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THE EFFECTS OF ANTIOXIDANTS ON THE FLAVOR AND COLOR STABILITY OF PRERIGOR FRESH PORK SAUSAGE

A Thesis

Presented for the

Master of Science

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ABSTRACT

Fresh prerigor pork sausage links and patties were processed with and without antioxidants to determine if antioxidants had any effect on color or flavor stability. BHA, BHT and citric acid were added for 0% or .003% in finished product. Each of the four treatments were stored at -18°C for 140 days or 4°C for 12 days. Treatments stored at 4°C were analyzed for oxidation (TBA), color, flavor, and psychrophile growth. Treatments stored at -18°C were analyzed for oxidation (TBA) and color.

Antioxidants did not reduce oxidation during 4°C storage but links had higher ($P<.05$) TBA values than patties. Links with antioxidants had higher ($P<.05$) TBA values than links with no antioxidants. Links had higher Hunter "a" values than patties, but contained 10% less fat. Patties had higher Hunter "L" values, most likely due to higher fat content. Storage temperature had no effect.

Antioxidants had no effect ($P>.05$) on flavor of links or patties. Consumer panelist flavor scores did not show significant differences between links and patties with or without antioxidants. Psychrophile counts (Log_{10}) indicated antioxidants may have had an antimicrobial effect.

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CHAPTER I

INTRODUCTION

The United States produces over 500 million kg of fresh sausage under federal inspection with pork sausage representing as much as 70% of this total (AMI, 1985). Demand for such products has grown steadily in recent years and there has been an increase in the number of producers. Pork sausage is produced conventionally by grinding postrigor meat, with salt and other spices being added during the mixing stage of production. Major concerns of the industry revolve around color and flavor stability of pork sausage products. Color and flavor are both important in consumer selection and satisfaction of the product.

Oxidative deterioration of pork sausage is a major concern because of the undesirable odor and flavor which becomes more intense and unpleasant as oxidation progresses (deMan, 1980). Oxidative reactions result in the formation of peroxides, aldehydes and free fatty acids with organoleptic changes being more closely related to the secondary products (aldehydes). Antioxidants can be used to effectively slow the oxidation process and increase the induction periods.

Industry and technological changes have stimulated increased interest in prerigor processing. Interest in prerigor pork processing has become more popular for reasons

of energy savings, speed of processing, superior binding properties, and other attributes (Kastner, 1977). Yet, little information has been reported about the color and flavor stability of prerigor pork sausage products when antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are incorporated into the product. Prerigor grinding of pork muscle has been shown to reduce the rate of lipid oxidation (Judge and Aberle, 1980). But the addition of salt and seasonings has been shown to have a prooxidant effect (Drerup et al., 1981). With the increase in popularity of prerigor processing, work needs to be conducted to determine if any benefits are derived from the use of antioxidants. The objective of this project was to determine the effect of antioxidants on the flavor and color stability of frozen and refrigerated prerigor processed pork sausage.

CHAPTER II

REVIEW OF LITERATURE

I. OXIDATION REACTION

Autoxidation or oxidation is a chemical reaction defined as the loss or transfer of electrons (Brown and Rogers, 1980). These reactions can occur in muscle food lipids and are recognized as an important factor affecting the quality and acceptability of foods with regard to flavor, odor, and color (Forrest et al., 1975; Brown and Rogers, 1980; Addis et al., 1985; Lillard, 1985). The characteristic flavor and odor of oxidized fat is caused by the presence of oxidative products, such as low molecular weight aldehydes, acids and ketones, that form during the oxidation and decomposition of the fatty acid molecules (Forrest et al., 1975; Brown and Rogers, 1980; Lillard, 1985).

The rate at which these fats are degraded is related to the degree of unsaturated fatty acids composing the lipid tissue. Polyunsaturated fats are more susceptible to autoxidation than are monounsaturated fats with saturated fatty acids being the most resistant to oxidation (Mark and Stewart, 1954; Brown and Rogers, 1980; Addis et al., 1985; Lillard, 1985). Discoloration can also be caused by the oxidation of heme pigments (Mark and Stewart, 1954). Both

lipid oxidation and heme pigment oxidation can accelerate each other but each can also proceed independently (Mark and Stewart, 1954). Following is a review of the chemical reactions involved in lipid oxidation and heme pigment oxidation.

Lipid Oxidation

Lipids are composed of fatty acids and other groups (Mark and Stewart, 1954) with unsaturated fatty acids being the initial substrate in lipid oxidation (Mark and Stewart, 1954; Lillard, 1985). The reaction initiates at carbon atoms adjacent to double bonds. Once the reaction is started, it proceeds by a chain reaction and is autocatalytic in that oxidation products catalyze the reaction and cause an increase in the reaction rate as oxidation proceeds (Mark and Stewart, 1954; Lillard, 1985).

The oxidation can be divided into three phases, initiation, propagation, and termination (Lillard, 1985). Initiation begins with the extraction of a hydrogen atom from carbon atoms adjacent to the double bonded carbon atoms (Figure 1) (Mark and Stewart, 1954). These reactions lead to the formation of free radicals with conjugated double bonds which in turn leads to the propagation phase (Lillard, 1985). Propagation begins when free radicals react with oxygen and extract hydrogen from other fatty acids to produce hydroperoxides and free radicals (Figure 1) (Lillard, 1985).

Products of oxidation that are formed during the propagation phase are referred to as primary or initial oxidation products but contribute very little to flavor and odors associated with oxidation (Mark and Stewart, 1954; Lillard, 1985).

(1) Initiation $2RH + O \longrightarrow 2R^{\cdot} + 2^{\cdot}OH$

(2) Propagation $R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot}$

$RO_2^{\cdot} + RH \longrightarrow RO_2H + R^{\cdot}$

(3) Termination $R^{\cdot} + R^{\cdot} \longrightarrow PR$

$R^{\cdot} + RO_2^{\cdot} \longrightarrow RO_2R$

$RO_2^{\cdot} + RO_2^{\cdot} \longrightarrow RO_2R + O_2$

RH = Generic unsaturated fatty acid.

O_2 = Molecular Oxygen.

$\cdot$ = Free Radical

FIGURE 1. The Oxidation Process.

The hydroperoxides, primary oxidation products, are very unstable and degrade by free radical mechanisms (Figure 1) to produce several classes of secondary oxidation products

(Keeney, 1962). These products are the major contributors to unacceptable odor and flavor (Lillard, 1985).

Myoglobin Oxidation

Color is recognized as one of the most important factors consumers use as a quality indicator of fresh red meats. Currently industry conditions have conditioned consumers to select fresh meat and processed meat items based on a bright cherry red color. This color is a direct contribution of myoglobin and its derivatives (Mark and Stewart, 1954).

