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THE ROLE OF NITRITE IN PREVENTING DEVELOPMENT OF WARMED-OVER FLAVOR IN COOKED MEAT FROM DIFFERENT SPECIES OF ANIMALS

By

Mohamad Hassan Fooladi

A THESIS

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ABSTRACT

THE ROLE OF NITRITE IN PREVENTING DEVELOPMENT OF WARMED-OVER FLAVOR IN COOKED MEAT FROM DIFFERENT SPECIES OF ANIMALS

by

Mohamad Hassan Fooladi

The present study was designed to determine the role of nitrite in development of warmed-over flavor in beef, pork and chicken, which was followed by TBA values and sensory panel scores. Samples with and without added nitrite were evaluated both before and after cooking at 0 days and after 48 hours storage at 4°C. The relationships between TBA numbers and total lipid and phospholipid levels were also followed to ascertain their significance to development of warmed-over flavor.

Added nitrite protected against autoxidation of cooked meat during storage at 4°C for 48 hours, causing a 6-fold reduction in TBA values for pork and a 2-fold reduction in beef and chicken. Sensory panel scores confirmed the protective effect of added nitrite in all three species. Total lipid levels were not significantly related

to warmed-over flavor development, but there was some evidence for involvement of phospholipids.

ACKNOWLEDGMENTS

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INTRODUCTION

When uncured cooked meat is stored for a relatively short period of time, it develops an undesirable taste and odor which is commonly referred to as warmed-over flavor (WOF). The objectionable stale rancid odor becomes especially noticeable after the cooked meat is refrigerated and then reheated again. The problem of warmed-over flavor has assumed much greater significance in recent years due to the rapid increase in fast food service facilities (airlines, vendors, and franchises) requiring the use of large quantities of precooked or partially-cooked meats and meat products.

Oxidative rancidity is a major cause of flavor deterioration in meat during storage (Turner et al., 1954; Timms and Watts, 1958). The lipids present in muscle tissue are responsible at least in part for problems related to product stability. Love and Pearson (1971) concluded that the phospholipids result in oxidative deterioration of cooked meat and the resulting rancid flavor, which develops rapidly during refrigerated storage. The relatively high content of unsaturated fatty acids in the phospholipid fraction appears to be responsible for development of warmed-over flavor.

Heme pigments have traditionally been considered as catalysts of lipid oxidation in meat. Metmyoglobin in raw

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meat and ferric denatured hemichromes in cooked meat have been implicated as the catalytically active forms of the muscle pigments. Younathan et al. (1959) showed that ferric hemochromogen is an active catalyst for oxidation of unsaturated fat, whereas, ferrous nitric oxide hemochromogen in cured meat does not catalyze lipid oxidation.

Zipser et al. (1964) reported that TBA numbers during storage of cured pork samples are lower than those for uncured pork. Liu and Watts (1970) and Sato and Hegarty (1971) concluded that the presence of nitrite in the meat products inhibits oxidation of the lipids, while off-flavor in uncured meat is due to oxidation of lipids. Thus, the present study was undertaken to investigate the role of nitrite in preventing development of warmed-over flavor in cooked meat from different species of animals.

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LITERATURE REVIEW

Oxidation of Lipids

The lipid components of foods are readily susceptible to autoxidation, which occurs slowly even at normal temperatures according to Waters (1971). He concluded that the deleterious effects of oxidation are more serious because peroxides produced by lipid oxidation can attack molecules of other types. He further reported that secondary reactions of autoxidation are responsible for undesirable changes in foods. Thus, exposure of food lipids to atmospheric oxygen causes extensive deterioration. As a result of autoxidation, unpleasant odors may develop in foods, and sometimes even toxic compounds may be produced (Holman, 1960; Lundberg, 1962). Kummerow (1962) and Matsuo (1962) have pointed out that peroxides of unsaturated fatty acids are toxic to animals.

In lipids containing unsaturated double bonds oxygen attacks at or near the unsaturated center, while in saturated fats breakdown may occur anywhere along the hydrocarbon chain, predominantly at the beta position (Ingold, 1962).

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Mechanism of Autoxidation

The generally accepted mechanism of lipid oxidation has been reviewed by Dugan (1961), Labuza (1971), and Sato and Herring (1973) and involves a free radical chain reaction. They indicated that the reaction proceeds in three stages:

(1) Initiation. This step involves the formation of a free radical species (unpaired electron) from an unsaturated fatty acid as shown below:

$$R_1H \xrightarrow[\text{(initiators)}]{\text{heat, light, metals}} R_1^{\cdot} + H^{\cdot}$$

(unsaturated fat) (free radical)

(2) Propagation. Free radicals combine with molecular oxygen (autoxidation) to form peroxide free radicals, which upon reaction with fatty acids, yield hydroperoxides and other free radicals. The free radicals are then available to continue the chain reaction in the following manner:

$$R_1^{\cdot} + O_2 \longrightarrow R_1OO^{\cdot} \quad \text{(peroxide free radical)}$$

$$R_1OO^{\cdot} + R_2H \longrightarrow R_1OOH + R^{\cdot}_2$$

(hydroperoxide)

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(3) Termination. Deactivation of the radical results in stable end products as illustrated below:

$$R^{\cdot} + R^{\cdot} \longrightarrow RR$$

$$R^{\cdot} + ROO^{\cdot} \longrightarrow ROOR$$

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow ROOR + O_2$$

$$R^{\cdot} + R_1 \longrightarrow RR_1$$

Free radical inhibitors (R_1) also include antioxidants.

The development of off flavors results from hydroperoxide degradation (Kaunitz, 1962; Lundberg, 1962). Although hydroperoxides are odorless, they are degraded through a series of scission and dismutation reactions to yield low molecular weight carbonyl compounds (aldehydes and ketones) and short chain fatty acids, which possess extremely low sensory threshold values (Keeney, 1962; Lea, 1962; Lundberg, 1962).

Factors which effect the rate of off-flavor development include the fatty acid composition of the lipids, temperature, light, metal catalysts, inhibitory compounds, and the availability of oxygen (Lea, 1962; Labuza, 1971). Ackman (1976) simplistically emphasized two major points in discussing lipid stability of foods. He first indicated the need to begin with high quality raw materials, and secondly, the need to optimize all handling and storage procedures.

Products of Lipid Oxidation

Hydroperoxides are the primary products of the reaction of oxygen with unsaturated lipids (Farmer et al., 1942; 1943). Decomposition of these primary products produces alcohols, aldehydes, ketones, acids, lactones, and unsaturated hydrocarbons, which are known as secondary degradation products (Lundberg, 1962). These compounds are highly susceptible to further oxidation (Keeney, 1962; Lundberg, 1962; Sherwin, 1972). According to Keeney (1962) and Lea (1962), the primary products (hydroperoxides and peroxides) are odorless, whereas, the rancid odors in oxidized fat are chiefly due to aldehydes, ketones and acids that are formed from the primary products.

Aldehydes are notoriously unstable compounds, and are susceptible to polymerization and condensation reactions. They may be oxidized by active oxygen in the autoxidative system to form carboxylic acids (Sato and Herring, 1973). Although the free aldehyde level in oxidizing fat is low, free aldehydes still cause major flavor problems because some of them (2-4-decadienal) have flavor thresholds of less than one part per billion (Keeney, 1962).

Catalysts of Lipid Oxidation in Meat

It has been generally accepted that hematin compounds, such as hemoglobin, myoglobin, and cytochromes in animal tissues, are catalysts for unsaturated fat oxidation (Lew

and Tappel, 1956).

Fox (1966) has reviewed the chemistry of meat pigments. He stated that myoglobin in fresh meat exists in three interconvertible forms, namely, oxymyoglobin, reduced myoglobin, and metmyoglobin. He concluded that oxymyoglobin imparts the desirable bright red color to meat, whereas, reduced myoglobin is purplish-red in color, and metmyoglobin is responsible for the undesirable brown to black discoloration occurring in fresh meat. Watts et al. (1966) stated that the balance between the different pigment forms is affected by the activity of enzymatic reducing systems in the meat and the oxygen concentration of the surrounding atmosphere. During cooking, the pigments are irreversibly converted to denatured ferric hemichromogens (Fox, 1966).

Brown et al. (1963) reported that ferric hemes are more active catalysts of lipid oxidation than ferrous hemes. The rapid oxidation of lipids in cooked meat has been attributed to catalysis by denatured ferric hemichromes (Younathan and Watts, 1959; Liu and Watts, 1970). The mechanism of the reaction is incompletely understood. Banks (1944) suggested that the active catalyst results from the combination of a fatty peroxide with an iron porphyrin. Maier and Tappel (1959) proposed that catalytically active hemes form unstable compounds with fat peroxides, which then decompose to give two free radicals, each of which in turn is capable of initiating autoxidation.

Tarladgis (1961) attributed the catalytic activity of

ferric hemoproteins to the paramagnetic character of the porphyrin bound iron. He suggested that the presence of five unpaired electrons in metmyoglobin produces a strong magnetic field that would favor the initiation of free radical formation. He further reported that the decomposition of hydroperoxides by a ferric porphyrin was mediated through the donation of an electron from the π cloud of the porphyrin ring.

According to Timms et al. (1958), oxidative rancidity in stored cooked meat, is higher in uncured than in cured meat. They suggested that differences in the heme pigments of cured versus uncured meat might be responsible for the differences in their oxidative behavior.

Waters (1971) stated that the nitric oxide complex of the heme containing compounds is structurally similar to organic nitrioxide free radicals (inhibitors of autoxidation) and have an unpaired electron which is more closely associated with the NO groups than with the iron. They suggested that in this way stereochemical and functional blocking of catalytic reactivity by heme containing systems may occur. Younathan and Watts (1959) hypothesized that the cured meat pigment, in which the 5th and 6th coordination position on the iron molecule are occupied by denatured globin and nitric oxide, respectively, would not be expected to react with a fat peroxide in the manner postulated for hematin or hemoglobin.

Even though heme pigments have traditionally been implicated as the major prooxidants in meat, there is

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evidence that non-heme iron may play an important role in accelerating oxidation of muscle lipids (Moskovits and Kielsmeier, 1960; MacLean and Castell, 1964; Sato and Hegarty, 1971). Moskovits and Kielsmeier (1960) demonstrated that contaminating iron fractions act as prooxidants in sausage. MacLean and Castell (1964) found that trace amounts of iron added to cod muscle produced a rancid odor. Sato and Hegarty (1971) showed that non-heme iron accelerated the oxidation of lipids in water extracted cooked meat. They also reported that myoglobin and hemoglobin failed to act as prooxidants in cooked meat.

The principles of metal ion catalysis in lipid oxidation have been reviewed by Ingold (1962; 1968) and Waters (1971). Lipids contain heavy metals originating from either metal activated enzymes (Ingold, 1962) or from direct contamination by contact with metals during processing (Patron, 1968). These authors concluded that heavy metals, notably iron and copper, which exist in several valency states, generally increase the rate of the oxidative reaction. Metals can also affect the rates of initiation and propagation and hydroperoxide degradation (Ingold, 1962).

