as by paper and column chromatography that the structure of TBA is altered upon acid-heat treatment. Such findings prompted Tarladgis, Pearson and Dugan (43) to introduce a new method for performing the TBA test in rancid food products without the need of acid and/or heat. They demonstrated that malonaldehyde, or the compounds responsible for the 530 mμ absorption peak upon reaction with

TBA, produced the typical reaction colors without the need of acid or heat. Their method was reported to be 10-100 times more sensitive than those involving acid or heat.

The principal difficulty encountered in testing materials other than pure fats with 2-thiobarbituric acid (TBA) is the formation of yellow interfering pigments (4). The pink color formed when rancid foods react with TBA has an absorption maximum at 530-535 mμ. However, interfering yellow and orange colors with absorption maxima at 450-460 mμ are frequently observed in TBA reaction solutions. Wilbur et al. (51) and Biggs and Bryant (3) used the TBA test to measure lipid oxidation, and attributed the yellow color to carbohydrate interference and showed that the yellow interfering color had an absorption maximum at about 450-460 mμ. Schmidt (30) described the yellow reaction product of TBA and formic acid and found that the absorption maximum was at 450 mμ.

Patton (29) found that glyceraldehyde and epihydrin aldehyde gave yellow pigments with an absorption maximum at 458 mμ after heating with an acid TBA solution. Keeney and Bassette (17), from the study of the browning reaction of milk products, reported that yellow pigments were formed by TBA and furfural compounds. Smith, Tinsley, and Buhl (37) described the two pigments that resulted when irradiated beef was heated with TBA as a glyoxal-TBA compound and a malonaldehyde-TBA compound. Landucci, Pouradier, and Durante (23) found that glycolic aldehyde, glyceric aldehyde, and dihydroxyacetone after reaction with TBA each gave an absorption maximum at 455 mμ.

Tarladgis and Watts (40) reported that the distillates from certain foods and from unsaturated fatty acids contain a compound that reacts with TBA to give a peak at 450 mμ. Koning and Silk (21) carried out the TBA determination on fish oils in a monophase system of ethyl alcohol to overcome errors inherent in the existence of a two-phase system between oil and water. They concluded that the reaction should run in the dark because daylight reduced the intensity of the TBA-malonaldehyde peak at 532 mμ and caused the appearance of a second maximum at 452 mμ.

Taüfel and Zimmerman (44) reported that the presence of ferric ion greatly increased the intensity of the yellow color formed by the reaction of TBA with aldehydes when the reactions were carried out at 70°C instead of the customary 100°C. Jacobson, Kirkpatrick, and Goff (13) used a single-phase solvent system consisting of iso-octane, n-propanol, and water to evaluate the relative potential usefulness of the absorption peaks at 452 and 532 mμ in the measurement of undesirable aldehyde flavors of fats. It was found that when the test was performed at 60°C, the yellow color or 452 mμ absorption peak became quite prominent, while the pink color representing the absorption at 532 mμ wavelength was considerably less intense at 60°C. Since the absorption spectra overlap each other, the determination of malonaldehyde in certain foods at 530-535 mμ will lead to erroneously high values.

Caldwell and Grogg (4) applied the TBA test to the petroleum ether extract of cereal and baked products, and removed the yellow interfering color by selective adsorption on a cellulose column. They eluted the pink color with pyridine and conducted the color measurement in this solvent. Yu and Sinnhuber (55) described a procedure using cellulose to separate the yellow interfering color from the red TBA pigment by two alternative methods which enabled them to obtain quantitative measurements of both pigments. The red TBA pigment was purified on a cellulose column by first washing away the yellow pigment with HCl and then eluting with NaOH. Determination of absorbance indicated that recovery of the red solution was 96.4% of the original concentration. The preferential adsorbance of cellulose for the red pigment allowed the yellow pigment to be separated, and its degree of interference measured by repeated shakings of the TBA reaction mixture with cellulose.

# EXPERIMENTAL METHODS AND PROCEDURE

## Preparation of Beef and Freeze-Drying

Beef (commercial grade, inside top round) was obtained from the Michigan State University Food Stores. It was cut into slices of approximately one-half an inch thick with a band saw. The beef was divided into four lots and each was treated in one of the following ways prior to freeze-drying:

I. The raw beef was diced into pieces of about 1" x 3/4" x 1/2".

II. The beef was broiled in an ordinary oven at 500°F for 10 minutes, turned over, and broiled at 250°F for 5 minutes. Then cubes of a size similar to the raw meat were cut.

III. The beef slices were placed in an electronic oven for 4 minutes (2 minutes on each side) before being diced.

IV. The beef was dropped into boiling water and cooked for 30 minutes at medium speed on an electric range. Then it was cut into pieces.

All four samples were frozen as single layers on trays at -20°F and then were freeze-dried concurrently in a Stokes freeze drier (Model 2003F-2, lot P65691, serial P65753) for 20 hours. During the final 16 hours of freeze-drying the gauge controlling the heating plate was set at 150 which was equivalent to a temperature of 42°C. The internal vacuum was observed to fall to a minimum pressure of 150 microns. After 20 hours had elapsed, the beef was removed from the freeze drier to be stored in aluminum foil and in stoppered glass bottles. The samples were kept in a laboratory drawer which had an average temperature throughout the storage period of 24.5°C.

## Measurement of Rancidity

Thiobarbituric acid (TBA) tests, moisture determinations, and odor analyses were run at intervals throughout an eight week period on freeze-dried beef samples from the various preparations and storages to find possible correlations. Readings were taken immediately after the beef was removed from the freeze drier.

The TBA method employed was the non acid-heat 15 hour treatment of Tarladgis *et al*. (43). In addition, an effort was made to compare different methods of measuring the TBA reaction on freeze-dried cooked beef stored in aluminum foil at 24.5°C over a period of 30 days. TBA determinations were performed by the 15 hour treatment, the distillation method of Tarladgis, Watts, Younathan, and Dugan (39) and by modifications of methods of Sinnhuber and Yu (35), and Jacobson *et al*. (3).

### Procedure for non-acid-heat 15 hour treatment.

A 10 g. sample of freeze-dried beef was blended with 80 ml. of distilled water in a Waring Blendor for 2 minutes at high speed. (The volume of distilled water was increased from 50 ml. as suggested by Tarladgis to 80 ml. in order to get better mixing from the Waring Blendor that was used.) The slurry was transferred quantitatively into a Buchner funnel by washing with an additional 20 ml. of distilled water. The meat residue was discarded and 1 g. of $(NH_4)_2SO_4$ was added to the filtrate which was again filtered, this time into a 100 ml. volumetric flask. Distilled water was added to complete the volume to 100 ml. Five ml. of the filtrate were pipetted into a glass vial and 5 ml. of 0.02M TBA in water were added. The vial was stoppered, and the mixture was allowed to stand for 15 hours in the dark at room temperature. The absorbancy was read with a Beckman DU spectrophotometer at 452 mμ and 530 mμ against a TBA reagent blank.

Distillation method procedure.

The procedure used was the same as that of Tarladgis *et al.* (39) except once again the volume of water used in blending was increased to obtain a better slurry. The final volume of liquid added before distillation was still 100 ml. The absorbances of the TBA reaction solutions were read at 452 mμ in addition to 538 mμ.

Sinnhuber and Yu procedure.

Pieces of freeze-dried beef were ground with a mortar and pestle and 0.25 g. of the mixture was weighed on an analytical balance. Then the method was followed as outlined by Sinnhuber and Yu. In addition to measurement of the absorbance at 535 mμ, a reading was also taken at 452 mμ.

Jacobson procedure.

Five g. of freeze-dried beef were mixed with 50 ml. of a reaction solution consisting of 50 parts of iso-octane, 27 parts n-propanol, and 3 parts of water in a Waring Blendor for 2 minutes at high speed. The slurry was transferred quantitatively into a Buchner funnel with a spatula. The meat residue was discarded and the filtrate obtained was filtered into a 50 ml. volumetric flask. More reaction solution was added to complete the volume to 50 ml. (The weight of the meat sample and the volume of the subsequent extract were exactly one half the amount of the 15 hour and distillation methods. Because of this, the volume of reaction solution needed was reduced.)

A 5 ml. aliquot of the extract was added into a test tube, followed by 5 ml. of 0.02M TBA dissolved in ethanol. The tubes were shaken unstoppered for a few minutes. After shaking the tubes were stoppered and heated at 60°C in a constant temperature water bath for 30 minutes. After cooling, the absorbances of the solutions were read at 452 mμ and 532 mμ with the Beckman DU against a blank consisting of the solvent mixture and TBA solution.

### Purification of TBA.

To 5 g. of TBA in a 400 ml. beaker was added 200 ml. of distilled water. The mixture was heated to 80°C to dissolve the TBA. Activated charcoal was added and the solution was filtered through a Buchner funnel. The filtrate was returned to a beaker placed in an ice bath in order to recrystallize the TBA. Then the contents of the beaker were filtered with a Buchner funnel. The filtration apparatus was allowed to run for one hour to give the TBA crystals time to dry. The TBA was stored in a brown bottle in the freezer until it was made into solution. The TBA reagent was freshly prepared in every case, on the day that it was used.