Meat color relates to the chemical state of the heme pigments of myoglobin (Brown and Rogers, 1980). Myoglobin contains a protein portion (globin) and a nonprotein portion (heme). Heme is composed of an iron atom and a porphyrin ring (Brown and Rogers, 1980). The porphyrin ring binds with four of the six binding sites located on the iron atom, with the fifth binding site occupied by a group of histidine residues of the polypeptide chain which forms the globin structure (Dryden and Birdsall, 1980). This leaves the sixth binding site open to react with atoms that can donate a pair of electrons. This sixth binding site determines the critical properties of myoglobin. Livingston and Brown (1981) examined the iron molecule and its light absorption capabilities and determined the color of the various myoglobin complexes. Three forms of myoglobin can be found in fresh meat: (1) deoxymyoglobin (mb), (2) oxymyoglobin (omb), and (3)

metmyoglobin (mb). Deoxymyoglobin is the purplish-red meat color that corresponds to the freshly cut red meat. Myoglobin at this stage contains iron in the ferrous (Fe^{2+}) state. The oxygenated form of myoglobin is known as oxymyoglobin and is characterized by the bright red color most consumers are familiar with. Iron remains in the ferrous state (Fe^{2+}) as long as there is an adequate supply of oxygen. Metmyoglobin results once the reducing compounds are no longer available and the iron is oxidized to a ferric state (Fe^{3+}) which in turn yields a brownish-grey meat color.

II. FACTORS AFFECTING OXIDATION

Catalyst

As mentioned earlier, the oxidation process begins with the initiation reaction. This reaction is not thermodynamically favorable therefore a catalyst is needed to begin the initial reaction (Liu and Watts, 1970). Examples of common catalysts are high temperature, light, enzymes, and transition metals. Of these, transition metals and light seem to play the most important role in oxidation so the focus will be in these areas with brief consideration given to the other factors.

Transition metals (e.g., Fe and Cu) have been shown to react with hydroperoxides to produce free radicals

$$TM^{1+} + ROOH \longrightarrow Fe^{2+} + OH^{-} + RO\cdot$$

$$TM^{2+} + ROOH \longrightarrow TM^{2+} + H^{+} + ROO\cdot$$

$$2ROOH \longrightarrow RO\cdot + ROO\cdot + H_2O$$

TM = transition metal

(Tichivangan and Morrissey, 1985)

The example shows the mechanisms of how transition metals catalyze lipid oxidation by reaction with ROOH producing free radicals. Fennema (1985) has shown that levels of transitional metals at 0.1 ppm can decrease the length of the induction period and increase the rate of oxidation. A means of reducing the catalytic effect of metal ions is to use chelating agents to sequester the metals and prevent/reduce the onset of the initiation reaction.

Heme and non-heme iron can catalyze lipid oxidation (Greene et al., 1971). These studies have shown that heme pigments may be more active oxidation catalysts in the ferric state and that non-heme pigments may be more active catalysts in the ferrous state. This would indicate that as the natural color reactions take place and the amount of metmyoglobin increases, there should be an increase in lipid oxidation (Liu and Watts, 1970).

Lighting is another catalyst that plays a major role in lipid oxidation. Light, according to Caukins et al. (1986), significantly increased the rate of oxidation but light source showed no significant effect. Watts (1954) and MacDouglas (1982) also reported that light accelerated rancidity development.

Temperature and enzymes are also factors that affect oxidation but are not as significant as metals and light. It is well known that as temperature increases the rate at which chemical reactions occur also increases. The same holds true for the oxidation reaction, but since most meat/meat products are processed or at least held at refrigeration or frozen temperatures temperature will not be considered as a factor.

The final catalysts are enzymes. Lipid oxidation in meat is thought to be non-enzymatic even though oxidizing enzymes are present in meat (Love, 1983). Enzymatic oxidation has a greater impact on color than on lipid oxidation, but in both cases is insignificant when compared to the effect of other catalysts (Lin and Hultin, 1977).

Antioxidants and Chelating Agents

Antioxidants act as blocking devices in the oxidative chain reaction. Their basic function is to act as hydrogen donors which in turn slow or delay the onset of oxidation.

In current industry practice, four different types of antioxidants and three types of chelating agents are used in the manufacturing of fresh pork sausage. The limited use of antioxidants and synergists is in response to U.S.D.A. regulations. U.S.D.A. currently allows the use of the following antioxidants in fresh pork sausage: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ) and the following chelating agents: citric acid, monoisopropyl citrate and monoglyceride citrate. Usage levels for antioxidants and chelating agents are also regulated by the following parameters. The maximum usage level for BHA, BHT and PG, individually, is 0.01% based on the fat content of the product or 0.02% if the antioxidants are used in combination. TBHQ can be used at levels of 0.02% only in combination with BHA and BHT. The maximum usage levels for citric acid, monoisopropyl citrate and monoglyceride citrate are 0.01%, 0.02%, and 0.02%, respectively. These levels are also based on product fat content. Chelating agents benefit antioxidants by combining with metal ions which in turn reduces the oxidative reactive capabilities of the meat system. Additional benefits from the chelating agents may be that they increase the functional time of the antioxidant and inhibit peroxide decomposition (Lillard, 1985).

Several researchers have examined the use and benefits of antioxidants and synergists in fresh meat systems. In each study antioxidants and synergists were investigated as a means of preventing/slowing lipid oxidation and/or as a means of color preservation. Following will be a more detailed look at these studies.

Iron and ethylenediaminetetraacetic acid (EDTA) and their effect on lipid oxidation was examined in a prerigor ground pork system. Tay et al. (1983) added varying levels of iron and EDTA and measured the extent of lipid oxidation using the 2-thiobarbituric acid test (TBA). EDTA added at 0.02%, 0.2%, and 2.0%, and in the presence of added iron, inhibited the oxidation rate. Additional work revealed that as concentrations of EDTA exceeded 0.002%, a negative correlation existed between concentrations and oxidation rate, but when concentration levels were low (0.002%), EDTA actually enhanced the rate of oxidation. Kwoh (1971) concluded that EDTA inhibited oxidation by complexing with the non-heme iron. Kwoh (1971) also studied the affect of ascorbic acid and concluded that ascorbic acid reacted with the myoglobin pigment which in turn indirectly reduced the rate of oxidation by inhibiting metmyoglobin formation. Mahoney and Graf (1986) revealed that ascorbic acid, along with BHA, BHT, PG and TBHQ, reacted with free radicals formed during oxidation to form a more stable compound which in turn would yield a

longer shelflife, as measured by lipid oxidation, when the concentrations of the antioxidants and synergists were increased.

Color has also been shown to be affected by the use of antioxidants. Govindarajan and Hultin (1977) used antioxidants, citric acid and ascorbic acid in lean ground beef and discovered that the initial slow oxidation of myoglobin was not affected but that the additives did extend the time before the onset of rapid pigment oxidation. Additional support was generated by Greene et al. (1971) when they slowed oxidation in refrigerated, overwrapped ground beef by adding ascorbic acid in combination with PG and BHA to a meat blend. Ascorbic acid at 0.05% and at 0.1% was also shown to increase the color shelflife of ground beef (Shivas et al., 1984). Other researchers determined the affect of lipid and pigment oxidation on each other. Govindarajan and Hultin (1977) examined lipid and pigment oxidation by adding fresh lipid extract and lipid extract from aged ground beef to a ground beef system. Fresh lipid extract added to the ground beef system increased pigment oxidation and TBA values while the aged lipid extract did not affect TBA values and slightly reduced myoglobin oxidation. These later researchers concluded that early lipid oxidation is the most important to pigment oxidation.