Role of Phospholipids in Development of Warmed-Over Flavor in Cooked Meat

Watts (1954; 1961) suggested that lipid oxidation of adipose tissue was responsible for meat rancidity. Timms and Watts (1958) noted that there is little, if any, correlation

between flavor changes occurring in cooked meat and the oxidation of neutral lipids. Younathan and Watts (1960) concluded that the phospholipids in cooked pork are more susceptible to oxidation than the neutral lipid fraction.

Campbell and Turkki (1967) reported that during the cooking of meat the neutral lipids are lost more readily than the phospholipids. Thus, the ratio of phospholipids to total lipids increases during cooking. Similar results were obtained by Hornstein et al. (1961), who concluded that off-flavor development in cooked meat is greater than in raw meat because of a higher phospholipid ratio. Hornstein et al. (1961) indicated that polyunsaturated fatty acids (C_{18}, C_{20} and C_{22}) with two to six double bonds are found in animal tissues and are mainly responsible for development of warmed-over flavor (Lea, 1962). Acosta et al. (1966) showed that the phospholipid fraction is implicated in the early stages of autoxidation in turkey meat.

A number of investigators (Watts, 1954; Lea, 1962; Love and Pearson, 1971) have concluded that the lability of the phospholipid fraction is a result of their high unsaturated fatty acid content. For example, 19% of the fatty acids in beef muscle phospholipids have four or more double bonds, while only 0.1% of the triglyceride fatty acids from beef show this degree of unsaturation (Hornstein et al., 1961).

Oxidation of polyunsaturated fatty acids is

accompanied by the destruction of both fat-soluble and water-soluble vitamins (Holman, 1960; Kummerow, 1962; Lundberg, 1962). The products of oxidation of polyunsaturated fatty acids and their subsequent degradation products impart objectionable flavors and odors to foods (Holman, 1960; Keeney, 1962; Lundberg, 1962).

Influence of Nitrite on Meat Flavor

Bailey and Swain (1973) stated that nitrite serves several purposes during meat curing, including color fixation and as an antibacterial agent, but perhaps the most important feature of nitrite is its influence on flavor.

The relationship of nitrite to flavor was first described by Brooks *et al.* (1940) in studying the use of nitrite in curing bacon and ham. Although they presented no taste panel data, these authors stated that the panel showed a preference for meat cured with nitrite. Barnett *et al.* (1965) reported an extensive study on the factors affecting cured ham flavor. They reported that nitrite improved flavor of cured ham, but the level did not greatly influence acceptability. Cho and Bratzler (1970) studied the effect of nitrite and smoke on the flavor of cured pork roasts, and concluded that the flavor was improved in the roasts cured with nitrite. Wasserman and Talley (1972) found that frankfurter flavor became less desirable upon elimination of sodium nitrite from the cure. Thus, results demonstrated

that cooked, uncured frankfurters have an unappetizing flavor.

Swain (1972) studied the effect of nitrite on the flavor of hams and found that a taste panel rated smoked cured hams, unsmoked cured hams, smoked uncured hams and unsmoked uncured hams in order of intensity for cured flavor.

Simon et al. (1972) also investigated the flavor of frankfurters produced with either beef and pork or from beef alone while using different levels of sodium nitrite (0, 39, 78, 156 ppm). In frankfurters containing both beef and pork, taste panel results indicated that both nitrite level and storage time were associated with taste acceptance. In both vacuum packaged and bulk packaged frankfurters taste acceptance decreased with time, but was not associated with the nitrite level. In frankfurters produced from beef alone the addition of nitrite improved the flavor. Although taste panel scores slowly decreased with storage time, they were not related to the nitrite level. Simon et al. (1972) reported that the flavor of uncured frankfurters can be improved by incorporation of antioxidant (0.01% by weight of BHA or BHT), but the taste acceptance is still not equal to that of frankfurters cured with sodium nitrite.

Improvement of TBA Values on Adding Nitrite

Bulk stored frankfurters produced from beef, pork and mechanically deboned chicken with a spice extract of rosemary as a substitute for nitrite were studied by MacNeil and Mast (1973). TBA values were used as being indicative of oxidative changes during 16 days storage at 45°F. Results indicated that there was a significant decrease in TBA values when either nitrite or the spice extract was included in the frankfurter formation. The TBA values support results obtained by panel flavor scores, which show that extracts of rosemary as well as nitrite inhibit oxidation (Chipault et al., 1956). Zipser et al. (1964) found a high correlation between TBA values and oxidative off flavor in cooked meat. Thus, the TBA procedure has been used routinely to measure off flavor development in cooked meat and fish.

Swain (1972) used TBA values to measure the changes in oxidative products after cooking and following storage at 7°C for hams cured with and without nitrite. The TBA values of the nitrite-treated hams were initially lower than comparable samples without nitrite and remained lower during storage up to 2 weeks.

Younathan and Watts (1959) reported that nitrite and sodium chloride acted synergistically to retard oxidation of lipids in cooked meat stored at refrigerated temperatures. They concluded that the difference in flavor between nitrite

cured and non-nitrite cured meat soon after cooking is probably due to development of warmed-over flavor caused by rapid oxidation of unsaturated fatty acids.

Comparison of the Volatiles from Cured and Uncured Meat

The volatile compounds from cooked meat or meat extracts were found to comprise carbonyl compounds, organic acids, alcohols, sulfur compounds and ammonia (Lillard and Ayres, 1969). The carbonyls and the sulfur-containing substances are believed to be the predominant contributors to meat flavor (Hornstein et al., 1960; Kramlich and Pearson, 1960; Bender and Ballance, 1961). Gas chromatographic examination of the volatiles from cured and uncured ham by Cross and Ziegler (1965) showed that hexanal and valeraldehyde were present in appreciable quantities in the uncured product, but were barely detectable in the volatiles of the cured meat. They assumed that these volatiles were derived by oxidative cleavage of unsaturated fatty acid residues, probably from linoleate. Although butyraldehyde, propionaldehyde and acetaldehyde were also more prevalent in uncured hams, the differences between the cured and uncured meat were less pronounced. The results were essentially the same on comparing cured and uncured beef or chicken. The branched chain aldehydes (isobutyraldehyde, isovaleraldehyde, and 2-methylbutyraldehyde) occurred to the same extent in both cured and uncured meat. Sulfur compounds in both cured and

uncured meat were H_2S and methanethiol. Cross and Ziegler (1965) concluded that curing with nitrite does not seem to contribute any volatile compounds other than nitrogen oxides that are not present in cooked uncured meat. Thus, different aromas of the cured and uncured meat depend on the spectra of carbonyl compounds derived by oxidation of fats. They stated that nitrite prevents the oxidation of unsaturated lipids by deactivating the hematin catalysts.

Swain (1972) also compared volatiles from cured and uncured hams, and reported that isobutanal and higher molecular weight aldehydes were more concentrated in the uncured than the cured samples. There was an even greater difference in the level of these compounds when the hams were stored for 8 hours at $4^{\circ}C$ prior to evaluation. They suggested that oxidation of unsaturated fatty acids in uncured hams was responsible for formation of high molecular weight aldehydes. These results supported the findings of Cross and Ziegler (1965) showing that nitrite retarded oxidation of unsaturated fatty acids.

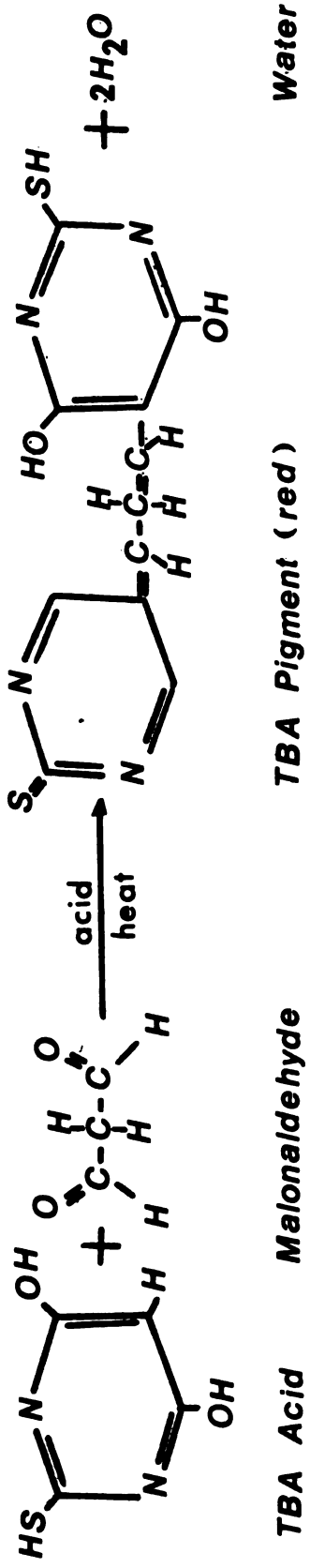
Piotrowski et al. (1970) concluded that there is a difference in the flavor of cured and uncured meat, which is associated with variation in the lipid phase.

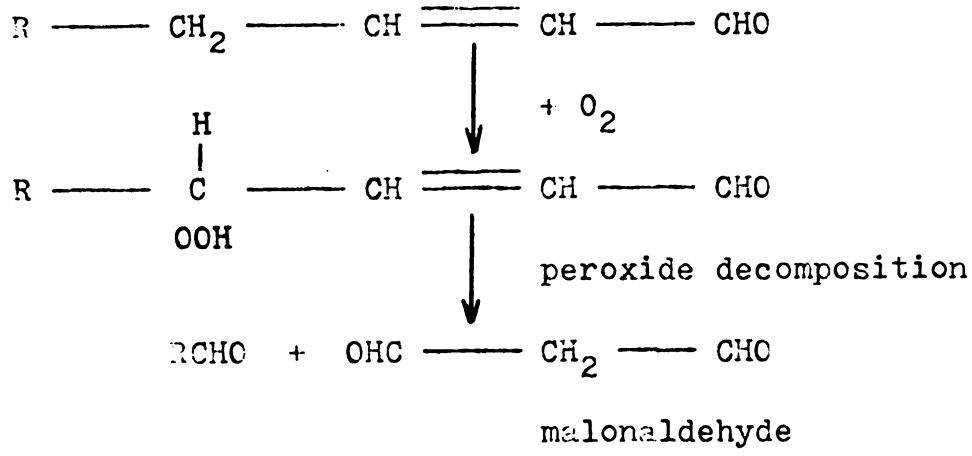
Thiobarbituric Acid Test

Sherwin (1968) reviewed the methods for determining the stability of fats and oils in foods. Current methodology available for evaluating the stability of lipids in foods has also been reviewed by Erickson and Bowers (1976). These workers concluded that the methods for determining lipid stability are based upon either measuring oxygen uptake, peroxide formation, or peroxide decomposition products.