### Separation of Pigments by a Chromatographic Column

A Pyrex chromatographic tube 300 x 10 mm was packed with an aqueous slurry of Whatman standard grade cellulose powder to a height of about 12 cm. using nitrogen pressure. A small wad of glass wool was placed over the open end of the capillary. Five ml. of the TBA reaction solution were carefully added to the column. After the solution had passed onto the column, 2 ml. of phenol solution (phenol 5 g, ethanol 15 ml, water 25 ml) were added. The column was washed with 0.1N HCl until all the yellow pigment had been removed. Finally, 0.1N NaOH was added to the column to elute the pink colored pigment. When the pink band was 2-3 cm above the bottom of the column, collection of the eluate was begun and it was collected to the mark in a 10 ml volumetric flask containing 1 ml of 1.2N HCl. After thorough mixing, the absorbance was measured at 530 mμ with a Beckman DU spectrophotometer and multiplied by 2 to compensate for dilution.

## Moisture Analysis

The freeze-dried beef samples were ground thoroughly with a mortar and pestle. No less than three cubes were selected for the determination as the difficulty in obtaining a sample representative of the whole was recognized. Approximately 3-4 g. of the ground meat were added to a 100 ml beaker that had been heated in an oven, cooled in a desiccator, and weighed on an analytical balance. The beaker and its contents were weighed on the analytical balance and put in a drying oven at a temperature of 105°F for 48 hours. After cooling for 30 minutes, the beaker was reweighed and the weight loss was considered to be due to loss of water. The decrease in sample weight, multiplied by 100, and divided by the initial sample weight gave the moisture content in percent.

## Organoleptic Evaluation (ODOR)

The freeze-dried beef was sliced into small pieces and rehydrated with distilled water before being presented to a panel. The panel, consisting of 6-8 judges, rated the intensity of rancid odor on the following scale: not detectable, just detectable, moderate, moderately strong, strong, and very strong (5). Numerical values of 1-6 (from not detectable to very strong) were assigned to the ratings given by the judges, and average sensory scores were calculated.

## RESULTS AND DISCUSSION

### Effect of Rehydration on Measurement of TBA Values

When a food product is analyzed for rancidity by the non acid-heat treatment of Tarladgis *et al*. (43), it is macerated with solvent in the Waring Blendor. Since a high percentage of moisture is removed from meat during the course of freeze-drying, an investigation was made of the effect of reconstitution, to determine the importance of replacing the water that was lost, before performing a TBA analysis. Three month-old samples of freeze-dried beef were allowed to stand for 10 minutes in water, in chloroform, and in a 1:1 mixture of chloroform-methanol. The solvents were held at room temperature. TBA control tests were run in each solvent with rehydration.

Table I. Effect of rehydration on TBA test.

| | ABSORBANCE | | | | | |
|---|---|---|---|---|---|---|
| | With rehydration | | | Without rehydration | | |
| Solvent | 452 mμ | 530 mμ | *530 mμ | 452 mμ | 530 mμ | *530 mμ |
| Water | .147 | .085 | .066 | .168 | .103 | .074 |
| Chloroform | .088 | .061 | .070 | .104 | .068 | .088 |
| Methanol-Chloroform (1:1) | .124 | .089 | .113 | .197 | .141 | .212 |

*Absorbance after separation of interfering pigments on cellulose column.

In every instance the absorbances at 452 mμ, 530 mμ, and 530 mμ after column separation of interfering pigments, were higher when the freeze-dried beef was not reconstituted before performing the TBA test (Table I). To obtain the highest absorption readings possible and still be consistent with the procedure outlined for the non acid-heat TBA test, it was decided to perform all future TBA determinations without rehydrating the freeze-dried samples.

It is worthy to note that the magnitude of the absorption readings varied with the different extraction solvents and were highest for the chloroform-methanol mixture. This maximizing effect of chloroform-methanol is understandable in view of its use in the breakage of lipid-protein bonds, which would presumably release more TBA reactable moieties.

## Removal of Interfering Pigments by a Chromatographic Column

According to Caldwell and Grogg (4), the principal difficulty encountered in testing materials other than pure fats with TBA is the formation of yellow interfering pigments. Many reports have noted the presence of yellow and orange colors with absorption maxima at 450-460 mμ in TBA reaction solutions. (See Review of Literature under the heading 'Limitations of the TBA Test' for a more complete discussion.)

Yu and Sinnhuber (55) described a procedure using a cellulose column to separate the yellow interfering color from the red TBA pigment. An effort was made to determine the percentage recovery of the cellulose-adsorbed TBA-malonaldehyde pigment. Recovery of the pink solution was equivalent to 95.0% of the original concentration, comparable to the 96.4% recovery reported by Yu and Sinnhuber (55). The TBA color was purified on a cellulose column whenever a TBA determination was performed.

Separation of the raw meat pigments from the TBA reaction mixture was an absolute necessity when the non acid-heat 15 hour method was performed on uncooked samples. The color of the pink TBA was completely masked by the raw meat pigments with the result that the absorption at 452 mμ was higher than the absorption at 530 mμ for uncooked samples stored in aluminum foil and in glass bottles (Table II). After passing the uncooked TBA reaction mixtures through the column, the pink color remained, which permitted a quantitative determination of absorbance to be made on TBA color from the raw samples.

Table II. Absorption values of TBA reaction performed on freeze-dried uncooked beef.

| Time of storage (weeks) | 452 mμ |  | 530 mμ |  | *530 mμ |  |
|---|---|---|---|---|---|---|
|  | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles |
| 0 | .618 | .618 | .438 | .438 | .030 | .030 |
| 2 | .760 | .728 | .540 | .517 | .067 | .060 |
| 4 | .796 | .803 | .530 | .546 | .044 | .060 |
| 5 | .727 | .863 | .458 | .585 | .048 | .052 |
| 6 | .748 | .815 | .450 | .553 | .056 | .069 |
| 8 | .780 | .850 | .480 | .550 | .058 | .071 |

*Absorbance after separation of interfering pigments on cellulose column.

Table III. Absorption values of TBA reaction performed on freeze-dried broiled beef.

| Time of storage (weeks) | 452 mµ | | 530 mµ | | *530 mµ | |
|---|---|---|---|---|---|---|
| | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles |
| 0 | .180 | .180 | .142 | .142 | .063 | .063 |
| 2 | .135 | .187 | .148 | .205 | .187 | .188 |
| 4 | .141 | .163 | .175 | .195 | .138 | .162 |
| 5 | .184 | .169 | .163 | .178 | .136 | .142 |
| 6 | .155 | .159 | .100 | .125 | .095 | .115 |
| 8 | .207 | .224 | .167 | .175 | .121 | .118 |

*Absorbance after separation of interfering pigments on cellulose column.

Table IV. Absorption values of TBA reaction performed on freeze-dried microwave cooked beef.

| Time of storage (weeks) | 452 mµ | | 530 mµ | | *530 mµ | |
|---|---|---|---|---|---|---|
| | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles |
| 0 | .072 | .072 | .077 | .077 | .061 | .061 |
| 2 | .056 | .085 | .188 | .189 | .225 | .206 |
| 4 | .106 | .112 | .204 | .211 | .182 | .184 |
| 5 | .112 | .111 | .141 | .199 | .119 | .182 |
| 6 | .108 | .124 | .119 | .140 | .123 | .127 |
| 8 | .140 | .128 | .144 | .138 | .106 | .105 |

*Absorbance after separation of interfering pigments on cellulose column.

Table V. Absorption values of TBA reaction performed on freeze-dried boiled beef.

| Time of storage (weeks) | 452 mμ | | 530 mμ | | *530 mμ | |
|---|---|---|---|---|---|---|
| | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles | Aluminum foil | Glass bottles |
| 0 | .054 | .054 | .063 | .063 | .068 | .068 |
| 2 | .128 | .094 | .259 | .208 | .290 | .270 |
| 4 | .121 | .123 | .224 | .277 | .210 | .248 |
| 5 | .107 | .106 | .163 | .224 | .188 | .224 |
| 6 | .108 | .138 | .142 | .225 | .144 | .223 |
| 8 | .140 | .130 | .183 | .219 | .157 | .196 |

*Absorbance after separation of interfering pigments on cellulose column.

The magnitude of the interference progressively increased in both the raw and cooked meat. Interfering pigments were present in the cooked samples but to a much lesser degree than in the uncooked meat. There were noticeable differences in the absorption at 452 mμ between the broiled samples and the samples prepared in the electronic oven and in boiling water as evidenced in Tables III, IV and V. The absorbances at 452 mμ were highest for the broiled meat but about equal for the boiled and electronic oven preparations. The broiled beef was not as well-done as that prepared by boiling and in the electronic oven following cooking, resulting in greater interference from unconverted pigments for the broiled meat.

From Table III it is observed that the absorbance at 452 mμ exceeded the absorbance at 530 mμ for the freeze-dried broiled beef at 0, 5, 6, and 8 weeks when stored in aluminum foil and at 0, 6, and 8 weeks when stored in bottles. The absorbance at 530 mμ was always higher than the absorbance at 452 mμ for the boiled and electronic oven samples regardless of storage (Tables IV and V). In every instance yellow colors separated and were washed from the column, justifying the value of a cellulose column in obtaining greater purification of the pink TBA color.

## The Effect of Cooking on Stability of Freeze-Dried Beef

Having established that it was not necessary to rehydrate freeze-dried meat, but that it was quite important to remove interfering pigments in the TBA analyses, the effect of cooking on the stability of freeze-dried beef was investigated. The curves in Figure 1 demonstrated a marked difference in the TBA number between freeze-dried cooked and uncooked beef. (In Figure 1 only the samples stored in aluminum foil are represented since they exhibited a pattern identical to that by the samples kept in bottles.) Only slight changes in TBA numbers occurred in raw meat after 8 weeks storage whereas the cooked samples showed greater fluctuation in values over the same period. The results are in agreement with Tims and Watts (45) who performed the TBA test on several meats both cooked and raw. They found that cooked meats gave increasingly higher TBA values on storage but observed no such increase in raw meat and concluded that oxidative rancidity was not proceeding as rapidly in uncooked meats as it was in cooked meats.