Prerigor Meat Systems

Prerigor processing is justified in today's industry because of two benefits: (1) energy savings from the reduced need for refrigeration, and (2) the improved functional characteristics of hot processed meat (West, 1983). Reduced refrigeration needs are self explanatory. Functional characteristics can be more detailed in that prerigor meat has (1) higher water binding capacity, (2) higher pH, (3) increases cooking yields, (4) has a juicier more tender texture, and (5) increases the solubility of salt soluble proteins (Cross et al., 1979; Reagan et al., 1981; West, 1983). Most researchers have also shown that the ultimate higher pH of hot boned processed meats is of great benefit. These benefits include the ability to attract and bind water and to display a more desirable red color (West, 1983; Contreras and Cillard, 1986).

Prerigor meat systems have also displayed a reduced rate of lipid oxidation over post-rigor meat systems. TBA tests were conducted on salted and unsalted, prerigor and post-rigor ground pork muscle to compare lipid oxidation rates (Judge and Aberle, 1980). Conclusions from these tests indicate that prerigor ground porcine muscles are more stable to lipid oxidation than are post-rigor ground porcine muscles. It is also interesting to note that the pro-oxidant effect of salt was evident but the prerigor salted sample

was much less susceptible to lipid oxidation than the post-rigor salted sample (Judge and Aberle, 1980). Since salt is one of the major ingredients in pork sausage it should be further investigated to determine to what extent it effects oxidation and color degradation.

Salt has a negative effect on the properties of meat color. In a lean ground beef system, a 3% NaCl concentration increased the rate of myoglobin oxidation (Govindarajan and Hultin, 1977). Huffman et al. (1980) also found that NaCl at levels of 0.5% to 0.75% significantly discolored restructured fresh pork chops. The above mentioned work was done on post-rigor meat but additional work done by Marriott et al. (1983) suggests that the same holds true for prerigor meat systems. Marriott et al. (1983) manufactured restructured pork chops from prerigor and post-rigor pork and found slightly higher color scores for unsalted prerigor pork chops when compared to salted prerigor pork chops, however, the color scores for the salted prerigor chops were not significantly different than the salted post-rigor chops.

It is evident that prerigor meat systems offer an advantage in lipid and pigment oxidation over a post-rigor meat system. This advantage results in longer color and flavor stability. It is also evident that these benefits can be reduced by addition of NaCl to the system. Further research is needed to determine if these reduced benefits could be minimized if a salt substitute of similar functional characteristics were used.

III. METHODS FOR ANALYZING OXIDATION

To better understand the effect of oxidation we must understand the methodology used to measure oxidation. Most methods used to measure oxidation are designed to quantify the primary, secondary, or teritary products of oxidation. In this section of the review, methods that are currently used as measures of oxidation will be discussed. Outlined in this section will be peroxide values, the TBA test, and oxygen absorption.

Peroxide Values

The first products to develop during lipid oxidation are hydroperoxides, also known as peroxides. Peroxides are then used to liberate iodine from potassium iodide which in turn is measured to determine the degree of oxidation in the fat sample. Results are then expressed as milliequivalents of iodine formed per kilogram of fat (Gardner, 1979; Weiss, 1983). This method has been used in several studies to measure oxidation in pork products. Bailey et al. (1973) followed the oxidation of subcutaneous fat from pork carcasses stored at -20°C over a nine month period and found that the peroxide values remained low over the course of the study. Owen and Lawrie (1975) measured peroxide values in pork muscle stored at -10°C over 10 weeks and found that they were significantly higher than those reported by

Bailey et al. (1973). Another interesting note is seen in a study conducted by Tsai et al. (1978). In this study, peroxide values and TBA values were collected on ground pork samples stored at 0°C for nine days. Results were very erratic with large deviations seen in perioxide values as storage time increased. Gardner (1979) offered an explanation for the erratic results mentioned previously in that peroxides can react with proteins thus making it difficult to extract the peroxides. When the samples were ground in the Tsai et al. (1978) study the protein surface area was greatly increased thus increasing the amount of peroxides reacting with protein.

TBA Test

The TBA test is used as a means of measuring malonaldehyde and other lipid oxidation products (Pryor et al., 1976). Malonaldehyde is a secondary oxidation product which reacts with 2-thiobarbituric acid (TBA) to form a red complex. This red complex is then measured at an absorption maximum of 532 NM and compared back to a known standard curve to establish Mg of malonaldehyde per KG of sample or TBA number (Pryor et al., 1976).

The most common means of performing the TBA test is the Tarladgis distillation method (Tarladgis et al., 1960). In this method malonaldehyde is liberated through an acid wash, collected during steam distillation, and the distillate

is then taken through the TBA color reaction. This method has been modified by adding antioxidants such as BHA, propyl gallate, citric acid, or EDTA (Rhee, 1978) to the distallate to prevent oxidation during the distillation process. Other methods of performing the TBA test include the TBA reaction directly with the food sample followed by extraction/filtration of the colored complex (Sinnhuber and Yu, 1958).

Although each method mentioned is an acceptable way of performing the TBA test, differences do exist between methods (Witt et al., 1970). The distillation method yields higher TBA values than the extraction/filtration process. According to Witt et al. (1970), differences in results from the two methods can be explained by incomplete extraction/ filtration of malonaldehyde since no heat was involved in the method and/or that the heat from the distillation process prompted the release of malonaldehyde from carbonyl compounds which produced increased quantities of aldehydes. Buttkus (1967) felt erratic TBA number could be caused by the reaction between malonaldehyde and amino acids or proteins. Once the reaction occurred, malonaldehyde was unavailable for the TBA color reaction.

One additional factor that affects TVA values is the presence of certain bacteria. Certain bacteria completely destroy some aldehydes (Brown et al., 1979). These aldehydes

contribute to the TBA color formed during reaction, thus, decreasing the amount of aldehydes will lower the color absorption value resulting in lower TBA values.

CHAPTER III

MATERIALS AND METHODS

Prerigor pork sausage patties were manufactured from deboned meat from sow carcasses approximately 1 hr. post slaughter. The sows weighed 204 to 318 kg at time of slaughter. Deboned meat was coarse ground through a 1.27 cm plate, followed by a final grind through a 0.40 cm plate before being mixed with seasoning and water in a 1200 lb Rietz ribbon mixer for approximately 45 seconds yielding a meat block at 40% fat. Meat was then stuffed into a barrier teepack poly film (5.1 cm diameter) using a Kartridge Pack vacuum stuffer. After stuffing, the product was chilled in a glycol chiller to an internal temperature of 0°C to 5°C, placed in a -28.8°C blast freezer and held 48 hours. The stuffed product was then sliced into 42.5 g patties and packed into a wax-lined paperboard carton with each carton containing eight patties. Cartons were then placed into corrugated cardboard boxes for frozen storage as described later.

Prerigor pork sausage links were manufactured from the previously mentioned deboned pork also. The deboned meat was chopped through a 3 blade bowl chopper for approximately three revolutions. Coarse chopped meat was then transferred

to a CO_2 injected ribbon mixer and mixed with seasoning and water. During mixing, CO_2 snow was injected into the blend to reduce the temperature to -5°C to 0°C. Coarse chopped meat was then final ground through a 0.40 cm plate, transferred to a continuous vacuum stuffer and stuffed into edible collagen Teepack casings (21 mm diameter). Strands were then cut into 28.35 g links at approximately 9.2 cm in length using Devro Z-Linker with a "B" cam. Link will then be quick frozen through a mechanical CO_2 freezer, packed into a foam tray at 12 links per tray, and overwrapped with a non-barrier Saran based film. Trays were then packed into corrugated cardboard boxes for frozen storage.