The thiobarbituric acid test (TBA) was classified as measuring the final reaction products of peroxide decomposition. This method has been used for determining the extent of lipid oxidation in foods under a variety of test conditions (Turner et al., 1954). The method is based on the development and quantitation of a red pigment formed by the condensation of one molecule of malonaldehyde and two molecules of 2-thiobarbituric acid (Sinnhuber et al., 1958). The condensation occurs as shown on the following page.

The chemistry of the pigment has been studied by a number of investigators (Sinnhuber et al., 1958; Tarladgis et al., 1962; Yu and Sinnhuber, 1962; Marcuse and Johansson, 1973). Maximum absorbance of the red pigment has been shown to occur at 530 to 535 nm (Sinnhuber and Yu, 1958). The proposed mechanism of malonaldehyde formation is by dismutation and scission of aldehydes generated during hydroperoxide degradation (Day, 1966). The reaction proposed by Day (1966) is shown on Page 18.

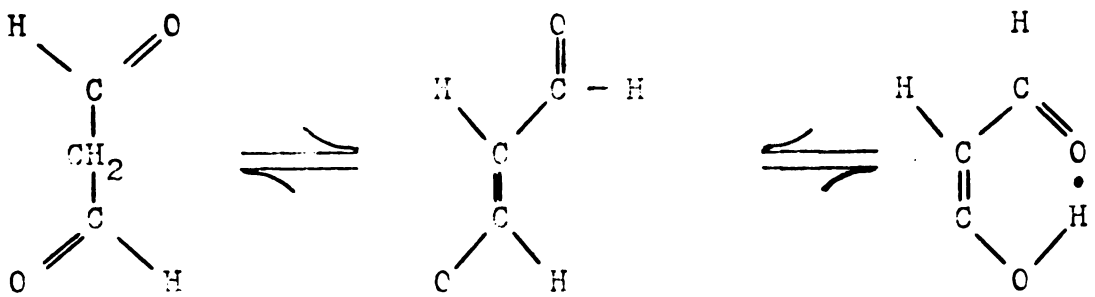

acid
heat
+2H2O
TBA Acid
Malonaldehyde
TBA Pigment (red)
Water



TBA numbers are quantified in mg of malonaldehyde per 1,000 g of sample as a result of the discovery that 1,1,3,3,-tetraethoxypropane (TEP) yields malonaldehyde upon acid hydrolysis (Sinnhuber and Yu, 1958). Kwon and Watts (1963) postulated that preformed malonaldehyde reacts with other food components and is not distillable. Thus, they proposed the term "distillable malonaldehyde" for use when describing the TBA test. After observing malonaldehyde in aqueous solution, Kwon and Watts (1964) concluded that malonaldehyde has the capacity to enolize from its diketo form (I) to its enolate anion (II), which is not volatile. A third volatile chelated form (III) is also possible (Kwon and Watts, 1964). These three forms are shown on the following page.

Kwon and Watts (1964) also indicated that in aqueous solution, almost all (96%) of the malonaldehyde is in the enolic form (II), and that the various forms are pH dependent. They also presented evidence that the enolic form

(II) occurs at pH > 7, while the chelated form (III) dominates at pH < 3. Therefore, Kwon and Watts (1964) indicated that maximum volatilization of free, preformed malonaldehyde occurs at pH < 3. The acid is added in order to free the malonaldehyde from possible combinations in food constituents.



(I) (II) (III)

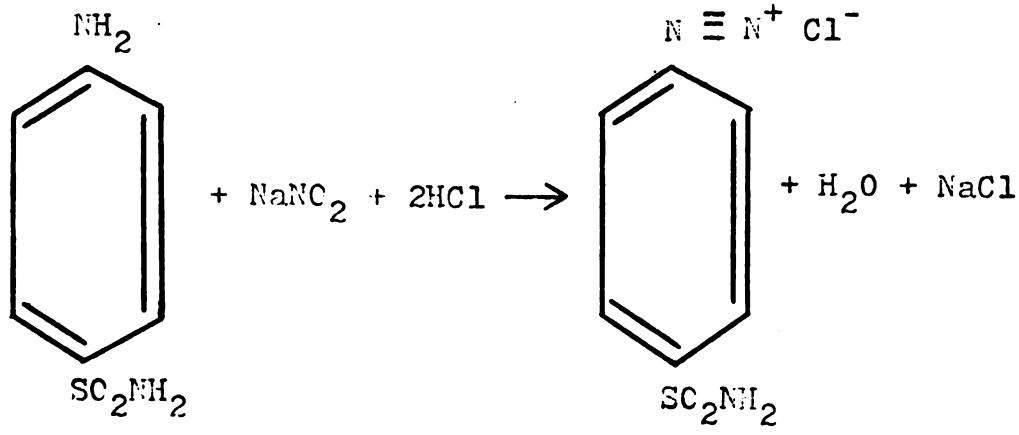
According to Tarladgis et al. (1960), malonaldehyde itself does not contribute to typical rancid odors, although a high correlation between malonaldehyde content and rancid odor has been noted (Zipser et al., 1964). The relationship may be limited to moist foods, especially to animal tissues (Kwon and Watts, 1964).

Pearson (1968) pointed out that the TBA test apparently measures the deterioration in both the extractable and non-extractable lipids. However, he further reported that relatively high TBA values may be found in some fresh samples, and yet in advanced stages of rancidity the TBA values may actually fall to zero or remain constant after reaching a maximum value.

Trace amounts of Fe^{+2} or Fe^{+3} have been reported to increase TBA values (Wills, 1964). Ascorbic acid has also been alluded to as a cause of high TBA values (Wills, 1966).

Hougham and Watts (1958) reported that the presence of 200 ppm of nitrite decreased the TBA value by 20-30%, but a concentration of less than 100 ppm did not interfere with the TBA test. Zipser and Watts (1962) stated that small amounts of nitrite ion are capable of significantly reducing TBA numbers in rancid meat, with the reduction increasing linearly with nitrite concentration. Nitrite interference with the TBA test takes place during the distillation step and is believed to be due to nitrosation of malonaldehyde.

Diazonium salt formation with sulfanilamide is utilized to bind the nitrite before beginning the TBA test (Zipser and Watts, 1962). The reaction is shown below.



Sulfanilamide

Diazonium salt

An advantage of the TBA test is that the fat does not need to be extracted from the rest of the muscle tissue (Tarladgis et al., 1960). Therefore, the TBA test would be expected to measure malonaldehyde produced from autoxidation occurring in all of the lipid fractions.

Off-flavor threshold values have been reported for TBA numbers in the range of 0.5 to 1.0 (Tarladgis et al., 1960; Watts, 1962). However, this range has not been firmly established. Jantawat and Dawson (personal communication) have reported threshold values for cooked chicken to be 4.0 mg malonaldehyde per 1,000 g of meat. Yu et al. (1969) stated that fish samples with a TBA value of 2.4 was judged to be acceptable, whereas, the samples with TBA values of 3.1 or greater were very rancid and unacceptable. Younathan and Watts (1959) have reported that TBA values for pork ranging from 0.46 to 0.60 are indicative of tissue rancidity.

Patton (1974) stated that the TBA test is highly sensitive and useful in monitoring lipid oxidation. However, Dugan (1976) stated that all objective methods available for determining lipid stability have their limitations. Therefore, sensory methods are necessary for absolute confirmation.

MATERIALS AND METHODS

Solvents and Chemicals

All solvents, chemicals and reagents were of analytical grade. Distilled deionized water was used throughout the study.

Source of Meat

Four 6 lb samples from each of three different species (chicken, beef and pork) were obtained for this study. The samples were used to determine the effect of nitrite on development of warmed-over flavor.

Chicken breast and thigh muscles were obtained 24 hrs postmortem. Pork semitendinosis and biceps femoris muscles were obtained at 24 hrs postmortem and were pooled and used for this study. Beef flank steak and hanging **tender** muscles were obtained at 24 hrs postmortem and were mixed together in the same proportion as they occur in the beef carcass.

All samples were trimmed of excess connective tissue and of subcutaneous adipose tissue. The chicken skin was removed from all chicken muscle samples.

Analytical Methods

Sample Preparation

The meat samples were chopped with a Hobart Silent Cutter, Model #84181D, for five minutes. During the chopping procedure, 5 g of sugar and 12 g of salt were dissolved in 200 ml of water, which was then mixed into the meat sample. After chopping, the sample was divided into two equal portions. One portion was mixed with 75 ml of water and chopped for an additional 5 minutes in the silent cutter. This was used as the control. The remaining 3 lb sample was mixed with 156 ppm of nitrite ion (as sodium nitrite) and the same volume of water and was chopped for an additional five minutes.

Each of the nitrite treated and control samples were divided into two portions. One portion was used raw and the other portion was cooked prior to chemical and sensory analyses.

Samples to be cooked were packaged in Cry-O-Vac bags and sealed. After weighing, the nitrite treated and control samples were placed in a boiling water bath separately and cooked until the meat reached an internal temperature of 70°C. After cooling at room temperature for 20 minutes, the samples were again weighed. Drip was then obtained by difference, by subtracting the weight of the cooked meat from the weight of the raw sample.

Zero-day samples from the raw and cooked meat with and

without added nitrite were analyzed immediately for TBA values, taste panel (raw samples were tested for aroma), and lipid analyses.

The remaining raw and cooked samples from the nitrite and control treatments were refrigerated for 48 hrs at 4°C. After storage, these samples were then analyzed for development of warmed-over flavor by the TBA test, taste panel and lipid analyses.

Measurement of Lipid Oxidation by the TBA Test

The distillation method of Tarladgis et al. (1960) was used for measuring TBA numbers. The distillation apparatus consisted of a 250 ml round bottom flask, which was attached to a Friedrich Condensor with a three-way connecting tube, and it was placed in an electric heating mantle. A duplicate 10 g sample of meat was homogenized with 50 ml of distilled deionized water for 2 minutes in a Virtis homogenizer at low speed. The homogenate was transferred quantitatively into a 250 ml round bottom flask by washing with 47.5 ml of distilled deionized water. The pH of the meat slurry was adjusted to 1.5 by the addition of 2.5 ml of 4 N HCl. Boiling chips were added and a small amount of Dow antifoam was sprayed into the flask to prevent foaming. The slurry was steam distilled using the highest setting on a power stat (the Superior Electric Company, Bristol, Connecticut) until 50 ml of the distillate were collected. The distillate was mixed and 5 ml were transferred to a 50 ml test

tube. Then, 5 ml of TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) were added. The tubes were stoppered and the contents mixed. The tubes were heated in a boiling water bath for 35 minutes. After cooling in cold water for 10 minutes, absorbance was read on a Beckman DU spectrophotometer at 538 nm against a blank containing distilled deionized water and TBA reagent. Absorbance readings were multiplied by a factor of 7.8 (Tarladgis et al., 1960). TBA values are expressed as mg malonaldehyde per 1,000 g of sample.