Since the TBA test is a sensitive test for the decomposition products of highly unsaturated fatty acids (6, 8) and can be performed directly on the meat tissue without the necessity of extracting the fat, it might be expected to measure the oxidation of highly unsaturated

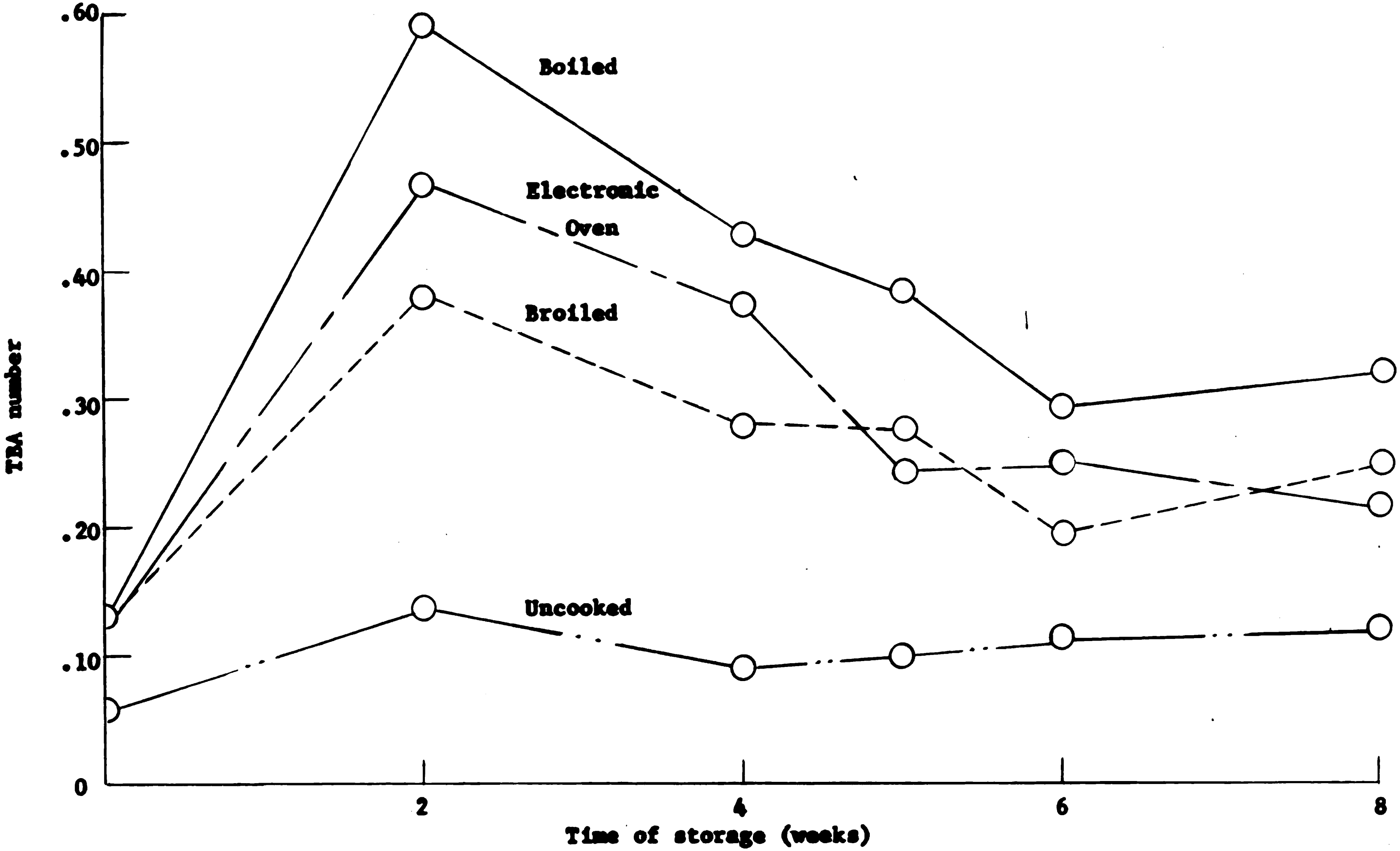


Fig. 1. The effect of cooking on stability of freeze-dried beef stored in aluminum foil.

protein bound phospholipids, not extractable by ordinary fat solvents (45).

Watts (49) has reviewed evidence for a reaction between unsaturated fats and heme pigments which accelerates both rancidity and loss of color. Tarladgis (41) postulated that the compound responsible for the color of cooked meats was a mixed denatured globin - water high-spin ferric porphyrin co-ordination complex similar to methemoglobin. He suggested that the catalytic activity of cooked meat pigments for lipid oxidation resulted from the transfer of electrons through water molecule bridges in the water-ferric ion complex. Little is known at the present time about conditions under which heme catalysis becomes important in the preservation of meat, but possibly denaturation of proteins during heating may free phospholipids and thus make them more susceptible to oxidative attack (45). It has already been mentioned that higher TBA absorption values were obtained with the solvent mixture of chloroform-methanol than for chloroform and for water (Table I).

Differences in the TBA numbers among the freeze-dried cooked beef samples were less pronounced, but still evident. The boiled beef had the highest values and the broiled meat the lowest (Figure 1). Microwave cooking did not appear to cause high TBA numbers since the electronic oven samples more closely approximated the broiled than the boiled samples. The severity of the cooking might be a strong factor in effecting the degree of the TBA reaction. Some of the raw meat pigment remained in the broiled samples after cooking, but 30 minutes in boiling water was sufficient to convert the myoglobin of the boiled meat to metmyoglobin.

## The Effect of Different Methods of Storage on Stability of Freeze-Dried Beef

Concurrent with the study of the effects of cooking was an investigation of storage in aluminum foil and in glass stoppered bottles using both the freeze-dried cooked and uncooked meat. It was noted that with the exception of the second week, the TBA numbers of the freeze-dried beef stored in glass bottles were higher than the meat kept in aluminum foil (Figure 2).

Tarladgis and Watts (40), in experiments with oxidized unsaturated fatty acids, observed that malonaldehyde production followed oxygen uptake very closely, reaching a peak at the same time the oxygen uptake started declining. They concluded that the malonaldehyde precursor did not accumulate as a stable end-product, but that after the peak was reached, more precursor was destroyed than produced. Oxygen seemed to be a limiting factor not only for the oxidation of the fatty acids but also for the destruction of the malonaldehyde precursor. As the supply of oxygen decreased or the rate of the oxidation of the fatty acids began to level off, the production of malonaldehyde as measured by the TBA test immediately dropped.

The availability of oxygen was probably an important factor in the storage studies conducted on freeze-dried beef. The better access of oxygen to the samples stored in aluminum foil than to those samples kept in glass bottles might have been the reason they reached higher TBA maximums before falling to lower values.

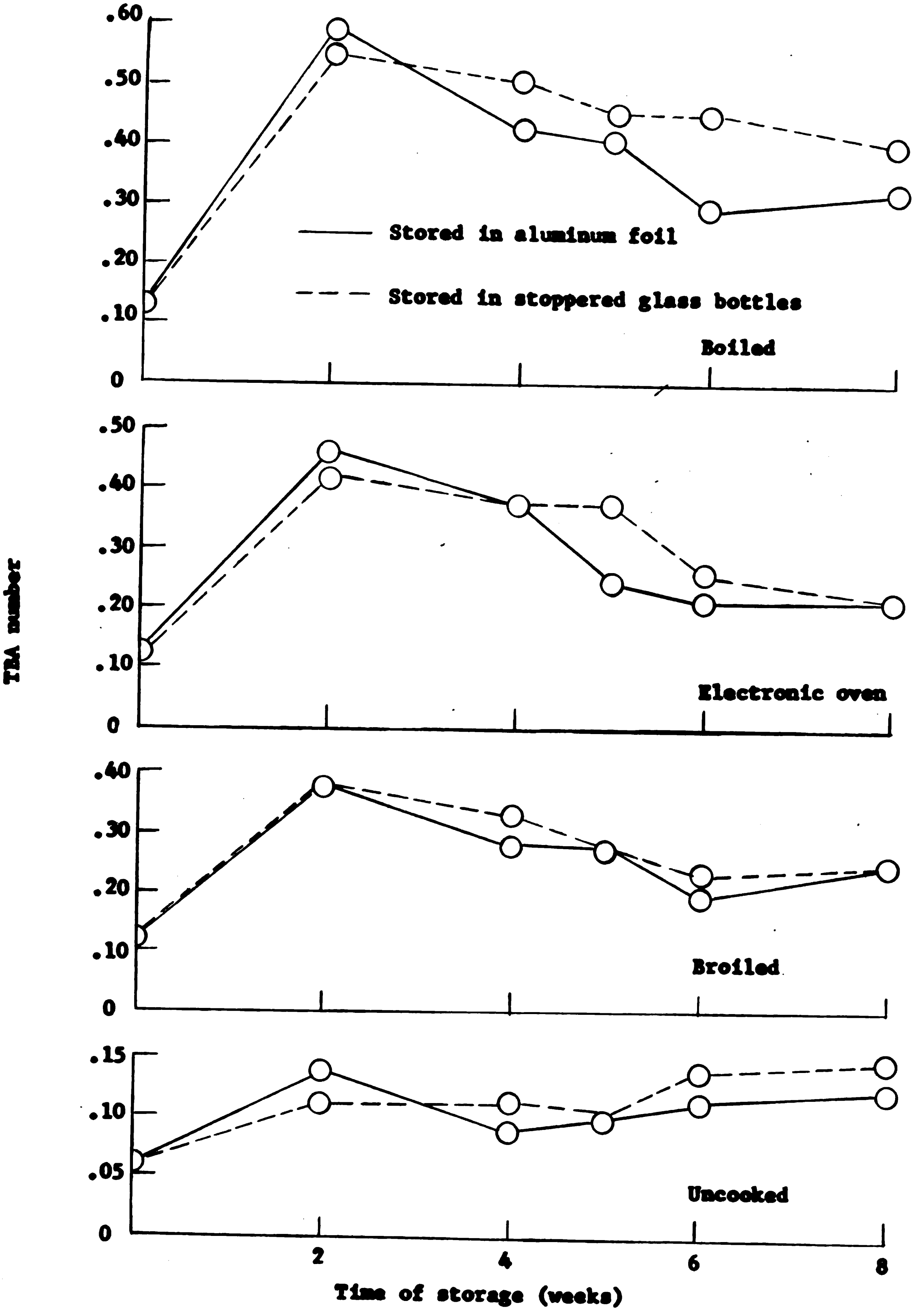


Fig. 2. The effect of storage on freeze-dried beef.