After the patties and links were packed, they were stored in a -28.8°C blast freezer for 48 hours. After 48 hours in the blast freezer, the links and patties were transferred into a 0°F freezer and stored for eight days to simulate industry distribution. After storage, patties and links were transported from the Nashville processing plant to the University of Tennessee Meat Lab and stored in a -28.9°C freezer for evaluation.

The experiment was divided into four treatments. Patties and links processed with seasonings containing salt, natural and oleoresin spices, sugar and monosodium glutamate but no other antioxidant were treatment 1 and treatment 3,

respectively (Table 1). Patties and links processed with seasonings containing salt, natural and oleoresin spices, sugar, and monosodium glutamate were treatments 2 and 4, respectively. Treatments 2 and 4 contained the antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and citric acid at 0.003% each in finished product (Table 1). Each treatment was then subjected to two separate storage conditions, refrigerated and frozen.

TABLE 1. Experimental Treatments

	Treatments [1]			
	1	2	3	4
BHA %	--	0.003	--	0.003
BHT %	--	0.003	--	0.003
Citric Acid	--	0.003	--	0.003

[1] 1 = Links without antioxidants; 2 = links with antioxidants; 3 = patties without antioxidants; 4 = patties with antioxidants.

Refrigerated samples (treatments 1-4) were prepared by removing product from the frozen storage and placing it in a refrigerated cooler at 4°C to simulate retail display temperatures. Total storage time for refrigerated display was 12 days with samples being pulled at the start and every day over the 12 day period for color and TBA analysis. Microbiological analysis was performed on samples every other day and sensory conducted on samples pulled at day zero and every fourth day of the study.

Frozen samples (treatment 1-4) were prepared using the following format. Samples frozen during normal processing at 0°F were labeled as frozen treatment Day 0 on the eighth day of actual frozen storage. After labeling, samples were returned to the 0°F freezer for holding. Total storage for frozen samples was 160 days. During the storage period, samples were pulled for each treatment at Day 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, and 140 for color and TBA analysis.

Color evaluations were made using a Hunter Color Difference Meter (Model D25-2). Color of pork sausage links was measured on four different sets (four links/set) of links. Color of pork sausage patties was measured on four different patties. Color was measured on all treatments for both storage conditions. Frozen samples of both patties and links were removed from the freezer and allowed to stand

at room temperature for approximately 1 hour prior to analysis. This step was necessary for frozen product to remove frost and allow the product to bloom prior to color analysis. After the 1 hour hold time for frozen samples, the frozen and refrigerated samples were analyzed under identical procedures. A pink plate (Standard #2167; L = 69.1; "a" = 22.0; "b" = 11.9) and a white plate (Standard #LS-13601; L = 91.0; "a" = -1.3; "b" = 1.6) were used to standardize the colorimeter. Four links were then placed over the opening with the flat side down and gently pressured to insure that the entire opening was covered. One patty was placed on the Hunter instrument port so that the patty covered the entire opening. "L" (light/dark), "a" (red/green), and "b" (yellow/blue) values were recorded by treatment which yielded four observations per treatment.

Oxidation was monitored over storage time for both the frozen and thawed samples. The method selected to monitor oxidation was the 2-thiobarbituric acid test (Talardgis et al., 1960) as modified by the addition of PG and EDTA (Rhee, 1978). Four pork sausage links and four pork sausage patties were randomly selected for analysis from each tray/carton across all treatments and each storage condition.

Each link and pattie sample was cut into approximately 2.54 cm pieces, frozen in liquid nitrogen and powdered in a Waring Blender. Two 10 g samples from each treatment and

storage condition were weighed into a Virtis Homogenizer cup. Five mL of 0.5% propyl gallate, 5 mL of a 0.5% EDTA, and 40 mL of deionized water were added to the 10 g powdered meat sample and blended for two min. using a Virtis Homogenizer. After homogenization, 2.5 mL of 4 N HCl was added to the sample and the entire mixture was transferred to a Kjeldahl flask. The homogenizer was washed with 47.5 mL of deionized water which was also transferred into the Kjeldahl flask. Boiling beads and 0.5 mL of Dow Antifoaming Agent was added to the flask. The flask was then heated, using a Kjeldahl apparatus, until 50 mL of distillate was collected. Five mL of distillate was then added to 5 mL of 0.02 m TBA and heated in a hot water bath for approximately 30 minutes for maximum color development. Samples were cooled for approximately 10 minutes and then the read for absorbance was determined using a Unicam SP1100 infrared spectrophotometer at 532 nm wave length. Absorption values were then recorded and malonaldehyde content determined. TBA numbers (mg ma/kg meat) were then calculated from the standard curve.

The standard curve was prepared by creating six tubes of solutions with known malonaldehyde concentration in each tube. Tetra-ethoxy propane (TEP) was diluted to 2×10^{-8} and mixed with distilled water in test tubes as shown in Table 2. Five mL of TBA reagent was then added to each tube

and heated in a water bath for 30 min. or until full color developed. Solutions were then cooled 10 min. and absorption was determined at 532 nm. Linear regression was used to calculate levels of malonaldehyde in the treatment samples.

TABLE 2. Standard Curve Preparation

Mls of $2NO^{-8}$ TEP	Mls of Distilled Water
0	5
1	4
2	3
3	2
4	1
5	0

Samples were also collected for microbial analysis. Aerobic plate counts for psychrophiles for each treatment during refrigerated storage were determined on samples used for TBA analysis at Day 0, 2, 4, 6, 8, 10, and 12 of storage. Samples were diluted with sterile peptone water using the Trekmar stomacher model 400. Psychrophiles were then plated and incubated on standard methods agar for 10 days at 4°C before being counted and recorded as LOG_{10} colony forming units.

All samples were prepared and counted in duplicate. Frozen samples were not subjected to microbial analysis.

Sensory analysis included consumer panels evaluating flavor for each treatment at Day 0, 4, 8, and 12 of refrigerated storage. In order to have all samples of all storage times available on one day, samples were transferred from frozen storage to refrigerated storage as follows: Day 12 refrigerated samples 12 days prior to testing, Day 8 refrigerated samples 8 days prior to testing, Day 4 refrigerated samples 4 days prior to testing, and Day 0 refrigerated sample the day of testing. Sausage samples from all treatments were cooked on the 176.7°C surface of a Sunbeam kitchen grill (Model RC) to an internal temperature of 56.9°C. Links were placed on the grill and turned every 2 to 3 min. for a total of 20 min. Patties were placed on the grill and cooked for approximately 3-1/2 to 4 min. on the first side and 3 to 3-1/2 min. on the second side. All samples were then quartered and held between 48.9°C and 54.4°C until served.

Samples were divided by product type. Patties were evaluated during one panel sitting and links were evaluated in the second panel sitting. Sampling was divided, because of the large number of samples to be evaluated, so that panelists evaluated links with and without antioxidants from Days 0, 4, 8, and 12 of storage. At the following meeting, panelists evaluated patties with and without antioxidants from

Day 0, 4, 8, and 12 of storage. In each panel, eight coded samples were presented singularly and in random order in covered petri dishes to each panelist. Panelist then evaluated the flavor of each sample using an eight point hedonic scale where 1 = dislike extremely and 8 = like extremely, as listed in Table 3.