According to studies by Younathan and Watts (1959), Hougham and Watts (1958), and Zipser and Watts (1962), nitrite interferes with the distillation step by nitrosation of malonaldehyde. Thus, for the nitrite treated samples a modified TBA test (Zipser and Watts, 1962) was used to bind the nitrite by formation of diazonium salt with sulfanilamide. Ten g of nitrite treated meat was blended with 49 ml distilled deionized water and 1 ml of sulfanilamide reagent (0.5% sulfanilamide in 20% HCl - v/v) using a Virtis homogenizer at low speed for 2 minutes. The mixture was quantitatively transferred to a 250 ml round bottom flask by washing with 48 ml of distilled deionized water. Then, 2 ml of 4 N HCl was added to bring the pH to 1.5. The remainder of the procedure for TBA analysis was carried out as described by Tarladgis et al. (1960).

Extraction of Total Muscle Lipid

The procedure of Folch et al. (1957) as modified by Igene (1976) was used to extract the total lipids from the muscle tissue. A 100 g sample was homogenized in a Waring blender and extracted three times with 500 ml of a chloroform-methanol mixture (2:1 - v/v). The extract and tissue residue were then transferred to a medium grade sintered glass funnel and filtered under vacuum. The homogenizer and the residue in the funnel were washed with an additional volume of chloroform-methanol and filtered. The extract was quantitatively transferred into a 1,000 ml separatory funnel and 10% by volume of distilled water was added and thoroughly mixed. The mixture was allowed to separate into two phases until the interface was clear. The lower phase was transferred to a 500 ml volumetric flask and evaporated in a vacuum Rotavapor-R (Buchi, Switzerland) at 20-30°C. When the volume of the total lipid extract was reduced to 10-20 ml, the extract was quantitatively transferred to a previously tared 100 ml volumetric flask by washing with an additional quantity of chloroform-methanol. The final extract was further evaporated under a stream of nitrogen until it reached a constant weight. The weight of the lipids was then obtained by difference.

Isolation of Phospholipids and Neutral Lipids

Separation of the phospholipids from the total lipids was accomplished using the method of Choudhury et al. (1960).

This method involves separation on activated silicic acid, in which neutral lipids are preferentially removed by washing with chloroform, followed by solubilization of the phospholipids with methanol.

A weighed amount of silicic acid (20-25 g) was activated for 16 hrs by drying in a 100°C oven. The lipid sample was then quantitatively transferred to a 125 ml Erlenmeyer flask containing the activated silicic acid. The contents were shaken and allowed to settle for 6 hrs. The mixture was then thoroughly stirred and filtered through a sintered glass funnel under vacuum. The silicic acid was washed six times with 50 ml portions of chloroform. The filtrate and washings were combined and evaporated using the Rotavapor-R as described previously.

The phospholipid fraction was determined by washing the silicic acid residue with six 50 ml portions of methanol. The filtrate and washings were combined and evaporated to a constant weight using the Rotavapor-R.

The percent total lipids, neutral lipids and phospholipids in the raw and cooked meat samples were calculated.

Sensory Evaluation

To determine flavor changes, the samples were evaluated at 0 day and after 48 hrs storage at 4°C by 3 trained panelists. At each evaluation time, the panelists were presented with four different coded samples (raw without nitrite, cooked without nitrite, raw with nitrite, cooked with

nitrite). The stored cooked samples were reheated in Cry-O-Vac bags in a boiling water bath for 20 minutes prior to evaluation. The raw samples were scored only for aroma by panelists. Score sheets were designed so that the samples were scored from 1 to 5 (1 = very pronounced warmed-over flavor and 5 = no warmed-over flavor). The score sheet used is given in Appendix Table III.

Statistical Treatment

Analysis of variance for TBA values and taste panel scores was calculated using a Michigan State University computer package program identified as MSU Stat System and run on a Control Data Corporation (CDC) 6500 computer. Correlation coefficients were calculated using a Texas Instruments programmable calculator, Model SR52. Factors analyzed were TBA values, taste panel scores, total lipids, phospholipids as percent of tissue, and phospholipids as a percent total lipid. The significance of the computed correlation coefficients was determined by using the distribution of "r" table given by Snedecor and Cochran (1973).

RESULTS AND DISCUSSION

TBA Values for Meat Samples With and Without Nitrite

Raw and cooked samples of chicken, pork and beef with and without nitrite were analyzed for TBA numbers initially (0 days) and again after 48 hrs storage at 4°C. Table 1 presents the mean squares of TBA numbers of the different species. Appendix Table I contains the raw data, and Appendix Table II contains the analysis of variance for the TBA values.

Table 1 indicates that with the exception of pork, lipid oxidation in muscles from different animals of the same species behaved differently. This agrees with the results of Fitzgerald and Nickerson (1939), who found that the keeping quality of chicken fat varied between individual birds. The difference in behavior of the meat from different animals found in the current study may be due to the variation in the environment or to the previous history of the individual animals.

Table 1 also indicates that in chicken, pork and beef, there is a significant difference ($P < .01$) between TBA values for the nitrite treated and non-nitrite treated samples. Cooking treatment as well as storage time were also shown to be factors which significantly influenced

Table 1. Mean Squares for TBA Values of Chicken, Pork and Beef (samples with and without added nitrite)

Source of Variance	Degrees of Freedom	Mean Squares		
		Chicken	Pork	Beef
Animals	3	5.43**	2.44	2.22**
Nitrite vs. no nitrite	1	67.91**	40.92**	5.48**
Raw vs. cooked	1	7.23*	16.71**	5.78**
Fresh vs. stored	1	0.75	35.84**	1.04*
Nitrite levels x cooking	1	36.16**	15.66**	16.77**
Nitrite levels x storage	1	2.57	15.08**	2.28**
Cooking x storage	1	9.24**	14.62**	4.28**
Nitrite x cooking x storage	1	1.10	11.15**	0.87*
Residual error	21	0.89	0.89	0.15
Total	31			

** Significant at the 0.01 level.
* Significant at the 0.05 level.

the TBA values of the samples. Table 1 shows that there is a significant interaction between nitrite levels (0 and 156 ppm) and cooking treatment for all three species. There was a significant interaction between nitrite levels and storage time (0 day and 48 hrs) for pork and beef samples but the interaction was not significant for chicken, while all three species have significant interactions between cooking and storage. The interaction between nitrite x cooking x storage is not significant in chicken, but it is significant for pork and beef samples.

TBA Values for Chicken

Inspection of the values in Table 2 indicates that the TBA values of raw chicken with and without nitrite initially were not statistically different. However, the effect is in the same direction as the other effect and is probably real even though not significant. In fresh cooked samples there was a significant difference ($P < .01$) between TBA values of the nitrite treated and control (non-nitrite treated) samples. The nitrite dramatically inhibited the effect of storage and cooking on the development of warmed-over flavor by preventing oxidation as shown by much lower TBA values.

The TBA values for fresh raw nitrite treated chicken were only about half that of the samples without nitrite. Although the small number of samples prevented the difference from being statistically significant, the magnitude of the mean difference suggests that the addition of

Table 2. TBA levels from Chicken Muscles Treated with and without Nitrite[a]

Sample	TBA Values[b]		Mean Difference[c]
	Without Nitrite	With Nitrite	
Raw at 0 day	2.52	1.36	1.16
Cooked at 0 day	3.58	1.06	2.52**
Raw after 48 hr	5.52	1.47	4.05**
Cooked after 48 hr	6.98	3.05	3.93**

(a) Thigh and breast muscle.

(b) Each value is the average from 4 different batches.

(c) ** Significant at the 0.01 level by LSD.

nitrite protected against autoxidation.

Upon cooking at 0 days, the samples without nitrite showed a considerable increase in TBA values, but the nitrite treated chicken did not change appreciably. In fact, the nitrite treated sample had a slightly lower TBA value after cooking.

Slight changes in TBA values occurred in raw nitrite treated meat during 48 hrs storage at 4°C, whereas, nitrite free raw samples consistently increased in TBA values, increasing from 2.52 (0 day) to 5.52 (48 hrs at 4°C). The high rate of oxidation in raw chicken may be due to the grinding process, since Sato and Hegarty (1971) reported that warmed-over flavor occurs in raw ground meat with about the same rapidity and to the same extent as in cooked meat. Thus, they postulated that any process that disrupts the muscle membrane system, such as cooking or grinding, will result in exposure of the highly labile lipid components to oxygen and other reaction catalysts, thus, accelerating autoxidation.

In both nitrite treated and in non-nitrite treated cooked chicken, there was a marked increase in TBA numbers during storage at 4°C for an additional 48 hrs. In spite of the relatively large increase in the TBA values for the samples with nitrite, it was still below the threshold level. Dawson (personal communication) stated that the threshold value for TBA numbers in chicken is approximately four. The data in Table 2 clearly show that nitrite protects

against autoxidation, as shown by the considerably higher value for the nitrite free sample (6.98) as compared to the sample with nitrite (3.05).

TBA Values for Pork

Table 3 compares lipid oxidation for pork with and without added nitrite. The TBA value of the raw sample without nitrite before storage (0 day) was 1.52, which was about twice as high as that of the sample containing nitrite. The TBA value increased slightly on cooking the samples without nitrite, whereas, the mean value for similarly treated samples containing nitrite decreased slightly. However, changes in the TBA values of the pork samples with added nitrite were negligible upon cooking.

After storage for 48 hrs at 4°C, raw pork had a mean TBA value of 2.48, which is well above the threshold level of one to two reported by Watts (1962). Similar samples with added nitrite had a TBA value of only 1.42, which is lower than the samples without nitrite but still approaches the threshold level.

Table 3 shows that cooked pork with added nitrite is much more resistant to lipid oxidation during storage as compared to the stability of nitrite free cooked pork under the same conditions. Control cooked pork (without nitrite) increased about five-fold in TBA numbers during storage, with a mean value of 7.85. On the other hand, storage and cooking resulted in a negligible change in the TBA value

Table 3. TBA Levels from Pork Muscles Treated with and without Nitrite[a]

Sample	TBA Values[b]		Mean Difference[c]
	Without Nitrite	With Nitrite	
Raw at 0 day	1.52	0.85	0.67
Cooked at 0 day	1.83	0.72	1.11
Raw after 48 hr	2.48	1.42	1.06
Cooked after 48 hr	7.85	1.64	6.21**

(a) Semitendinosis and biceps femoris muscle.

(b) Each value is the average from 4 different animals.

(c) ** Significant at the 0.01 level by LSD.

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for pork containing added nitrite, with a TBA value of 1.64.

Table 3 indicates that although addition of nitrite reduced TBA values for both raw and cooked pork initially and during storage, the greatest inhibitory effect of nitrite on development of warmed-over flavor occurred during storage of the cooked samples. In this case, there was a significant ($P < .01$) difference between TBA values for nitrite free (7.85) and pork with added nitrite (1.64).