## Moisture Analysis

Moisture determinations were conducted on freeze-dried beef samples from the various cooking preparations and storages to discover if a correlation could be made with the TBA test. The moisture content for all samples (see Figures 3a and 3b) was at a minimum initially, then began to increase before it reached a value that was relatively constant. The percentage moistures were higher for those samples (both cooked and uncooked) that were stored in aluminum foil than for those that were kept in stoppered glass bottles. Obviously the greater access of air, which contained water vapor, to the samples wrapped in aluminum foil accounted for their higher moisture values. The tightness of the screw-on-caps was more effective in preventing the passage of air in and out of the bottles.

It can be observed from Figure 2 and Figures 3a and 3b that the TBA number and the percent moisture do not necessarily increase accordingly. Although the samples stored in aluminum foil had higher moistures than did those kept in bottles, the samples from the glass bottles had higher TBA numbers. A possible explanation for this observation is that the decomposition of malonaldehyde and/or its precursors might be better facilitated at higher moistures. Then, too, an interaction could exist between the water in the freeze-dried meat and TBA reactables sufficient to prevent comparable TBA numbers at different moisture levels.

A definite limitation to the following of moisture changes rested in the difficulty of obtaining meat chunks that were representative of the sample as a whole. The amount of moisture contained in a chunk was certain to be dependent upon such factors as the size, shape, thickness,

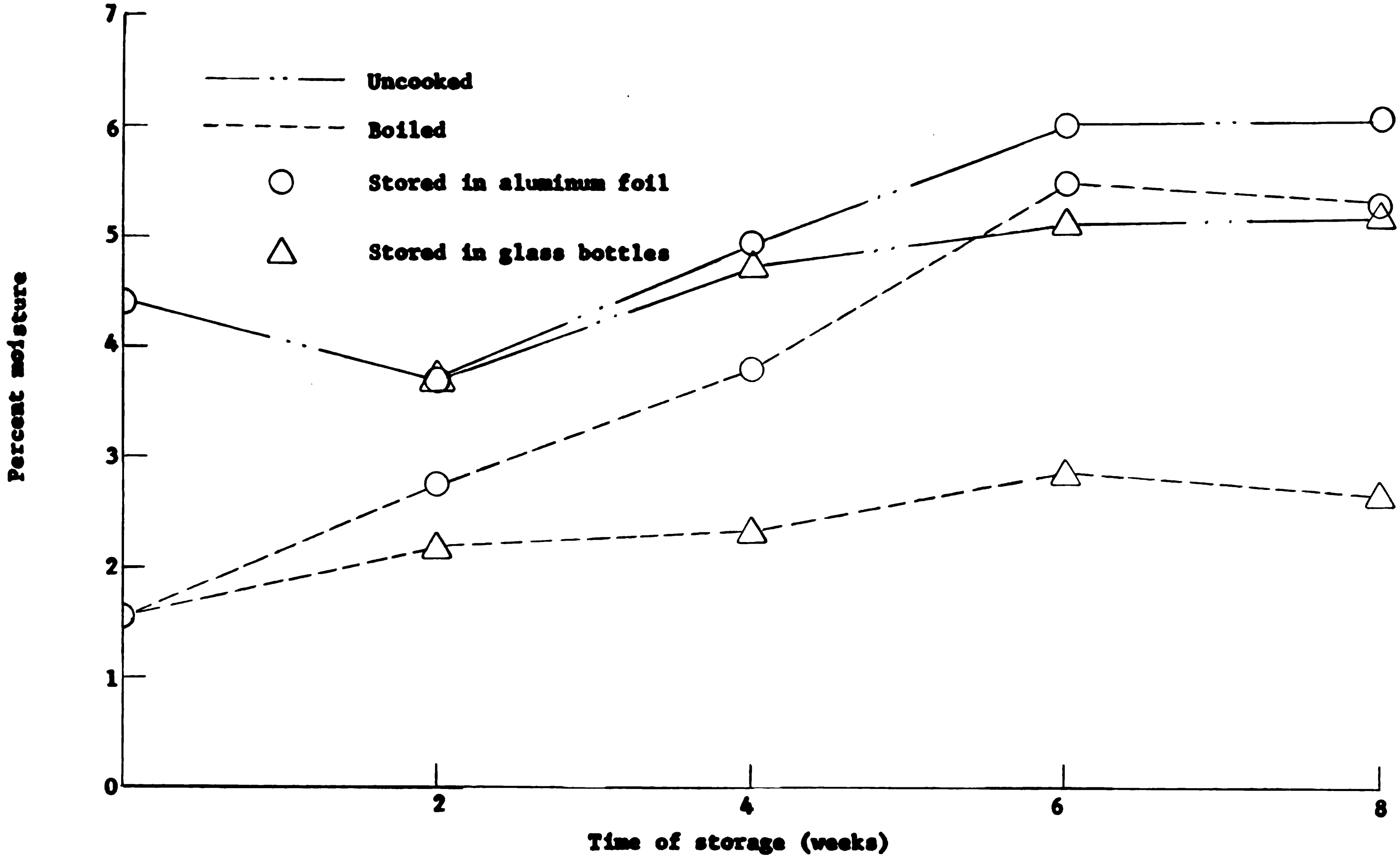


Fig. 3a. Moisture content of freeze-dried beef.

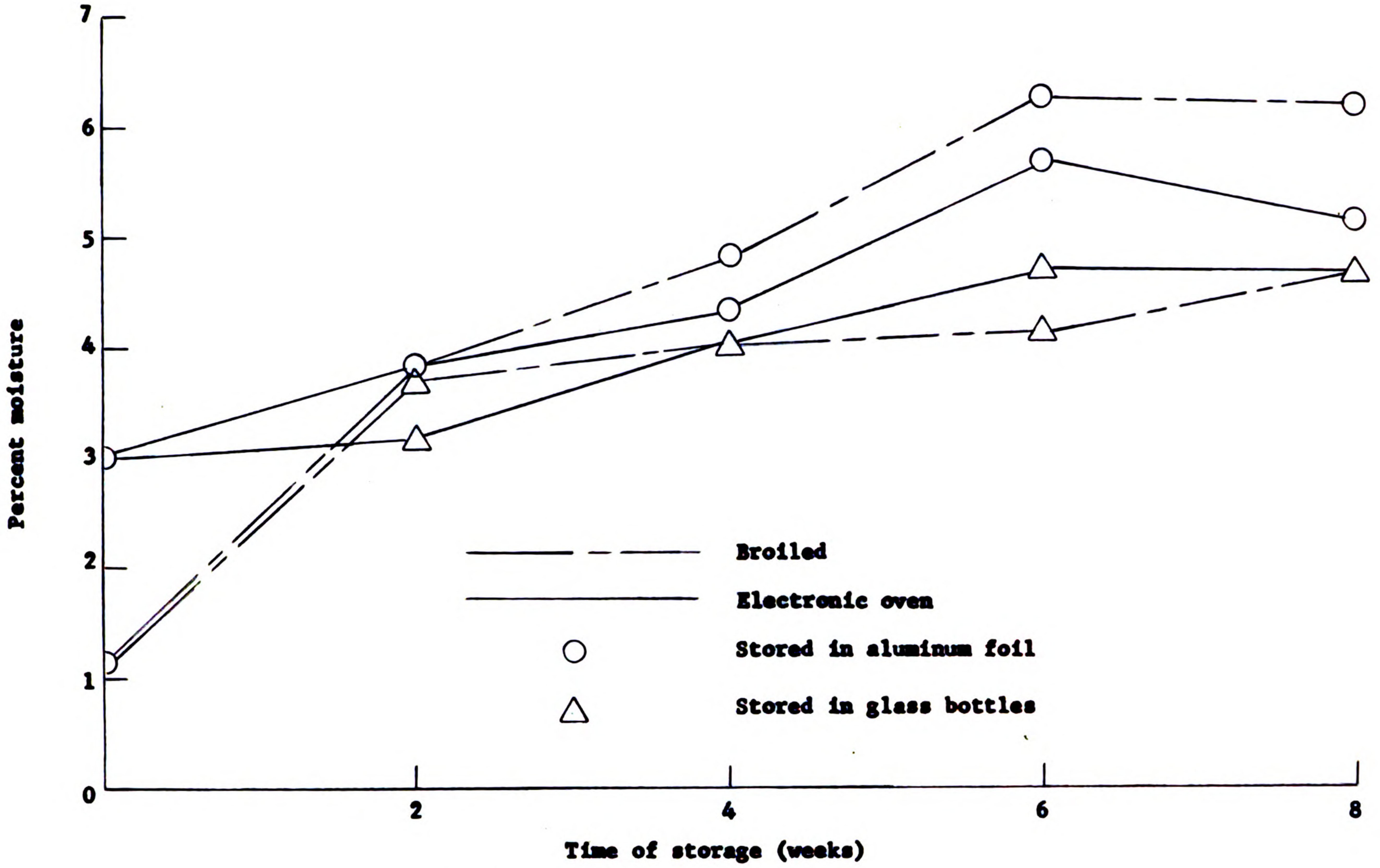


Fig. 3b. Moisture content of freeze-dried beef.

surface area, fat and tissue composition of the meat particle.