After sampling, each panelist completed a questionnaire concerning demographics. The questionnaire dealt with gender, and age of panelist and frequency of eating sausage, and the form of product most frequently used.

All data were analyzed by analysis of variance with treatment, product, and storage as independent variables (Mendenhall, 1979). Nonsignificant interactions were used as part of the error term to test the model (Mendenhall, 1979; SAS, 1985). Student Newman-Keuls mean separation was used to establish range of all means as well as differences between any pair of means (Mendenhall, 1979).

TABLE 3. Sensory Scorecard

Sausage Scorecard

Panelist Number _____ Sample Number _____

You will receive 8 samples followed by a questionnaire. After testing each of the samples, please take a bite of apple to clear your palate.

Please taste the sample of sausage and describe how well you like the flavor of the sample.

Like Extremely	_____
Like Very Much	_____
Like Moderately	_____
Like Slightly	_____
Dislike Slightly	_____
Dislike Moderately	_____
Dislike Very Much	_____
Dislike Extremely	_____

CHAPTER IV

RESULTS AND DISCUSSION

Results and discussion will be presented initially by storage condition because most of the major emphasis of this study was focused on retail products. It is important to mention prior to discussion of any results that differences did exist in TBA values between pork sausage links and patties. The significant differences could have been caused by variation in chemical properties of the meat block, variation in seasoning formulation, or by other, unknown processing effects. Analysis of variance tables for the dependent variables may be found in the appendix.

Mean TBA values for refrigerated sausage links and patties by treatment are shown in Table 4. Mean TBA values for the 12 day refrigerated storage time were significantly different between link and patty products, with links having a higher TBA value than the patties. In addition, the antioxidants did not significantly affect the mean TBA value of either product over the 12 day refrigerated storage period.

As mentioned earlier, the TBA test is an indirect measure of oxidation, measuring malonaldehyde, a secondary product of the oxidation reaction in food products. Therefore, as oxidation proceeds through the steps of initiation, propagation, and termination, increased levels of malonaldehyde during

the propagation phase of the oxidation process can be expected. To better understand the effect of antioxidants on the meat system, TBA values within each treatment were analyzed as a function of storage times to determine if the antioxidants increased the initiation phase of the oxidation process.

TABLE 4. Mean[1] TBA Values by Treatment for Pork Sausage Products Stored 12 Days at 4°C

Treatment[2]	TBA Value[3]
1	0.490[a]
2	0.456[a]
3	0.197[b]
4	0.229[b]

[1]n = 24.

[2]Treatments: 1 = link without antioxidant; 2 = link with antioxidant; 3 = patty without antioxidant; 4 = patty with antioxidant.

[3]TBA Values: Expressed as mg malonaldehyde/kg meat.

[ab]Means in the column with different superscripts are significantly different at $P<.05$.

Table 5 shows differences between TBA values within treatments across refrigerated storage time. Generally, the TBA values increased significantly over the first 2 to 3 days of storage and the product treated with antioxidants (treatments 2 and 4) had slightly lower peak TBA values during the initial days of storage. Table 5 shows that peak TBA values were delayed approximately one day in products treated with antioxidants (treatments 2 and 4) compared to the products with no antioxidants (treatments 1 and 3). Figure 2 illustrates the TBA values by treatment and shows that as storage time increased there was a gradual decline in TBA values, after the first peaks occurred at 2 or 3 days of storage, in all treatments except links with antioxidants (treatment 2). Treatment 2 TBA values peaked on the third day of storage, then decreased until day 7 when they started increasing to a slightly higher peak at day 9 followed by a decline to the end of the storage period. This is not consistent with data reported in the literature (Drerup et al., 1981). Possible explanations for the decrease in TBA values could be that prerigor meat was used to make the product, malonaldehyde can react with itself resulting in poor extraction and lower TBA values, or bacteria could be destroying malonaldehyde which would result in lower TBA values.

Results of the Hunter color evaluations ("L", "a", and "b" values) for each treatment are presented in Table 6.

TABLE 5. Mean[1] TBA Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at 4°C

Day[4]	Treatment[3]			
	1	2	3	4
1	0.189^{b}	0.202^{b}	0.018^{b}	0.003^{b}
2	0.706^{a}	0.542^{ab}	0.390^{a}	0.187^{ab}
3	0.513^{ab}	0.575^{a}	0.310^{a}	0.265^{a}
4	0.462^{ab}	0.501^{ab}	0.249^{ab}	0.237^{a}
5	0.443^{ab}	0.495^{ab}	0.351^{a}	0.300^{a}
6	0.312^{ab}	0.425^{ab}	0.353^{a}	0.308^{a}
7	0.396^{ab}	0.364^{ab}	0.237^{ab}	0.261^{a}
8	0.433^{ab}	0.480^{ab}	0.206^{ab}	0.185^{ab}
9	0.534^{ab}	0.582^{a}	0.204^{ab}	0.183^{ab}
10	0.495^{ab}	0.472^{ab}	0.128^{ab}	0.130^{ab}
11	0.507^{ab}	0.483^{ab}	0.169^{ab}	0.171^{ab}
12	0.482^{ab}	0.519^{ab}	0.173^{ab}	0.126^{ab}

[1] $n = 2$.

[2] TBA Values: Expressed as mg malonaldehyde/kg meat.

[3] Treatments: 1 = link with antioxidants; 2 = link without antioxidants; 4 = pattie with antioxidants; 3 = pattie without antioxidants.

[4] Time in days product stored at 4°C.

[ab] Means in the same column with different superscripts are significantly different at $P<.05$.

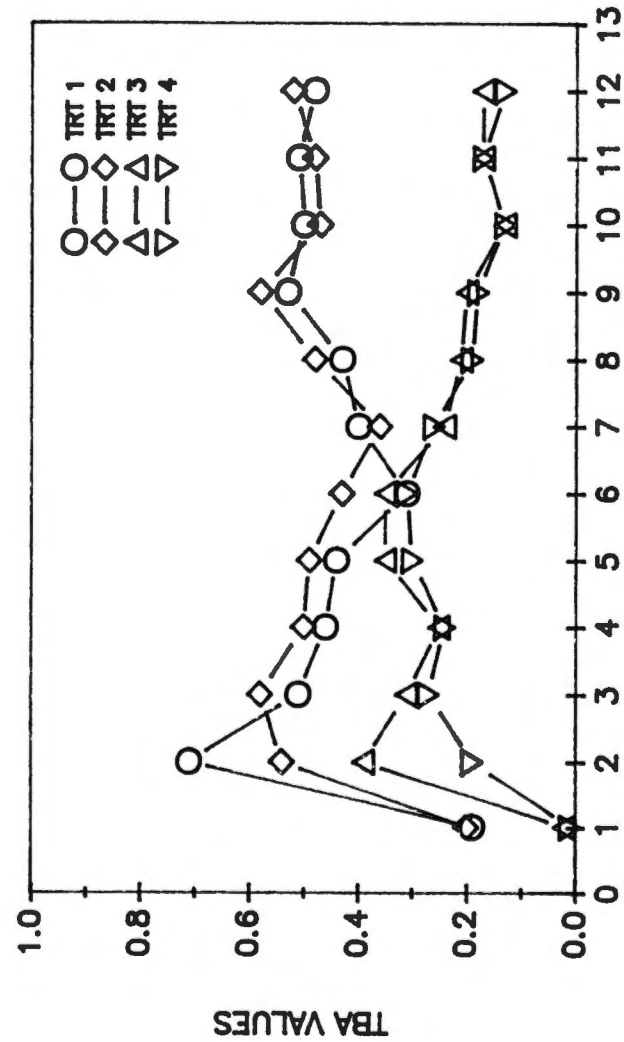

FIGURE 2. Mean TBA Values for Treatment and Storage Time for Pork Sausage Products Stored at 4°C.