TBA Values for Beef

The oxidative process is not limited to meats containing a relatively high percentage of unsaturated fatty acids (chicken and pork), but also occurs in beef which contains a lower proportion of polyunsaturated fatty acids (Timms and Watts, 1958). Table 4 indicates there was a significant difference between the TBA values of beef with and without added nitrite both in the raw and cooked state. This was true both initially and after 48 hrs storage at 4°C.

Addition of nitrite significantly ($P < .01$) reduced lipid oxidation in cooked beef during storage (TBA values of 2.06 vs. 4.12). However, the variance estimate for beef is much smaller than that of chicken and pork. Thus, the smaller variance resulted in lesser differences in TBA values being significant. The TBA value for nitrite treated beef is just above the threshold level. Thus, results indicate that nitrite partially retarded lipid oxidation due to cooking and storage.

The data in Tables 2, 3 and 4 indicate that rate of

Table 4. TBA Levels for Beef Muscles with and without Nitrite[a]

Sample	TBA Values[b]		Mean Difference[c]
	Without Nitrite	With Nitrite	
Raw at 0 day	0.92	0.66	0.26*
Cooked at 0 day	1.07	0.75	0.32**
Raw at 48 hr	1.84	1.17	0.67**
Cooked at 48 hr	4.12	2.06	2.06**

(a) Composite sample of flank steak and hanging tender muscles.

(b) Each value is the average from 4 different animals.

(c) * Significant at 0.05 level by LSD.

(d) ** Significant at 0.01 level by LSD.

oxidation varies for the different species, with chicken and pork being the most rapid, followed by beef. This is in agreement with results reported earlier by Witte et al. (1970).

The ability of nitrite to retard lipid oxidation also differs between species. Pork and chicken, which have the highest lipid oxidation rate upon cooking and storage, are stabilized the most by the addition of nitrite. Oxidation of pork and chicken is reduced to below the threshold level, while beef with a lower level of lipid oxidation is less inhibited by nitrite and is reduced to just above the threshold value. These results agree with Younathan and Watts (1959) who have shown that resistance of cured meats to oxidation varies between species.

Sensory Panel Scores for Nitrite Treated and Non-nitrite Treated Chicken, Pork and Beef

Both raw and cooked samples of meat prepared with and without nitrite were presented to trained judges at 0 day and after 48 hrs storage at 4°C to evaluate for development of warmed-over flavor. Mean squares from analysis of variance are presented in Table 5. Appendix Table IV contains the raw data, and Appendix Table V contains the analysis of variance for taste panel scores.

Table 5 indicates that there was a significant ($P < .05$) difference in flavor/aroma scores between samples with and without

added nitrite in both pork and beef. There was also a significant interaction ($P < .05$) between the nitrite levels (with and without nitrite) and cooking treatments (raw or cooked) on the flavor/aroma scores produced in chicken. Storage time had a highly significant effect on flavor/aroma ratings of all three species. All other interactions were not significant.

Sensory Scores for Chicken

Table 6 presents sensory panel scores for nitrite treated and non-nitrite treated chicken, and shows the approximate level of significance for the mean differences using the Least Significant Difference (LSD) method as described by Snedecor and Cochran (1973). The data indicate that the aroma of the raw chicken at 0 day had no significant off-odor, regardless of whether or not nitrite had been added to the samples.

When the samples were cooked, there was essentially no difference in the scores of the untreated and nitrite treated samples, as both samples were scored as having slight to no warmed-over flavor. Raw chicken did not produce off-flavor during storage, regardless of whether or not nitrite had been used in the sample.

Table 6 shows that cooked chicken is readily subject to development of warmed-over flavor when stored under refrigeration. When nitrite was added, the flavor of the cooked samples did not change during storage. In the cooked

Table 5. Mean Squares Taste Panel Scores from Chicken, Pork and Beef (samples with and without added nitrite)

Source of Variance	dif.	Mean Squares		
		Chicken	Pork	Beef
Animals	3	0.33	0.61	0.74
Nitrite vs. no nitrite	1	0.29	1.53*	1.39*
Raw vs. cooked	1	0.09	1.93*	10.89**
Fresh vs. stored	1	4.72**	1.24*	2.33*
Nitrite x cooking	1	5.8 **	0.78	0.49
Nitrite x storage	1	0.16	0.41	0.01
Cooking x storage	1	0.16	0.27	0.128
Nitrite x cooking x storage	1	1.50	0.59	0.05
Residual error	21	0.68	0.27	0.31
Total	31			

** Significant at the 0.01 level.
* Significant at the 0.05 level.

Table 6. Taste Panel Scores for Chicken Samples and the Significance of Mean Differences

Sample	Taste Panel Scores[a]		Mean Difference
	Without Nitrite	With Nitrite	
Raw at 0 day	4.74	4.74	0
Raw after 48 hr	4.33	4.17	-0.15
Cooked at 0 day	4.16	4.83	0.67
Cooked after 48 hr	3.16	4.41	1.25*

(a) Each value is the average from 4 different animals.

(*) Indicates significance at 0.05 level by LSD.

samples after storage at 4°C for 48 hrs, there was a significant difference in the values for the untreated and nitrite treated chicken. Off-flavor development in the nitrite free sample was in the moderate warmed-over flavor range and was accompanied by high TBA values (Table 3), while nitrite treated chicken was scored in the slight to no warmed-over flavor range and was accompanied by lower TBA numbers.

Sensory Scores for Pork

The data in Table 7 demonstrate that raw pork did not have any off odor at 0 day, regardless of whether or not nitrite was added. Furthermore, pork did not produce distinguishable off odor during storage and both nitrite treated and control (without nitrite) samples were scored as having no warmed-over flavor. Even after cooking, there was no significant difference between the flavor/aroma of samples with or without nitrite, since both were judged as having slight to no warmed-over flavor. Nitrite free cooked pork produced moderate warmed-over flavor during 48 hrs storage at 4°C, whereas, the flavor of similar samples with added nitrite did not change upon storage and were scored as having very slight to no warmed-over flavor. Thus, cooked pork with added nitrite was signficantly ($P < .05$) preferred over the similarly treated samples without nitrite. This suggests that cooked pork is very susceptible to development of warmed-over flavor, while the addition of nitrite definitely retarded development of off flavor.

Table 7. Taste Panel Scores for Pork Samples and Significance of Mean Differences by LSD

Sample	Taste Panel Scores		Mean Difference
	Without Nitrite	With Nitrite	
Raw at 0 day	4.66	4.75	0.14
Raw after 48 hr	4.58	4.58	0
Cooked at 0 day	4.28	4.58	0.3
Cooked after 48 hr	3.24	4.58	0.34*

(*) Significant at the 0.05 level.

Sensory Scores for Beef

Data from Table 8 also indicate that there was no significant difference in the sensory scores of raw beef in the presence or absence of sodium nitrite, as neither sample had any off odor at 0 day. When the nitrite free beef was cooked, there was a decline in the sensory panel scores, and it was judged as having moderate warmed-over flavor. On the other hand, similar samples treated with added nitrite were scored as having slight warmed-over flavor.

Raw samples of beef, both with and without nitrite after 48 hrs storage at 4°C, were judged as having very slight off odor. After storage, there was a significant difference between flavor of nitrite treated and non-nitrite treated cooked beef; however, nitrite did not greatly retard the development of warmed-over flavor. In this case, the non-nitrite treated beef was scored as having moderate warmed-over flavor, while the nitrite treated samples were judged as having moderate to slight warmed-over flavor.

Total Lipid and Phospholipid Levels In Muscles from Different Species

Extraction of total lipids was carried out using a chloroform and methanol extraction procedure modified from the technique described by Folch *et al.* (1957). Separation of the phospholipids from the total lipids involved fractionation on a silicic acid column. The neutral lipids were

Table 8. Taste Panel Scores for Beef Samples and Significance of Mean Difference by LSD

Sample	Taste Panel Scores[a]		Mean Difference
	Without Nitrite	With Nitrite	
Raw at 0 day	4.91	4.74	-0.17
Raw after 48 hr	4.49	4.41	-0.08
Cooked at 0 day	2.99	4.07	1.08**
Cooked after 48 hr	2.99	3.83	0.84**

(a) Each score is average from 4 different animals.

(*) Significant at 0.05 level by LSD.

(**) Significant at 0.01 level by LSD.

preferentially removed by washing with chloroform followed by subsequent removal of the phospholipids with methanol.

Table 9 gives the mean values for total lipids and for neutral lipids as a percentage of tissue and for phospholipids, both as a percentage of total lipid and as a percentage of tissue. Appendix Table VI presents the raw data.

Table 9 shows the percentage of total lipids in chicken (2.85) and pork (3.51) were fairly similar; however, the total lipid level in beef (7.22%) was much higher. The percentage of neutral lipids in the tissues were very similar for chicken (2.14) and pork (2.77), while beef contained over two-fold more neutral lipids. The data in Table 9 also show that the percentage of phospholipid to total lipid increases as the percentage of lipid decreases. For example, beef, which contained 7.22% total lipid expressed as percent of tissue, contained only 10.40% phospholipid when expressed as a percentage of total lipid. This can be compared to corresponding values for chicken of 2.85 and 23.57%, respectively. This is in general agreement with the premise of Dugan (1971) that the percentage of phospholipid to total lipid increases from less than 10% to nearly 70% as total lipid decreases from 5 to 1%.

Table 9 also reports the average levels of phospholipid as a percentage of tissue for the three species. The mean phospholipid levels were 0.66% for chicken and pork and 0.73% for beef. These values are within the range of 0.5 to 1.0% phospholipid as a percentage of muscle, which

Table 9. Total Lipid, Neutral Lipid and Phospholipid Levels for Chicken, Pork and Beef Samples (a)

Sample	Total Lipids (% Tissue)	Neutral Lipids (% Tissue)	Phospholipid (% Lipid)	Phospholipid (% Tissue)
Chicken[b]	2.85	2.14	23.57	0.66
Pork[c]	3.51	2.77	19.66	0.66
Beef[d]	7.22	6.09	10.40	0.73

(a) Each value is an overall average of raw samples only for 4 different animals, regardless of presence of nitrite or storage time (0 day and 48 hrs).

(b) Breast and thigh muscles were mixed together.

(c) The semitendinosis muscle was mixed with the bicep femoris.

(d) Composite sample of flank steak and hanging tender muscles.

was reported earlier by Dugan (1971).

Correlation of TBA Values and Sensory Panel Scores

The correlation coefficients between TBA values and sensory scores for chicken, pork and beef were determined as described by Snedecor and Cochran (1973). The TBA values utilized in the calculations are shown in Appendix Table I, while the sensory panel scores are found in Appendix Table IV. Table 10 presents the calculated correlation coefficients.

Table 10. The Correlation Coefficients of TBA Values and Taste Panel Scores

Sample	No. of Samples	"r" Value
Chicken	32	-0.71**
Pork	32	-0.57**
Beef	32	-0.36*

** Significant at the $<$ 0.01 level.

* Significant at the $<$ 0.05 level.