### Organoleptic Evaluation (ODOR)

Detection of lipid oxidation in foods organoleptically is considered the most sensitive and reliable method, though it does not lend itself well to quantitative measurements (19). Researchers have successfully correlated the TBA reaction with the development of off odors and flavor losses. In order to discover if there was any relationship between the subjective factors of quality and the TBA test on freeze-dried beef, a panel was organized to rate samples of freeze-dried beef from the various treatments concurrent with the measurement of their TBA numbers.

The results of the odor evaluation are illustrated in Figures 4a and 4b. It is evident that the sensory scores of all samples increased throughout the storage period, indicating that off odors had developed. Panel members distinctly preferred the odor of the freeze-dried uncooked beef to the odor of the freeze-dried cooked beef. This finding correlated with the results of the TBA test for the TBA numbers of the uncooked samples were significantly lower than those of the cooked (Figure 1).

In general, those samples that were stored in stoppered bottles received better ratings than did those in aluminum foil, although the curves in Figures 4a and 4b criss-cross in several places. Here the TBA test was at variance with the panel for the TBA numbers were higher for the samples in the stoppered bottles (Figures 3a and 3b). The differences between the samples stored in aluminum foil and those in bottles were not very pronounced either for the TBA test or for the odor

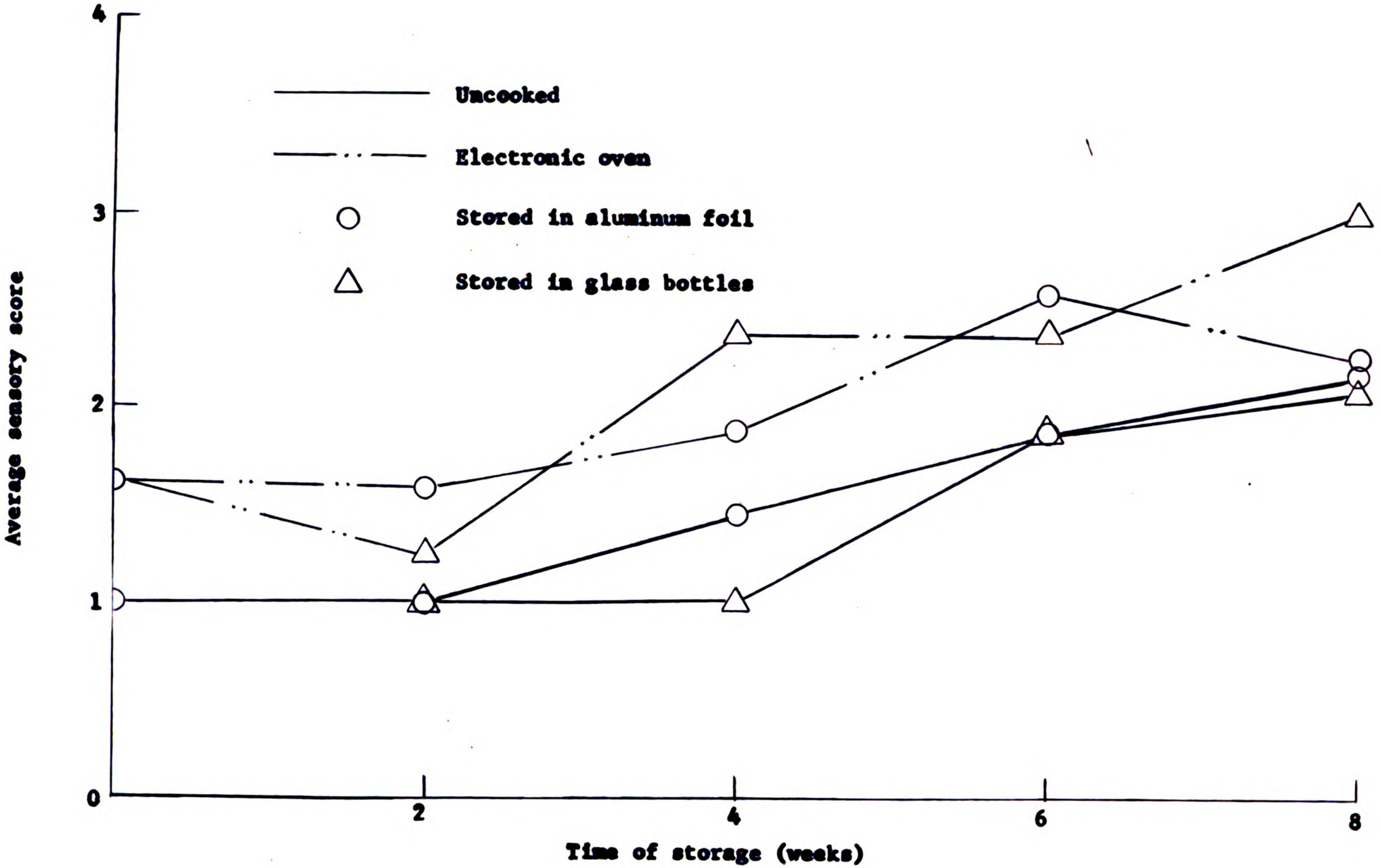


Fig. 4a. Organoleptic evaluation of freeze-dried beef.

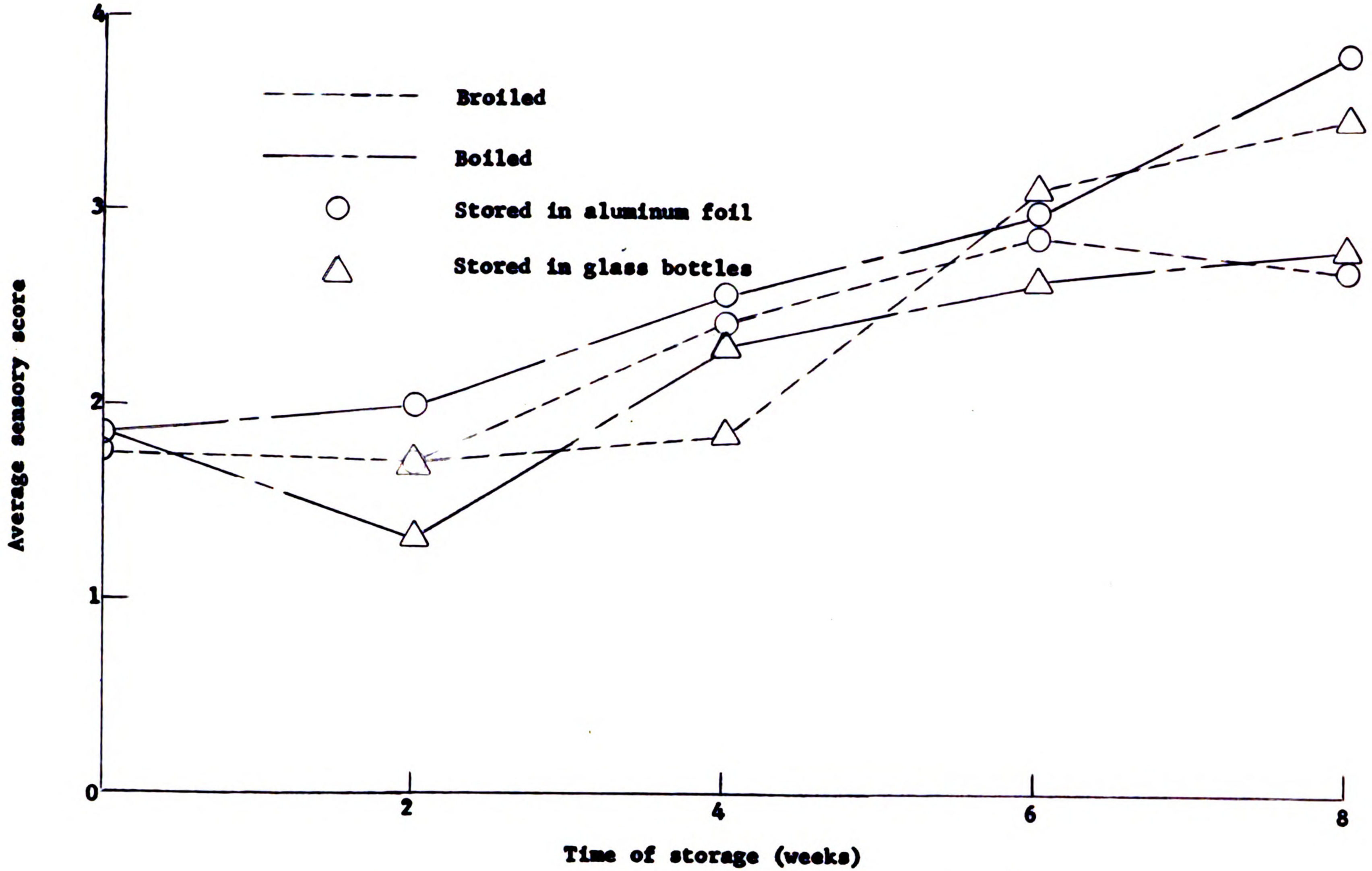


Fig. 4b. Organoleptic evaluation of freeze-dried beef.

evaluation. Even a professionally trained panel, not to mention a panel composed of members with little previous experience in determining off odors, would have undoubtedly experienced difficulty in detecting differences of such slight magnitude. At times, panel members appeared rather inconsistent as individuals in reporting their opinions on the same samples from one observation to another. But they unanimously agreed that unpleasant changes were occurring in the freeze-dried beef regardless of its method of preparation and storage.