TABLE 6. Mean Hunter Color Values by Treatment for Pork Sausage Products Stored 12 Days at 4°C

Variable	Treatment[1] 1	2	3	4
Hunter "L" Value[2]	49.3[a]	50.2[b]	55.3[c]	57.1[d]
Hunter "a" Value[3]	15.3[c]	14.1[b]	9.0[a]	9.2[a]
Hunter "b" Value[4]	12.2[bc]	12.3[c]	11.5[a]	12.0[b]

[1]Treatments: 1 = links with no antioxidants; 2 = links with antioxidants; 3 = patties with no antioxidants; 4 = patties with antioxidants.

[2]Hunter "L" values denote lightness or darkness; 100 = white, 0 = black.

[3]Hunter "a" values denote redness/greenness; higher positive numbers indicate greater redness.

[4]Hunter "b" values denote yellowness/blueness; higher positive numbers indicate greater yellowness.

[ab]Means in the same row with different superscripts are significantly different at $P<.05$.

The mean Hunter "L" values were significantly different among all treatments. Compared with treatments containing no antioxidants, treatments containing antioxidants had a higher Hunter "L" value during the early stages of refrigerated storage and maintained a lighter appearance throughout the storage period (Table 7). It was interesting to note that severe color degradation occurred from storage day 11 to 12 of the study across all treatments with the exception of treatment 2 (Figure 3). In general, antioxidants seemed to aid in maintaining a lighter color but did not delay the onset of color degradation (Figure 3).

Hunter "L" values also differed between product type. Table 6 and Figure 3 show that links had significantly lower "L" values than the patties. A combination of two factors can be used to explain this difference. First, according to the processor, the links were produced with a fat content of approximately 30% while the patties were produced with a fat content of approximately 40%. Second, the seasoning used to manufacture links contained a ground red pepper while the patty seasoning contained a crushed red pepper. The net result is the ground red pepper used in the links caused the product to take on a red tint across the lean and fat particles which reduced the whiteness of the product and lowered the "L" value. Therefore, with both a lower fat content and the effect of seasoning on color, the links should have had a lower "L" value than the patties.

TABLE 7. Mean[1] Hunter "L" Values[2] for Treatment and Storage Time for Pork Sausage Products Stored at 4°C

Day[4]	Treatment[3] 1	2	3	4
1	48.3^{b}	49.5^{ab}	56.2^{a}	57.0^{a}
2	48.1^{b}	49.7^{ab}	56.2^{a}	57.0^{a}
3	49.1^{b}	50.5^{ab}	55.2^{a}	57.9^{a}
4	50.0^{ab}	52.8^{a}	54.9^{a}	58.0^{a}
5	49.7^{ab}	50.4^{ab}	55.9^{a}	57.8^{a}
6	49.4^{ab}	50.4^{ab}	57.4^{a}	57.3^{a}
7	49.9^{ab}	51.1^{ab}	57.2^{a}	58.1^{a}
8	48.5^{b}	50.9^{ab}	53.7^{a}	56.6^{a}
9	48.4^{b}	49.5^{ab}	54.7^{a}	57.2^{a}
10	49.7^{ab}	49.2^{b}	56.9^{a}	57.8^{a}
11	52.2^{a}	50.9^{ab}	56.2^{a}	57.4^{a}
12	47.0^{b}	49.8^{ab}	49.9^{b}	53.0^{b}

[1] n = 4.

[2] Hunter "L" values denote lightness or darkness; 100 is perfect white, 0 is black.

[3] Treatments: 1 = link without antioxidants; 2 = link with antioxidants; 3 = pattie without antioxidants; 4 = pattie with antioxidants.

[4] Day represents time in storage at 4°C.

[ab] Means in the same column with different superscripts are significantly different at $P<.05$.

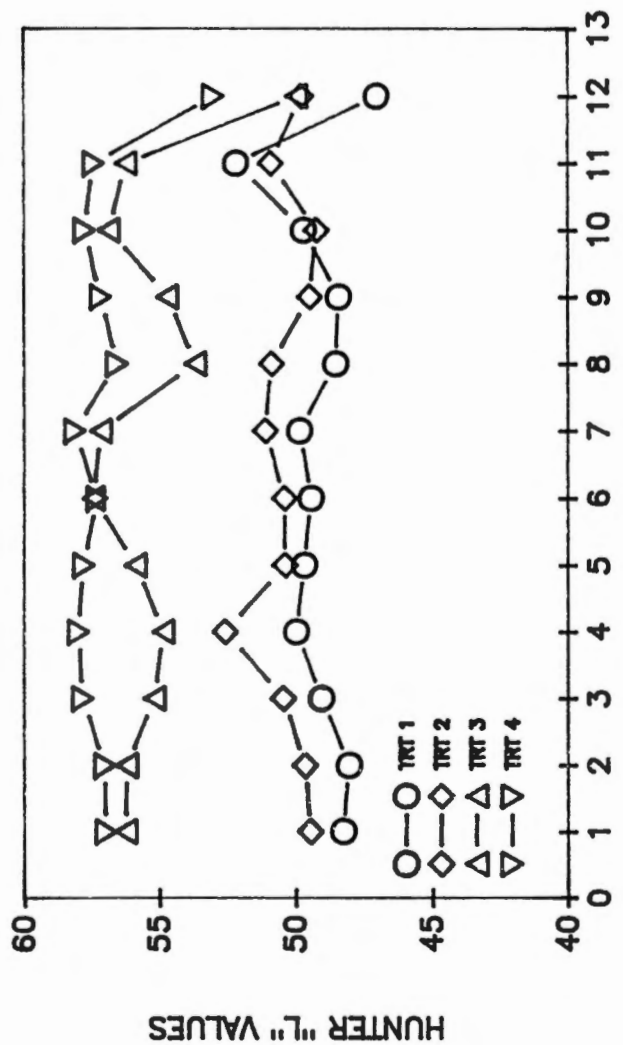

FIGURE 3. Mean Hunter "L" Values for Treatment and Storage Time for Pork Sausage Products Stored at 4°C.

Hunter "a" values were significantly different among treatments when averaged across refrigerated storage (Table 6). Links without antioxidants (treatment 1) had significantly higher Hunter "a" values than links treated with antioxidants (treatment 2). These results mean links without antioxidants maintained a more intense redness throughout refrigerated storage than did links treated with antioxidants. Greene et al. (1971) and Shivas et al. (1984) reported that ground beef treated with antioxidants had less darkening (a redder color) than untreated ground beef. This data is contradictory to those reports but may be explained when Hunter "a" values are evaluated by day and treatment across storage time (Table 8).