All three species showed a significant negative correlation between TBA values and sensory panel scores. This suggests that in chicken, pork and beef, regardless of treatment, the samples with high TBA values tended to be scored lower than those with low TBA values. This further confirms the existence of a relationship between warmed-over flavor and sensory panel scores. In general, these results agree with those of Zipser et al. (1964), who found a high correlation between TBA values and development of oxidative off flavor in cooked meats.

The significance of the correlation coefficients between TBA numbers and sensory evaluation scores were tested for significance using the Z-test as described by Snedecor and Cochran (1973). It was found that the correlation coefficients for chicken and beef were significantly different ($P < .05$), whereas, the "r" values for chicken and pork and for pork and beef were not significantly ($P < .05$) different. The coefficient of determination for chicken was 50.4%, which indicated that over 50% of the variation in panel scores could be accounted for by a corresponding change in TBA values. For beef, on the other hand, only 12.9% of the variation in panel scores could be accounted for by a similar change in TBA numbers. Thus, results verify the greater degree of oxidative deterioration in chicken as compared to beef, while pork was intermediate between the other two.

Relationship of Total Lipid and Phospholipid Levels to TBA Values

The level of lipids in samples with and without nitrite (Appendix Table VI) were correlated separately against their corresponding TBA values (Appendix Table I). The lipid measurements subjected to correlation analysis with TBA values were total lipid and phospholipid as a percentage of tissue and phospholipid as a percentage of total lipid.

Table 11 presents the correlation coefficients between the TBA values and total lipids for samples with and without nitrite.

The relationships between total lipid levels and TBA numbers were not statistically significant ($P < .05$) for any of the three species (i.e., beef, pork and chicken). These results suggest that total lipid level is not an important contributor to TBA values. Thus, total lipids do not appear to be involved in development of warmed-over flavor. Conversely, Wilson (1974) reported that both porcine red and white muscle showed a significant positive correlation ($P < 0.10$) between TBA numbers and total lipid levels.

Table 12 shows that the TBA values and phospholipid levels as a percentage of muscle tissue were significantly ($P < .05$) related for pork samples without nitrite. This relationship suggests that high levels of phospholipids are major determinants for warmed-over flavor development in pork. However, addition of nitrite retarded oxidation of these lipids in pork muscle. All other correlation

Table 11. Correlation Coefficients between TBA Values and Total Lipid Levels

Sample	Samples Without Nitrite		Samples With Nitrite	
	No. of Sample	"r" Values	No. of Sample	"r" Values
Chicken	14	0.35	16	0.34
Pork	14	0.06	12	0.30
Beef	14	0.07	10	0.23

*Significant at the < 0.05 level.

Table 12. The Correlation Coefficient of TBA Values and Phospholipids as Percent of Tissue

	Samples Without Nitrite		Samples With Nitrite	
Sample	No. of Sample	"r" Values	No. of Sample	"r" Values
Chicken	14	0.41	16	-0.04
Pork	14	0.63*	12	0.14
Beef	13	0.17	10	0.30

(*) Indicates significance at the 0.05 level.

coefficients were not statistically significant. In contrast to these results, Wilson (1974) found a significant negative correlation ($P < .05$) between TBA values and phospholipids as a percentage of muscle tissue. This was true in both red and white muscle from pork and suggested that there was an inverse relationship between TBA values and phospholipid levels as a percentage of tissue.

Table 13 shows the correlation coefficients between TBA values and phospholipids as a percentage of lipid. The data show that the TBA numbers and the phospholipid levels were significantly ($P < .05$) correlated for the nitrite treated chicken, but the relationship was negative. Thus, results suggest that the TBA values increased in the nitrite treated chicken as the level of phospholipids (as percentage of lipids) declined. All other correlations were not significant. In contrast, Wilson (1974) found that the TBA values and the levels of phospholipid as a percentage of total lipid were significantly ($P < .05$) correlated for red muscles from pork, although the relationship was negative.

Dugan (1971) reported that the composition of phospholipids varies between animals and for different carcass locations. Lea (1957) stated that the phosphatidylethanolamine fraction accounts for very small percentage of the total phospholipids, whereas, phosphatidylcholine comprises the bulk of the phospholipids. He found that oxidation of phosphatidylcholine was only of the order of one hundredth of phosphatidylethanolamine. He further reported

Table 13. The Correlation Coefficient of TBA Values and Phospholipids as Percent of Total Lipids

Sample	Samples Without Nitrite		Samples With Nitrite	
	No. of Sample	"r" Values	No. of Sample	"r" Values
Chicken	14	0.24	16	-0.49*
Pork	14	0.43	12	-0.38
Beef	13	0.21	9	0.52

(*) Significant at the 0.05 level.

that the cephalin ("Kephalin") content of commercial lecithin has antioxidant effects based upon the inactivation of traces of catalytically active metals, such as Fe or Cu. Thus, in the present study the lower rate of oxidation at higher levels of phospholipid as a percentage of total lipid in chicken is postulated to be due to a change in phospholipid composition, more specifically, to a decline in the proportion of phosphatidylethanolamine and a corresponding increase in the proportion of the more oxidatively stable phospholipids such as phosphatidylecholine.

SUMMARY AND CONCLUSIONS

The studies reported herein were designed to ascertain the role of nitrite in development of warmed-over flavor in chicken, pork and beef. Samples with and without added nitrite were evaluated by TBA numbers and panel aroma/flavor scores before and following cooking at 0 days and after 48 hours storage at 4°C. In addition, the relationship between TBA numbers and total lipid and phospholipid levels were determined in an effort to ascertain their relative contributions to warmed-over flavor development.

The most dramatic change in TBA values for all three species occurred during refrigerated storage (48 hours at 4°C) of the nitrite-free cooked meat. Cooked meat without added nitrite had a 5-fold higher TBA value for pork and was 2-fold higher for beef and chicken than similarly treated samples containing nitrite. Thus, the addition of nitrite protected against oxidative changes during the storage of cooked meat.

Sensory panel scores confirmed the protective influence of the addition of nitrite upon development of warmed-over flavor in meat from all three species. However, the higher coefficient of determination verifies the greater magnitude of warmed-over flavor development in chicken as compared to

beef, whereas, pork was intermediate between chicken and beef.

Total lipid levels were not significantly correlated with TBA values for any of the three species, which suggests that total lipids are not major contributors to warmed-over flavor development. However, the correlation coefficient between TBA numbers and phospholipids as a percentage of tissue was significant ($P < .05$) in nitrite free pork, but was not significant in pork containing nitrite. Thus, results suggest that nitrite blocks the autoxidation of the phospholipids.

A significant negative relationship ($P < .05$) was obtained between TBA values and phospholipids as a percentage of total lipid in chicken with added nitrite. It is postulated that the lower rate of autoxidation at higher levels of phospholipids may be due to an increase in the relative amounts of the more oxidatively stable phospholipids, such as phosphotidylcholine.

BIBLIOGRAPHY

BIBLIOGRAPHY

Ackman, R. G. 1976. Prediction of fat stability. In "Proceedings of a symposium: Objective methods for food evaluation". Nat'l Acad. Sci., Washington, D.C., p. 272.

Acosta, S. O., Marion, W. W., and Forsythe, R. H. 1966. Total lipids and phospholipids in turkey tissue. Poultry Sci. 45:169.

Bailey, M. E. and Swain, J. W. 1973. Influence of nitrite on meat flavor. Proc. Meat Ind. Res. Conf. p. 29.

Banks, A. 1944. A method for studying the effect of antioxidants on the oxidation of aqueous suspensions of unsaturated fatty acids. J. Soc. Chem. Ind. (London) 63:8.

Barnett, H. W., Nordin, H. R., Bird, H. D. and Rubin, L. J. 1965. A study of factors affecting the flavor of cured ham. 11th European Meeting of Meat Research Workers. Cited by Wasserman, A. E. and Talley, F. J. Food Sci. 37:536 (1972).

Bender, A. E. and Balance, P. E. 1961. A preliminary examination of the flavor of meat. J. Sci. Food Agr. 12:683.

Brooks, J., Haines, R. B., Moran, T., and Pace, J. 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Dept. Sci. and Indust. Res., Food Invest. Spec. Report No. 49 (Great Britain).

Brown, W. D., Harris, L. S., and Olcott, H. S. 1963. Catalysis of unsaturated lipid oxidation by iron protoporphyrin derivatives. Arch. Biochem. Biophys. 101:14.

Campbell, A. M. and Turkki, P. R. 1967. Lipids of raw and cooked ground beef and pork. J. Food Sci. 32:143.

Chipault, J. R., Mizuna, G. B., and Lundberg, W. O. 1956. Antioxidant properties of spices in foods. Food Technol. 10:209.

Cho, L. C. and Bratzler, L. J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35:668.

Cross, C. K. and Ziegler, P. 1965. A comparison of the volatile fraction from cured and uncured meat. J. Food Sci. 30:610.

Choudhury, R., Roy, B., and Arnold, L. K. 1960. The determination of the neutral oil content of crude vegetable oil. J. Amer. Oil Chem. Soc. 37:87.

Day, E. A. 1966. Autoxidation of milk lipids. J. Dairy Sci. 43:1360.

Dugan, L. R., Jr. 1961. Development and inhibition of oxidative rancidity in foods. Food Technol. 15(4):10.

Dugan, L.R.,Jr. 1971. Fats. In "The science of meat and meat products". Price, J. F. and Schweigert, B. S. (eds.). W. H. Freeman and Co., San Francisco, Calif., p. 133.

Dugan, L. R., Jr. 1976. Prediction of fat stability. In "Proceedings of a symposium: Objective methods for food evaluation". Nat'l Acad. Sci., Washington, D.C., p. 265.

Erickson, D. R. and Bowers, R. H. 1976. Objective determination of fat stability in prepared foods. In "Proceedings of a symposium: Objective methods for food evaluation". Nat'l Acad. Sci., Washington, D.C., p. 133.

Farmer, E. H. and Sundralingam, A. 1942. The course of autoxidation reactions in polyisopresence and allied compounds. I. The structure and reactive tendencies of the peroxides of simple olefins. J. Chem. Soc. p. 121.

Farmer, E. H. and Sutton, D. A. 1943. The course of autoxidation reaction in polyisopresence and allied compounds. IV. The isolation and constitution of photochemically formed methylaleate peroxides. J. Chem. Soc.,p. 119.

Fitzgerald, G. A. and Nicherson, J. T. R. 1939. Problems arising during holding of poultry prior to evisceration and freezing. Proc. 7th World's Poultry Congress, Cleveland, Ohio, p. 505.

Folch, J., Lees, M., and Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497.

Fox, J. B., Jr. 1966. The chemistry of meat pigments. J. Agr. Food Chem. 14:207.