Turner, Paynter, Montie, and Bessert (46) obtained a positive correlation between taste test acceptability scores and the TBA values of wieners and pork patties. Younathan and Watts (52) reported that rancid odors which developed in stored cooked meats correlated with an increase in TBA values.

When average sensory scores for the uncooked samples were plotted (graph not shown) against their TBA numbers, the TBA numbers for the uncooked samples increased as the average sensory scores increased. But when the average sensory scores for the cooked samples were plotted against their TBA numbers the reverse effect existed. The TBA numbers were higher when the average sensory scores were lower. Thus a positive correlation was observed between the TBA numbers and the organoleptic scores for the freeze-dried uncooked beef but not for the freeze-dried cooked beef. It has already been suggested in the section on the effect of cooking (page 19) that denaturation of proteins during heating may free phospholipids and make them more susceptible to oxidative attack (45). In this way cooking of meat could contribute to the development of off odors.

## Comparison of TBA Methods

Mention has already been made that TBA numbers reached a maximum by the second week of storage for the freeze-dried cooked beef (Figure 2). Younathan and Watts (53) found that the TBA test for cooked pork increased rapidly to a maximum in approximately 4 days, but they reported that raw samples showed no appreciable change in TBA values with storage. Tarladgis and Watts (40) interpreted similar experimental evidence from cooked meats and fishery products as an indication that TBA values could be correlated with oxygen uptake. If this were true, they concluded, the malonaldehyde precursor did not accumulate as a stable end product.

To investigate further the early maximums observed in the TBA curves, beef was cooked, freeze-dried, and stored in aluminum foil for 30 days. TBA determinations were performed on the cooked meat by: (1) the non acid-heat 15 hour method of Tarladgis _et al_. (43); (2) the distillation method of Tarladgis _et al_. (39); (3) the Sinnhuber and Yu method (35); and (4) a modification of the method of Jacobson _et al_. (13). The results are illustrated in Figure 5. Of the four methods tested, only the method of Sinnhuber failed to show a maximum or near maximum absorption on the second determination (the 5th or 6th day) for at least two of the three samples.

Most workers have reported values of the TBA reaction in arbitrary absorbance units, which, in view of the diversity and empirical nature of the methods employed, cannot be compared from one laboratory to another (39). Sinnhuber and Yu (35) proposed the use of 1,1,3,3-tetraethoxypropane (TEP) as a standard for the quantitative determination of malonaldehyde with TBA (acid hydrolysis of TEP yields malonaldehyde)

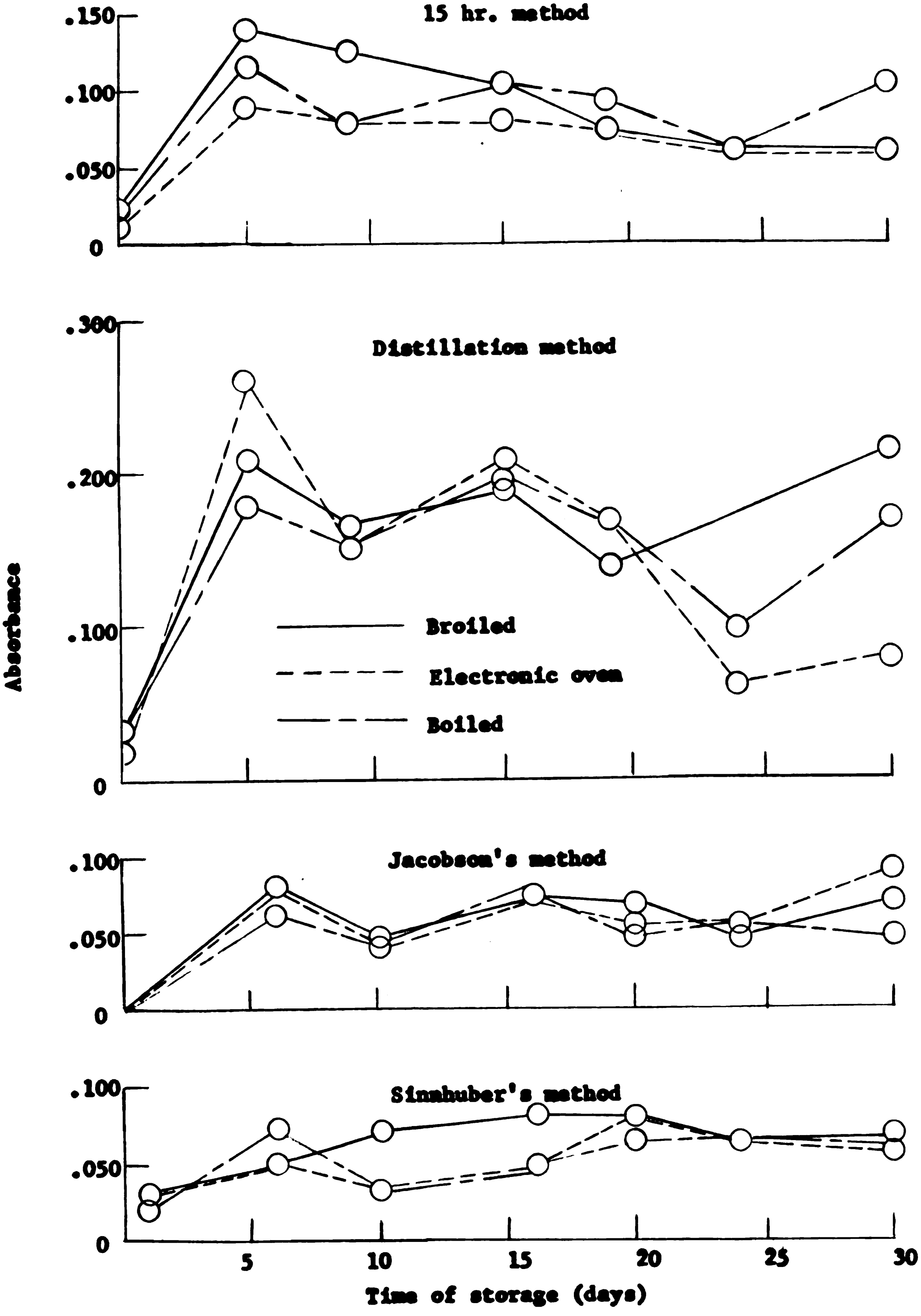


Fig. 5. A comparison of absorption curves for different TBA methods.

and expressed their results in terms of the "TBA number", defined as mg. of malonaldehyde per 1,000 g. of sample. Tarladgis *et al.* (43) prepared a 1,1,3,3-tetraethoxypropane (malonaldehyde) standard curve without acid to reduce acid-heat degradation of the TBA structure and converted their absorbance readings to TBA numbers by a formula.

Several attempts were made to duplicate the malonaldehyde standard curve of Tarladgis *et al.* (43) and to obtain the sensitivity, reported to be of the order of $10^{-9}$ moles. It was, however, only possible to read the absorbance at concentrations of malonaldehyde above $1x10^{-7}$ moles (Figure 6).

TBA numbers obtained from the 15 hr. method and the method of Sinnhuber are plotted in Figure 7. The values of the latter are significantly greater, contrary to what might be expected from a study of Figure 5. This follows from a consideration of the size of the meat sample used to develop the data. The method of Sinnhuber and Yu uses a sample of 0.25 g. while the equivalence of 10 g. samples were used for the comparison tests. It would thus appear that the real worth of any TBA test lies not in the absolute units it yields, but rather with respect to the position of its values within limits previously defined for that particular TBA test.