Initial Hunter "a" values for links without antioxidants (treatment 1) were higher than the initial Hunter "a" values for links treated with antioxidants (treatment 2). Figure 4 indicates that Hunter "a" values for these two treatments followed the same general trend when graphed across storage time. The significant difference could be attributed to the initially higher Hunter "a" values plus the fact that the different Hunter "a" values between the two treatments increased in the later storage times. This can be further justified by the line followed by the patties (Figure 4). Both patties with and without antioxidants had similar initial Hunter "a" values and followed a very similar line across

TABLE 8. Mean[1] Hunter "a" Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at 4°C

Day[4]	Treatment[3]			
	1	2	3	4
1	15.5^{bcd}	15.1^{a}	10.2^{a}	10.2^{a}
2	15.4^{bcd}	15.0^{a}	10.0^{ab}	10.4^{a}
3	14.9^{dc}	14.5^{abc}	10.2^{a}	10.2^{a}
4	15.2^{bcd}	13.8^{abcd}	9.5^{abcd}	9.6^{a}
5	15.7^{abc}	14.1^{abcd}	9.7^{abc}	9.7^{abc}
6	15.2^{bcd}	14.0^{abcd}	8.5^{d}	8.7^{c}
7	14.7^{dc}	13.4^{bcd}	8.7^{dc}	8.9^{bc}
8	16.4^{ab}	15.1^{a}	9.5^{abcd}	9.6^{abc}
9	16.6^{a}	14.8^{ab}	9.1^{bcd}	9.1^{bc}
10	14.8^{dc}	12.9^{d}	7.3^{e}	7.5^{d}
11	14.1^{dc}	13.0^{cd}	7.4^{e}	7.7^{d}
12	14.3^{dc}	13.2^{cd}	8.0^{de}	9.9^{ab}

[1] $n = 4$.

[2] Hunter "a" values denote redness/greenness; higher numbers indicate greater redness.

[3] Treatments: 1 = link without antioxidants; 2 = link with antioxidants; 3 = pattie without antioxidants; 4 = pattie with antioxidants.

[4] Day represents time in storage at 4°C.

[a-e] Means in the same column with different superscripts are significantly different at $P<.05$.

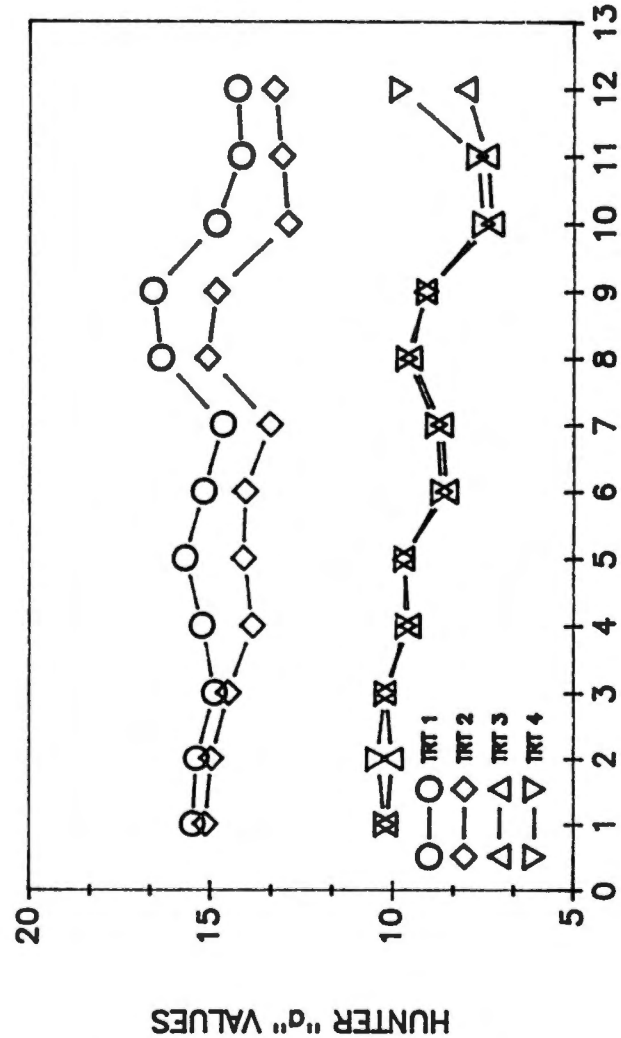

FIGURE 4. Mean Hunter "a" Values for Treatments and Storage Times for Pork Sausage Products Stored at 4°C.

storage time resulting in no significant differences in the Hunter "a" value between treatments 3 and 4 (Table 6).

Significant differences also existed between product Hunter "a" values. Links had higher ($P<.05$) Hunter "a" values than the patties. This was explained earlier by the seasoning and fat differences between products. Links containing ground red pepper and a lower fat content should have had the higher Hunter "a" value.

Mean Hunter "b" values also were significantly different between treatments (Table 6). Patties without antioxidants (treatment 3) had lower ($P<.05$) Hunter "b" values than did patties with antioxidants (treatment 4). Hunter "b" values were not significantly different between link products. Hunter "b" values for patties were lower than for links, however, the Hunter "b" values for patties with antioxidants (treatment 4) were not different ($P>.05$) from Hunter "b" values for links without antioxidants (treatment 1).

Differences between patties without antioxidants and patties with antioxidants could be attributed to initial Hunter "b" values (Table 6). Patties without antioxidants (treatment 3) had lower Hunter "b" values at the beginning of the study than patties with antioxidants (Table 9, Figure 5). The low Hunter "b" values were maintained throughout the storage time (Table 9). Figure 5 better illustrates this point and shows that the Hunter "b" values of both treatments

TABLE 9. Mean[1] Hunter "b" Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at 4°C

Day[4]	Treatments[3]			
	1	2	3	4
1	12.4^{a}	12.5^{a}	11.7^{ab}	12.1^{ab}
2	12.3^{a}	12.5^{a}	11.6^{ab}	12.0^{ab}
3	11.4^{a}	12.1^{a}	11.7^{ab}	12.6^{a}
4	12.3^{a}	12.6^{a}	12.0^{a}	12.1^{ab}
5	12.4^{a}	12.5^{a}	11.4^{ab}	12.0^{ab}
6	11.8^{a}	12.6^{a}	11.4^{ab}	11.9^{ab}
7	12.1^{a}	12.2^{a}	10.8^{c}	12.1^{ab}
8	12.6^{a}	12.6^{a}	11.5^{ab}	12.5^{a}
9	12.6^{a}	12.5^{a}	11.7^{ab}	11.9^{ab}
10	12.1^{a}	11.9^{a}	10.7^{c}	11.3^{b}
11	12.0^{a}	12.3^{a}	11.2^{c}	11.5^{b}
12	12.2^{a}	12.1^{a}	12.0^{a}	12.2^{ab}

[1] $n = 4$.

[2] Hunter "b" values denote yellowness/blueness; higher numbers indicate greater yellowness.

[3] Treatments: 1 = link without antioxidants; 2 = link with antioxidants; 3 = patties without antioxidants; 4 = patties with antioxidants.

[4] Day represents time in storage at 4°C.

[abc] Means in the same column with different superscripts are significantly different at $P<.05$.