Holman, R. T. 1960. Enzymes and polyunsaturated fatty acids. In "Food Enzymes". H. W. Schultz (ed.). Avi Publ. Co., Westport, Conn., p. 75.

Hornstein, I. and Crowe, P. F. 1960. Flavor studies on beef and pork. J. Agr. Food Chem. 8:494.

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

Hougham, D. and Watts, B. M. 1958. Effect of variations in curing salts on oxidative changes in radiation sterilized pork. Food Technol. 12:681.

Igene, J. O. 1976. Effects of dietary fat and vitamin E upon the stability of meat in frozen storage. M.S. Thesis, Michigan State University, East Lansing, Michigan.

Ingold, K. U. 1962. Metal catalysis. In "Symposium on foods: Lipids and their oxidations". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 93.

Ingold, K. U. 1968. Principals of metal catalyzed lipid oxidation. In "Metal catalyzed lipid oxidation". R. Marcuse (ed.). SIK, Goteberg, Sweden. SIK-Rapport nr 240, p. 11.

Kaunitz, H. 1962. Biological effects of autoxidized lipids. In "Lipids and their oxidation". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 269.

Keeney, M. 1962. Secondary degradation products. In "Lipids and their oxidation". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 79.

Kramlich, W. E. and Pearson, A. M. 1960. Separation and identification of cooked beef flavor components. Food Res. 25:712.

Kummerow, F. A. 1962. Toxicity of heated fats. In "Lipids and their oxidation". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport,Conn., p. 294.

Kwon, T. W. and Watts, B. M. 1963. Determination of malonaldehyde by ultraviolet spectrophotometry. J. Food Sci. 28:627.

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

Labuza, T. P. 1971. Kinetics of lipid oxidation in foods. Crit. Rev. Food Technol. 2:355.

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

Lea, C. H. 1962. The oxidation deterioration of food lipids. In "Symposium on foods; Lipids and their oxidation". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 3.

Lew, Y. T. and Tappel, A. L. 1956. Antioxidant and synergist inhibition of hematin-catalyzed oxidative fat rancidity. Food Technol. 10:285.

Lillard, D. A. and Ayres, J. C. 1969. Flavor compounds in country cured hams. Food Technol. 23:251.

Liu, H. P. and Watts, B. M. 1970. Catalysts of lipid peroxidation in meat. 3. Catalysts of oxidative rancidity in meats. J. Food Sci. 35:596.

Love, J. D. and Pearson, A. M. 1971. Lipid oxidation in meat and meat products. A review. J. Amer. Oil Chem. Soc. 48:547.

Lundberg, O. 1962. Mechanisms of lipid oxidation. In "Lipids and their oxidation". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 31.

MacLean, J. and Castell, C. H. 1964. Rancidity in lean fish muscle. I. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. J. Can. Fisheries Res. Board 21:1345.

MacNeil, J. H. and Mast, M. G. 1973. Frankfurters without nitrates or nitrites. Food Prod. Devel. 7:36.

Maier, V. P. and Tappel, A. L. 1959. Products of unsaturated fatty acid oxidation catalyzed by hematin compounds. J. Amer. Oil Chem. Soc. 36:12.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading. II. TBA reactivity of different aldehyde classes. J. Amer. Oil Chem. Soc. 50:387.

Matsuo, N. 1962. Nutritional effects of oxidized and thermally polymerized fish oils. In "Lipids and their oxidations". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 321.

Moskovits, V. G. and Kielsmeier, E. W. 1960. An effect of iron and sodium chloride on flavor in sausage. Proc. 12th Res. Conf., Amer. Meat Institute Found., University of Chicago, Illinois, p.121.

Patron, A. 1968. Oxidation through metal catalysis and flavor stability of foods containing animal fats. In "Metal catalyzed lipid oxidation". R. Marcuse (ed.). SIK, Goteberg, Sweden. SIK-Rappert nr. 240, p. 171.

Patton, S. 1974. Malonaldehyde, lipid oxidation, and the thiobarbituric acid test. J. Amer. Oil Chem. Soc. 51(3):114.

Pearson, D. 1968. Application of chemical method for the assessment of beef quality. III. Methods related to spoilage. J. Sci. Food Agric. 19:553.

Piotrowski, E. G., Zaika, L. L., and Wasserman, A. E. 1970. Studies on aroma of cured ham. J. Food Sci. 35:321.

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

Sato, K. and Herring, H. K. 1973. The chemistry of warmed over flavor in cooked meats. In "Proc. 26th Ann. Recip. Meat Conf." Amer. Meat Sci. Assn., p. 64.

Sherwin, E. R. 1968. Methods for stability and antioxidant measurement. J. Amer. Oil Chem. Soc. 45:632A.

Sherwin, E. R. 1972. Antioxidants for food fats and oils. J. Amer. Oil Chem. Soc. 49:468.

Simon, S., Ellis, E. D., MacDonald, B. D., Miller, D. G., Waldman, R. C.,and Westberg, D. O. 1972. Influence of nitrite on quality of packaged frankfurters. 18th Ann. Meeting European Meat Research Workers, University of Guelph, Guelph, Ontario, Canada. Cited by Bailey, M. E. and Swain, J. W., Proc. Meat Ind. Res. Conf. (1973).

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

Snedecor, G. W. and Cochran, W. G. 1973. Statistical methods. 6th ed., The Iowa State University Press, Ames, Iowa.

Swain, J. W. 1972. Volatile flavor constituents of pork cured with and without nitrite. Ph.D. Thesis, University of Missouri, Columbia, Mo.

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

Tarladgis, B. G. 1961. A hypothesis for the mechanism of the heme-catalyzed lipid oxidation in animal tissues. J. Amer. Oil Chem. Soc. 38:479.

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

Timms, M. I. and Watts, B. M. 1958. The protection of cooked meats with phosphates. Food Technol. 12:240.

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

Wasserman, A. E. and Talley, F. 1972. The effect of sodium nitrite on the flavor of frankfurters. J. Food Sci. 37:536.

Waters, W. A. 1971. The kinetics and mechanism of metal-catalyzed autoxidation. J. Amer. Oil Chem. Soc. 48: 427.

Watts, B. M. 1954. Oxidative rancidity and discoloration in meat. Adv. Food Res. 5:1.

Watts, B. M. 1961. The role of lipid oxidation in lean tissues in flavor deterioration of meat and fish. In "Proceedings flavor chemistry symposium". Campbell Soup Co., Camden, New Jersey, p. 83.

Watts, B. M. 1962. Meat products. In "Symposium on Foods: Lipids and their oxidation". H. W. Schultz, E. A. Day and R. C. Sinnhuber (eds.). Avi Publ. Co., Westport, Conn., p. 202.

Watts, B. M., Kendrick, J., Zipser, M., Hutchins, B., and Saleb, B. 1966. Enzymatic reducing pathways in meat. J. Food Sci. 31:855.

Wills, E. D. 1964. The effect of inorganic iron on the thiobarbituric acid method for the determination of lipid peroxides. Biochem. Biophys. Acta 84:475.

Wills, E. D. 1966. Mechanism of lipid peroxide formation in animal tissues. Biochem. J. 99:667.

Wilson, B. R. 1974. Effect of total lipids and phospholipids on warmed-over flavor measured by TBA analysis in muscle from several species. M.S. Thesis, Michigan State University, East Lansing, Mich.

Witte, V. C., Krause, G. F. and Bailey, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. 35:582.

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

Younathan, M. T. and Watts, B. M. 1960. Oxidation of lipids in cooked pork. Food Res. 25:538.

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

Yu, T. C., Landers, M. L., and Sinnhuber, R. O. 1969. Storage life extension of frozen silver salmon steaks. Food Technol. 23:1602.

Zipser, W. M. and Watts, B. M. 1962. A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. Food Technol. 16(7):102.

Zipser, W. M., Kwon, T. W.,and Watts, B. M. 1964. Oxidative changes in cured and uncured frozen cooked pork. J. Agr. Food Chem. 12:105.

APPENDIX

APPENDIX TABLE I

TBA Values for Chicken, Beef and Pork

Sample No.		Species					
		Chicken		Beef		Pork	
		0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr
Raw Without $NaNO_2$	1	1.75	3.78	1.38	2.41	0.80	3.14
	2	2.89	6.84	1.13	2.46	2.04	2.66
	3	3.21	7.59	0.15	0.39	1.34	2.00
	4	2.24	3.79	1.04	2.12	1.91	2.12
mean		2.51	5.50	0.92	1.84	1.52	2.48
Raw With $NaNO_2$	1	1.36	0.87	0.92	1.68	0.21	1.26
	2	0.70	0.70	1.15	1.42	1.95	2.02
	3	2.15	3.01	0.10	0.44	0.24	0.73
	4	1.24	1.32	0.48	1.16	1.01	1.69
mean		1.36	1.47	0.66	1.17	0.85	1.42
Cooked Without $NaNO_2$	1	4.10	8.13	1.92	4.96	0.81	5.61
	2	2.89	5.68	1.34	3.84	2.23	6.23
	3	4.23	8.89	0.18	3.43	1.64	9.99
	4	3.12	5.23	0.86	4.25	2.66	9.60
mean		3.58	6.98	1.07	4.12	1.84	7.85
Cooked With $NaNO_2$	1	1.09	1.92	1.18	1.74	0.28	0.24
	2	0.65	4.36	1.27	1.97	1.41	1.73
	3	1.62	4.23	0.14	1.74	0.35	1.74
	4	0.91	1.72	0.41	2.81	0.87	2.83
mean		1.06	3.07	0.75	2.06	0.73	1.64

APPENDIX TABLE II-A

Table of Analysis of Variance for Chicken.
Dependent Variable is TBA Value

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Animals	16.300	3	5.433	6.054**
Nitrite vs no nitrite	67.919	1	67.919	75.687**
Raw vs cooked	7.239	1	7.239	8.066*
0 day vs 48 hr storage	0.756	1	0.756	0.842**
Nitrite levels x cooking	36.167	1	36.167	40.304
Cooking x storage	9.245	1	9.245	10.302**
Nitrite x storage	2.576	1	2.576	2.871
Nitrite x cooking x storage	1.102	1	1.102	1.228
Residual error	18.844	21	0.897	
Total	160.151	31		

* Significant at the 0.05 level.
** Significant at the 0.01 level.

APPENDIX TABLE II-B

Table of Analysis of Variance for Pork.
Dependent Variable is TBA Value

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Animals	7.325	3	2.441	2.718
Nitrite vs no nitrite	40.928	1	40.928	45.550**
Raw vs cooked	16.718	1	16.718	18.609**
Nitrite levels x cooking	15.666	1	15.660	17.437**
0 day vs 48 hr storage	35.849	1	35.849	39.903**
Nitrite x storage	15.083	1	15.083	16.789**
Cooking x storage	14.620	1	14.620	16.273**
Nitrite x cooking x storage	11.151	1	11.150	12.412**
Residual error	18.866	21	0.898	
Total	176.21	31		

* Significant at the 0.05 level.
** Significant at the 0.01 level.