## General Discussion

This study was designed to follow changes in the deterioration of freeze-dried beef by the 2-thiobarbituric acid (TBA) test. A search of the literature revealed many different TBA methods and various modifications of these methods making the test applicable to such

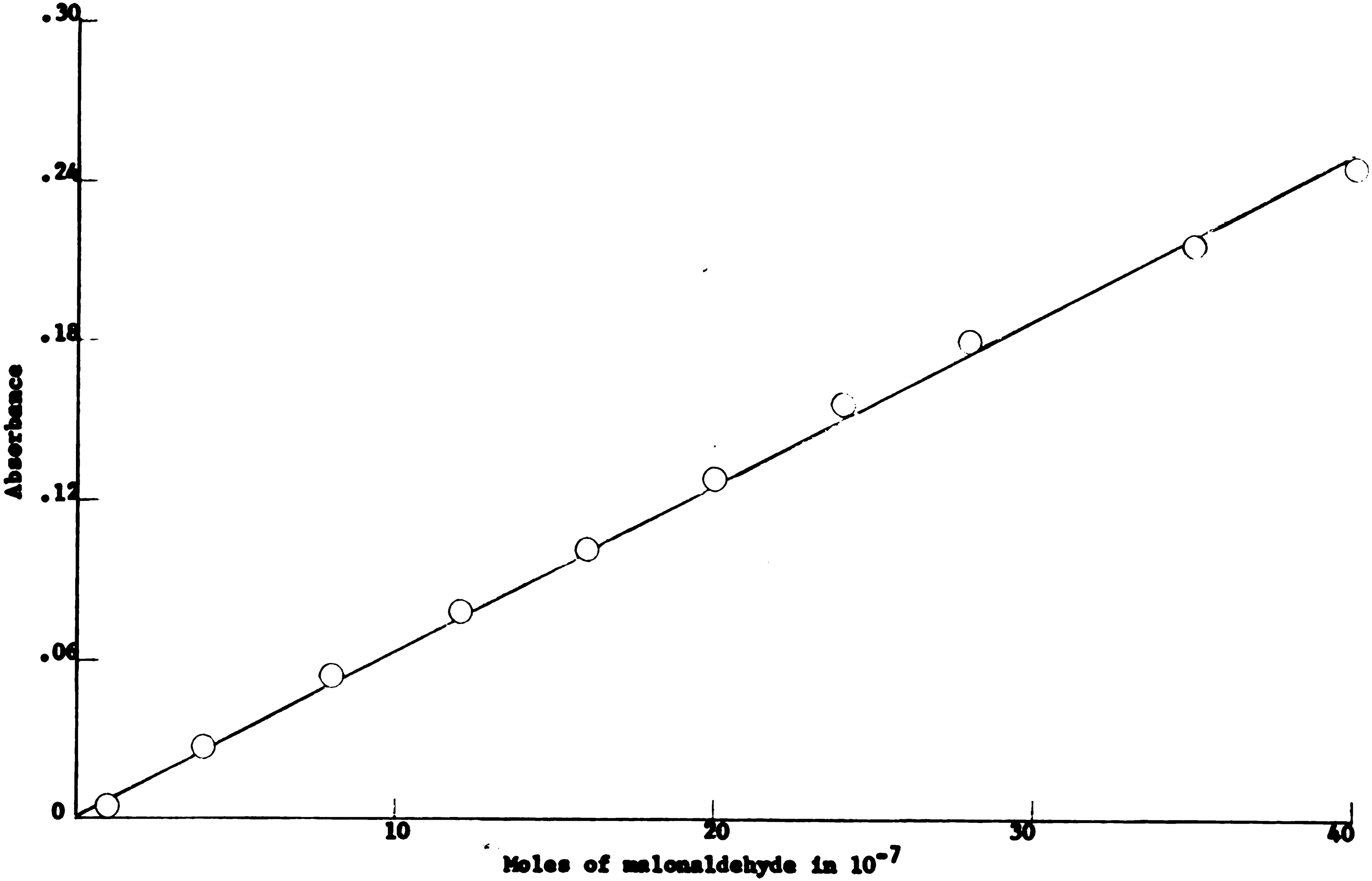


Fig. 6. Malonaldehyde standard curve

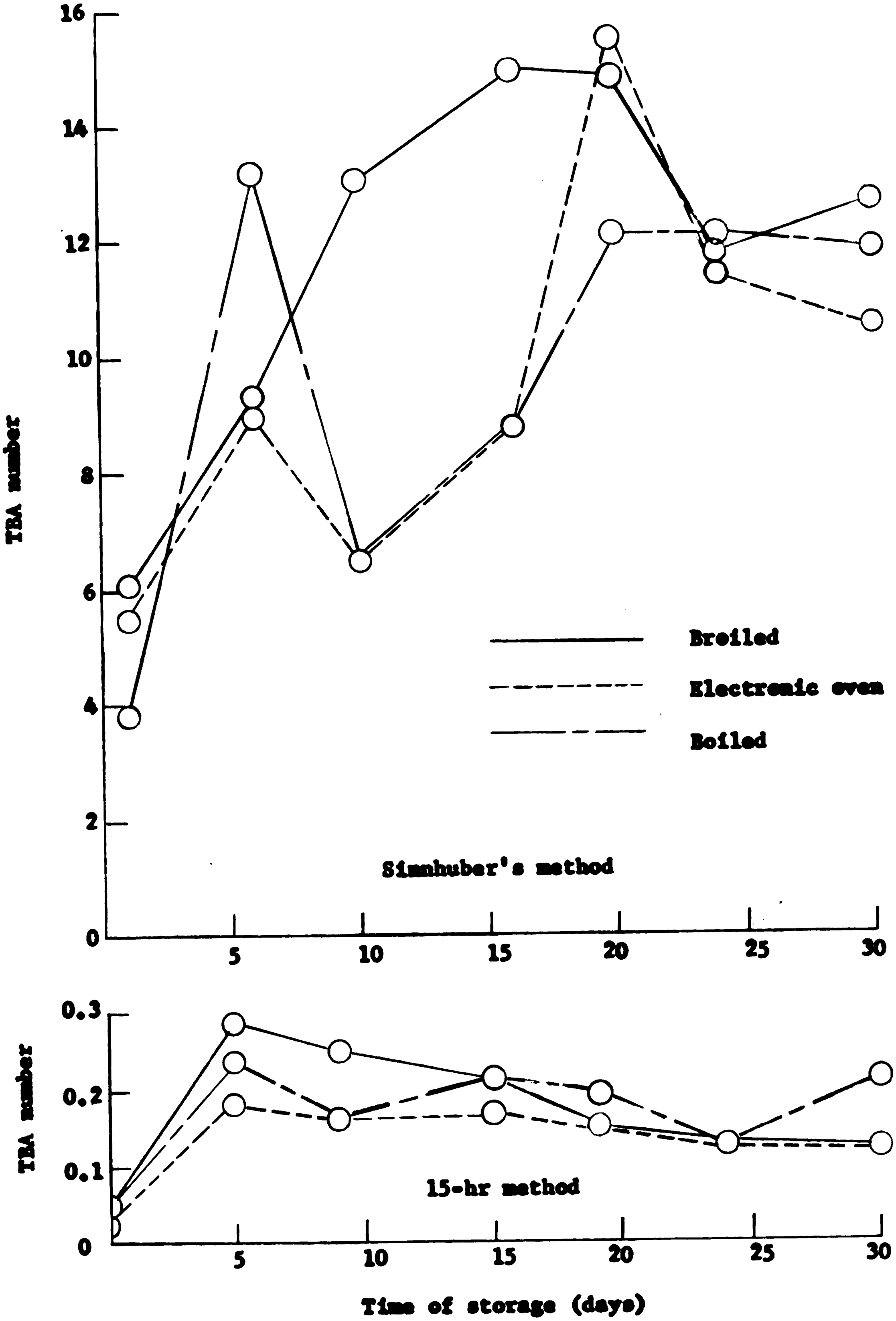


Fig. 7. A comparison of TBA methods.

diverse foods as meats, fats and oils, dairy products, fishery products, and cereal and baked products. No report relating the TBA test to freeze-dried meat was uncovered. The non acid-heat 15 hour method was selected for most of the preliminary work in view of evidence that the structure of TBA was altered upon acid-heat treatment. All of the other TBA methods utilized either acid, heat, or both simultaneously.

Investigation of the development of rancidity in freeze-dried beef established that rehydration of freeze-dried meat was unnecessary prior to conducting the TBA test, and that interfering pigments in TBA reaction solutions, reported by many workers to have absorption maxima at 450-460 mμ, should be removed by column separation when performing a TBA analysis.

Moisture determinations, odor evaluations, and studies on the effect of cooking and storage on the stability of freeze-dried beef were conducted. Interrelationships were found to exist. Uncooked samples had lower TBA numbers and received better average sensory scores than did cooked samples. It was suggested that cooking of meats resulted in the denaturation of proteins, liberating phospholipids for oxidative attack (45), and that the catalytic activity of cooked meat pigments on lipid oxidation was related to the formation of a coordination complex of water and ferric ion, which enhanced the transfer of electrons (41). Both suggestions were supported by the observation that the boiled samples, which received the most severe cooking, had the highest TBA numbers for all the samples.

Samples stored in aluminum foil had the higher moisture content, while the samples stored in glass bottles recorded higher TBA numbers. The better access of air to the aluminum foil samples was offered as an explanation since it contained water vapor that would be expected to increase the percentage of moisture, and oxygen which could react with unsaturated fatty acids. It was discovered, in experiments with oxidized unsaturated fatty acids, that malonaldehyde production followed oxygen uptake very closely (40). Possibly, either the decomposition of malonaldehyde and its precursors or an interaction between the water or other moieties in freeze-dried beef and TBA reactables was better facilitated at higher moisture values to produce lower TBA numbers for the aluminum foil samples.

Rancidity measurements of freeze-dried beef by the TBA test were disappointing since the TBA value reached a peak early and afterward fell in a way that was analogous to other reports (40, 53). The early TBA maximum on freeze-dried beef was investigated by other TBA methods and still found to exist. It would seem that freeze-dried beef is capable of tying up malonaldehyde and its precursors sufficiently to reduce the effectiveness of the TBA determination. If it were measuring oxidative deterioration, the magnitude of the TBA number would be expected to progressively increase throughout storage.

Even though the application of the TBA test to freeze-dried beef was largely unsuccessful, certain findings are worthy of consideration. Freeze-dried uncooked beef had better keeping quality than freeze-dried cooked beef. The more severe the cooking process, the greater was the deterioration. This would indicate that attention should be given to the cooking process prior to freeze-drying. The ability of oxygen to cause rancidity may depend not only on the quantity of oxygen present but also on the moisture content of the freeze-dried beef. Needless to say, storage under vacuum would have real value in eliminating changes which might be due to differences in the oxygen-moisture ratio.

# SUMMARY AND CONCLUSIONS

The development of rancidity in freeze-dried cooked and uncooked beef was followed by the 2-thiobarbituric acid (TBA) test. Preliminary work established that freeze-dried beef should not be reconstituted before performing a TBA analysis since lower values resulted. Interfering pigments present in TBA reaction solutions were effectively removed by separation on a cellulose column.

Moisture determinations, odor evaluations, and studies of the effect of cooking and storage on the stability of freeze-dried beef indicated that they were interrelated. Uncooked samples had lower TBA numbers and better sensory scores than did cooked samples. The TBA numbers of the samples prepared in an electronic oven or broiled in an ordinary oven were lower than were those of the samples boiled in water. Thus the severity of cooking appeared to be a factor in deterioration since the boiled samples were subjected to the most extreme conditions of all the cooked samples prior to freeze-drying. Samples with the lower moisture percentages (those stored in stoppered glass bottles as opposed to those stored in aluminum foil) had higher TBA numbers. This suggested that TBA reactables were more tightly bound or were possibly altered structurally by water and/or other moieties present in freeze-dried beef at higher moisture contents.

The application of the TBA test to freeze-dried cooked beef was largely unsuccessful because the TBA numbers of samples tested reached an early maximum and then fell to lower levels. It appeared that either freeze-dried beef was capable of tying up malonaldehyde and its precursors or else it was successful in hindering malonaldehyde production by allowing competing reactions to occur so as to reduce the overall effectiveness of the TBA test in following the development of rancidity in freeze-dried cooked beef.

# BIBLIOGRAPHY

1. Andersson, K., and C. E. Danielson. 1961. Storage changes in frozen fish: A comparison of objective and subjective tests. Food Technol. 15, 55.

2. Bernheim, F., M. L. C. Bernheim, and K. M. Wilbur. 1947. The reaction between thiobarbituric acid and the oxidation products of certain lipides. J. Biol. Chem. 174, 257.

3. Biggs, D. A., and L. R. Bryant. 1953. The thiobarbituric acid test for butterfat oxidation. Can. J. Technol. 31, 138.

4. Caldwell, E. F., and B. Grogg. 1955. Application of the thiobarbituric acid test to cereal and baked products. Food Technol. 9, 185.

5. Caul, J. F. 1957. "The profile method of flavor analysis." Advances in Food Research. 7, 5. Academic Press Inc., New York, N.Y.

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

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

8. Dunkley, W. L. 1951. Evaluation of the thiobarbituric acid test as a measure of oxidized flavor in milk. Food Technol. 5, 342.

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

10. Flosdorf, E. W. 1945. Drying by sublimation. Food Ind. 17, 22.

11. Flosdorf, E. W. 1949. Freeze-Drying. Reinhold Publishing Corp., New York, N.Y.

12. Fridriksdottir, S. 1953. The application of the thiobarbituric acid test to the determination of oxidative rancidity in frozen herring. Special Problem. Cornell Univ. Library, Ithaca, N.Y.

13. Jacobson, G. A., J. A. Kirkpatrick, and H. E. Goff, Jr. 1961. A study of the applicability of a modified thiobarbituric acid test to flavor evaluation of fats and oils. Presented at the American Oil Chemists' Society meeting, in Chicago, Illinois.

14. Jennings, W. G., W. L. Dunkley, and H. G. Reiber. 1955. Studies of certain red pigments formed from 2-thiobarbituric acid. Food Research. 20, 13.

15. Kawahara, F., and H. J. Dutton. 1952. Volatile cleavage products of autoxidized soybean oil. J. Am. Oil Chem. Soc. 29, 372.

16. Kawahara, F., H. J. Dutton, and J. C. Cowan. 1952. Volatile cleavage products of autoxidized methyl linolenate. J. Am. Oil Chem. Soc. 29, 633.

17. Keeney, M., and R. Bassette. 1959. Detection of intermediate compounds in the early stages of browning reaction in milk products. J. Dairy Sci. 42, 945.

18. Kenaston, C. B., K. M. Wilbur, A. Ottolenghi, and F. J. Bernheim. 1955. Comparison of methods for determining fatty acid oxidation produced by ultraviolet irradiation. J. Am. Oil Chem. Soc. 32, 33.

19. King, R. L. 1962. Oxidation of milk fat globule membrane material. I. Thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. J. Dairy Sci. 45, 1165.

20. Kohn, H. I., and M. Liversedge. 1944. On a new aerobic metabolite whose production by brain is inhibited by apomorphine, ergotamine, epinephrine, and menadione. J. Pharmacol. 82, 292.

21. Koning, A. J. de, and M. H. Silk. 1963. The 2-thiobarbituric acid reagent for determination of oxidative rancidity in fish oils. J. Am. Oil Chem. Soc. 40, 165.

22. Kurtz, G. W., E. F. Price, and S. Patton. 1951. Studies on 2-thiobarbituric acid as a reagent for detecting milk fat oxidation. Paper M-38. Presented at the 46th Annual Meeting of the American Dairy Science Association, Knoxville, Tenn. Abstracted in J. Dairy Sci. 34, 484.

23. Landucci, J. M., J. Pouradier, and M. Durante. 1955. Research on the aldehydes present in gelatin. Bull. Soc. Chim. France 22, 857.

24. Lea, C. H. 1957. Deteriorative reactions involving phospholipids and lipoproteins. J. Sci. Food Agric. 8, 1.

25. Palmateer, R. E., T. C. Yu, and R. O. Sinnhuber. 1960. An accelerated oxidation method for the estimation of the storage life of frozen seafoods. Food Technol. 14, 528.

26. Patton, S., M. Keeney, and G. W. Kurtz. 1951. Compounds producing the Kreis color reaction with particular reference to oxidized milk fat. J. Am. Oil Chem. Soc. 28, 391.

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

28. Patton, S., and G. W. Kurtz. 1955. A note on the thiobarbituric acid test for milk lipid oxidation. J. Dairy Sci. 38, 901.

29. Patton, S. 1960. Response of epihydrin aldehyde and glyceraldehyde in the thiobarbituric acid test for fat oxidation. Food Research. 25, 554.

30. Schmidt, H. 1959. Concerning the thiobarbituric acid-methin dyestuffs. Fette Seifen Anstrichmittel. 61, 881.

31. Schwartz, M. G., and B. M. Watts. 1956. Application of the thiobarbituric acid test as a quantitative measure of deterioration in cooked oysters. Food Research. 22, 76.

32. Shepherd, R. G. 1948. A specific analytical method for certain pyrimidines. Anal. Chem. 20, 1150.

33. Sidwell, C. G., H. Salwin, M. Benca, and J. H. Mitchell. 1954. The use of thiobarbituric acid as a measure of fat oxidation. J. Am. Oil Chem. Soc. 31, 603.

34. Sidwell, C. G., H. Salwin, and J. H. Mitchell. 1955. Measurement of oxidation in dried milk products with thiobarbituric acid. J. Am. Oil Chem. Soc. 32, 13.

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

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

37. Smith, N. L., I. J. Tinsley, and E. C. Buhl. 1960. Thiobarbituric acid test in irradiation sterilized beef. Food Technol. 24, 317.

38. Tappel, A. L. 1956. Freeze-dried meat. II. The mechanism of oxidative deterioration of freeze-dried beef. Food Research. 21, 195.

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

40. Tarladgis, B. G., and B. M. Watts. 1960. Malonaldehyde production during the controlled oxidation of pure, unsaturated fatty acids. J. Am. Oil Chem. Soc. 37, 403.

41. Tarladgis, B. G. 1962. Interpretation of the spectra of meat pigments. I. Cooked meats. J. Sci. Food Agric. 9, 481.

42. 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.

43. 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. II. Formation of the TBA-malonaldehyde colored complex without acid-heat treatment. Unpublished data and private communication.

44. Taüfel, H. C. K. and R. Zimmerman. 1961. Über den chemismus der farbreaktion von 2-thiobarbitursäure mit carbonyl-verbindungen. Fette Seifen Anstrichmittel. 63, 226.

45. Tims, M. J., and B. M. Watts. 1958. Protection of cooked meats with phosphates. Food Technol. 12, 240.

46. Turner, E. W., W. D. Paynter, E. J. 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.

47. Watts, B. M., and D. Peng. 1947. Rancidity development in raw versus precooked frozen pork sausage. J. Home Ec. 39, 88.

48. Watts, B. M., D. Peng, and E. A. Kline. 1948. Precooking pork for freezing storage. J. Home Ec. 40, 579.

49. Watts, B. M. 1954. "Oxidative rancidity and discoloration in meat." Advances in Food Research. 5, 1. Academic Press Inc., New York, N.Y.

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

51. Wilbur, K. M., F. Bernheim, and O. W. Shapiro. 1949. The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. Arch. Biochem. 24, 305.

52. Younathan, M. T., and B. M. Watts. 1959. Relationship of meat pigments to lipid oxidation. Food Research. 24, 728.

53. Younathan, M. T., and B. M. Watts. 1960. Oxidation of tissue lipids in cooked pork. Food Research. 25, 538.

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

55. Yu, T. C., and R. O. Sinnhuber. 1962. Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid reaction. Food Technol. 16, 115.

56. Zipser, M. W., and B. M. Watts. 1961. Oxidative rancidity in cooked mullet. Food Technol. 15, 318.

57. Zipser, M. W., J. Dupont, and B. M. Watts. 1962. Extraction of lipids from oxidizing mullet. J. Food Sci. 27, 135.

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