APPENDIX TABLE II-C

Table of Analysis of Variance for Beef.
Dependent Variable is TBA Value

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Animals	6.681	3	2.227	14.57**
Nitrite vs no nitrite	5.486	1	5.486	35.91**
Raw vs cooked	5.788	1	5.788	37.89**
0 day vs 48 hr storage	1.047	1	1.047	6.85**
Nitrite levels x cooking	16.776	1	16.776	109.82*
Cooking x storage	2.284	1	2.284	14.95**
Nitrite x storage	4.285	1	4.285	28.05**
Nitrite x cooking x storage	0.874	1	0.874	5.72*
Residual error	3.207	21	0.1527	
Total	46.432	31		

* Significant at the 0.05 level.
** Significant at the 0.01 level.

APPENDIX TABLE III

Sensory Panel Score Sheet

Name:_____ Species:_____

Date:_____ Storage Time:_____

	Code Number				
1	Very pronounced WOF				
2	Pronounced WOF				
3	Moderate WOF				
4	Slight WOF				
5	No WOF				

APPENDIX TABLE IV

Taste Panel Scores[a]

Sample No.		Species					
		Chicken		Beef		Pork	
		0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr
Raw Without Nitrite	1	5.00	4.33	5.00	4.33	4.66	4.66
	2	4.33	4.00	5.00	4.33	4.33	4.33
	3	4.66	4.33	4.66	4.66	5.00	4.33
	4	5.00	4.66	5.00	4.66	4.66	5.00
mean		4.74	4.33	4.91	4.49	4.66	4.58
Raw With Nitrite	1	4.66	3.66	4.66	5.00	5.00	4.66
	2	5.00	4.66	5.00	5.00	4.00	4.33
	3	5.00	4.33	4.33	3.00	5.00	4.33
	4	4.33	5.00	5.00	4.66	5.00	5.00
mean		4.74	4.41	4.74	4.41	4.74	3.49
Cooked Without Nitrite	1	4.33	2.66	2.33	3.33	4.00	2.00
	2	4.33	3.33	3.00	2.00	3.66	4.00
	3	3.66	3.00	3.00	3.00	5.00	2.66
	4	4.33	3.66	3.66	3.66	4.33	4.33
mean		4.16	3.16	2.99	2.99	4.24	3.24
Cooked With Nitrite	1	4.66	4.33	3.66	4.00	4.33	3.66
	2	5.00	4.66	3.66	3.00	4.33	5.00
	3	4.66	4.33	4.66	3.33	5.00	4.66
	4	5.00	4.33	4.33	5.00	4.66	5.00
mean		4.83	4.41	4.07	3.83	4.58	4.58

(a) Each value is the average score from 3 different judges.

APPENDIX TABLE V-A

Table of Analysis of Variance for Chicken.
Dependent Variable is Taste Panel Score

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Animals	1.009	3	0.336	0.488
Nitrite vs no nitrite	0.290	1	0.290	0.422
Raw vs cooked	0.093	1	0.093	0.135
0 day vs 48 hr storage	4.720	1	4.720	6.856**
Nitrite levels x cooking	5.805	1	5.805	8.433**
Cooking x storage	0.163	1	0.163	0.238
Nitrite x storage	0.163	1	0.163	0.238*
Nitrite x cooking x storage	1.509	1	1.509	2.192
Residual error	14.455	21	0.688	
Total	28.212	31		

* Significant at the 0.05 level.
** Significant at the 0.01 level.

APPENDIX TABLE V-B

Table of Analysis of Variance for Pork.
Dependent Variable is Taste Panel Score

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Animals	1.849	3	0.616	2.204
Nitrite vs no nitrite	1.535	1	1.535	5.490*
Raw vs cooked	1.838	1	1.838	6.573*
0 day vs 48 hr storage	1.244	1	1.244	4.449
Nitrite levels x cooking	0.784	1	0.784	2.804*
Cooking x storage	0.416	1	0.463	1.488
Nitrite x storage	0.279	1	0.279	0.998
Nitrite x cooking x storage	0.591	1	0.591	2.110
Residual error	5.873	21	0.279	
Total	14.412	31		

* Significant at the 0.05 level.
** Significant at the 0.01 level.

APPENDIX TABLE V-C

Table of Analysis of Variance for Beef.
Dependent Variable is Taste Panel Score

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Animals	2.226	3	0.742	2.342
Nitrite vs no nitrite	1.390	1	1.390	4.462*
Raw vs cooked	10.890	1	10.892	34.964**
0 day vs 48 hr storage	2.338	1	2.338	7.505
Nitrite levels x cooking	0.497	1	0.497	1.596*
Cooking x storage	0.012	1	0.012	0.039
Nitrite x storage	0.128	1	0.128	0.413
Nitrite x cooking x storage	0.055	1	0.055	0.177
Residual error	6.542	21	0.311	
Total	24.084	31		

* Significant at the 0.05 level.
** Significant at the 0.01 level.

APPENDIX TABLE VI-A

Total Lipid, Neutral Lipid and Phospholipid Levels as a Percentage of Tissue and as a Percentage of Lipid in Chicken

Sample No.		Total Lipid (% of tissue)		Neutral Lipid (% of tissue)		Phospholipid (% of tissue)		Phospholipid (% of lipid)	
		0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr
Raw	1	2.70	3.01	1.96	1.93	0.70	0.71	25.30	23.50
Without	2	----	2.53	----	1.81	----	0.65	-----	25.69
Nitrite	3	3.27	3.34	2.59	2.65	0.67	0.67	20.40	20.05
	4	2.71	2.68	2.10	2.09	0.66	0.64	24.30	23.88
Raw	1	2.79	2.75	2.07	2.08	0.65	0.63	23.20	23.20
With	2	2.48	2.50	1.80	1.70	0.69	0.69	28.10	27.70
Nitrite	3	3.27	3.27	2.50	2.72	0.67	0.64	20.40	19.50
	4	2.69	2.77	2.06	2.07	0.66	0.65	24.80	23.60
Cooked	1	3.36	3.62	2.27	3.74	0.75	0.84	22.30	23.20
Without	2	----	3.19	----	2.26	----	0.82	-----	25.70
Nitrite	3	4.03	3.96	3.20	3.19	0.78	0.82	19.35	20.70
	4	3.26	3.33	2.53	2.56	0.78	0.79	23.90	23.90
Cooked	1	3.32	3.44	2.35	2.41	0.69	0.77	20.71	22.30
With	2	3.18	3.14	1.88	2.03	0.97	0.74	30.70	23.50
Nitrite	3	3.64	3.82	2.98	3.11	0.63	0.75	17.50	19.60
	4	3.50	3.09	2.48	2.38	0.79	0.71	22.50	23.20

APPENDIX TABLE VI-A (continued)

Animal	% Drip Lost During Cooking	
	Without Nitrite	With Nitrite
Chicken 1	8.56	12.24
Chicken 2	6.66	23.00
Chicken 3	15.00	13.00
Chicken 4	18.00	18.00

APPENDIX TABLE VI-B

Total Lipid, Neutral Lipid and Phospholipid Levels as a Percentage of Tissue and as a Percentage of Lipid in Pork

Sample No.		Total Lipid (% of tissue)		Neutral Lipid (% of tissue)		Phospholipid (% of tissue)		Phospholipid (% of lipid)	
		0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr
Raw	1	6.16	5.77	5.40	4.97	0.75	0.79	12.70	13.69
Without	2	3.53	3.39	2.82	2.67	0.74	0.64	20.90	18.87
Nitrite	3	3.00	2.83	2.00	2.18	0.63	0.62	21.00	21.90
	4	3.14	3.14	2.25	2.37	0.75	0.71	23.00	22.60
Raw	1	----	----	----	----	----	----	-----	-----
With	2	3.51	3.26	2.59	2.64	0.62	0.61	17.66	18.85
Nitrite	3	2.80	2.82	2.17	2.28	0.63	0.64	22.50	23.01
	4	3.17	2.72	2.31	2.23	0.69	0.46	21.76	16.91
Cooked	1	----	----	----	----	----	----	-----	-----
Without	2	4.98	5.14	4.05	4.09	0.77	0.90	15.46	17.50
Nitrite	3	4.68	4.13	3.37	3.29	0.91	1.01	19.44	24.95
	4	5.10	4.17	3.67	3.04	1.08	0.99	21.17	23.74
Cooked	1	----	----	----	----	----	----	-----	-----
With	2	4.97	4.96	3.91	3.43	0.90	1.10	18.10	22.17
Nitrite	3	4.43	4.82	3.42	3.62	0.96	1.09	21.60	22.85
	4	4.58	4.77	3.37	3.60	1.08	1.04	23.58	21.80

APPENDIX TABLE VI-B (continued)

Animal	% Drip Lost During Cooking	
	Without Nitrite	With Nitrite
Pork 1	31.98	25.00
Pork 2	30.00	29.59
Pork 3	38.80	29.50
Pork 4	30.50	31.50

APPENDIX TABLE VI-C

Total Lipid, Neutral Lipid and Phospholipid Levels as a Percentage of Tissue and as a Percentage of Lipid in Beef

Sample No.		Total Lipid (% of tissue)		Neutral Lipid (% of tissue)		Phospholipid (% of tissue)		Phospholipid (% of lipid)	
		0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr	0 Day	48 Hr
Raw	1	6.75	6.80	6.24	6.30	0.45	0.50	6.60	7.30
Without	2	8.03	8.14	6.89	6.86	0.66	0.71	8.28	8.78
Nitrite	3	7.82	8.49	6.76	6.28	0.91	0.85	11.70	10.02
	4	6.16	6.35	5.14	4.99	0.83	0.84	13.60	13.22
Raw	1	----	----	----	----	----	----	-----	-----
With	2	----	7.52	----	6.62	----	0.75	-----	-----
Nitrite	3	8.24	8.15	6.85	6.56	0.54	1.08	6.65	13.25
	4	5.52	6.00	4.46	5.18	0.67	0.80	12.13	13.33
Cooked	1	8.10	8.00	7.48	----	----	----	-----	-----
Without	2	11.23	----	9.10	----	1.12	----	10.02	-----
Nitrite	3	8.52	7.85	7.21	6.63	0.86	1.13	10.16	14.40
	4	6.87	7.99	5.66	6.57	0.83	1.00	12.08	12.51
Cooked	1	----	----	----	----	----	----	-----	-----
With	2	----	10.83	----	9.03	----	1.08	-----	9.97
Nitrite	3	8.40	8.81	6.56	7.28	0.95	0.90	11.30	10.20
	4	8.41	7.77	7.08	6.82	1.05	0.98	12.48	12.70

APPENDIX TABLE VI-C (continued)

Animal	% Drip Lost During Cooking	
	Without Nitrite	With Nitrite
Beef 1	20.00	20.00
Beef 2	28.00	28.00
Beef 3	9.89	6.20
Beef 4	30.00	14.00