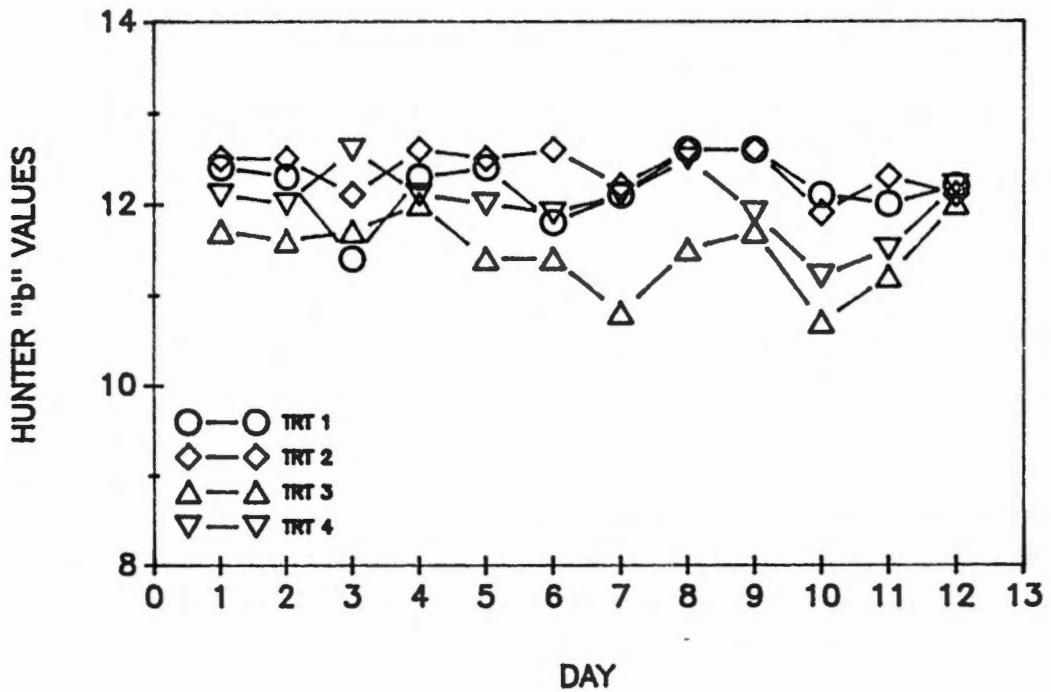

FIGURE 5. Mean Hunter "b" Values for Treatments and Storage Times for Pork Sausage Products Stored at 4°C.

generally followed the same trend across storage time. Compositional differences between patties and links related to color differences have been mentioned earlier and will not be detailed further.

Microbiological growth patterns for psychrophiles are shown in Figure 6. All treatments indicate a normal growth curve with the exception of patties treated with antioxidants (treatment 4). Patties treated with antioxidants had much lower psychrophile counts indicating antioxidants could have an antimicrobial affect, but link products did not follow this trend. The normal growth patterns could be contributing to the abnormal TBA values since psychrophiles will use malonaldehyde during metabolism resulting in lower TBA values (Smith and Alford, 1969; Brown et al., 1979).

Analysis of the frozen products also revealed significant differences in mean TBA values between treatments (Table 10). Differences ($P<.05$) were found between link and patty treatments (treatments 1 and 2 vs. treatments 3 and 4, respectively) and between the links with antioxidants vs. links without antioxidants. Patties had significantly lower mean TBA values than links and links without antioxidants had a lower ($P<.05$) mean TBA value than links with antioxidants.

TBA values were analyzed across frozen storage times within treatments to determine if the initiation phase of oxidation was prolonged. Table 11 shows that significant

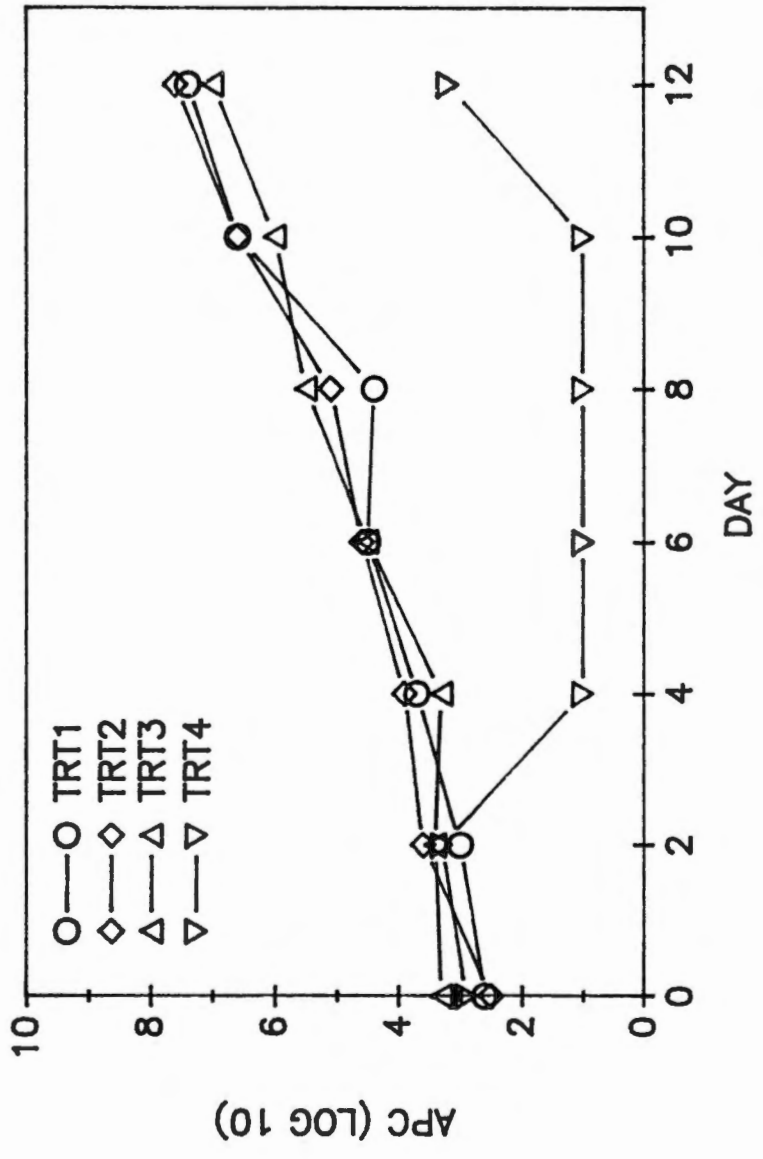

FIGURE 6. Psychrophile Counts (LOG_{10}) for Treatments and Storage Times for Pork Sausage Products Stored at 4°C.

TABLE 10. Mean[1] TBA Values by Treatment for Pork Sausage Products Stored up to 140 Days at -18°C

Treatment[2]	TBA Value[3]
1	0.856[b]
2	1.001[c]
3	0.474[a]
4	0.465[a]

[1]n = 30.

[2]Treatment: 1 = links without antioxidants; 2 = links with antioxidants; 3 = patties without antioxidants; 4 = patties with antioxidants.

[3]TBA Value: Expressed as mg malonaldehyde kg/meat.

[abc]Means in the same column with different superscripts are significantly different at P<.05.

TABLE 11. Mean[1] Frozen TBA Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at -18°C

Day[4]	Treatment[3] 1	2	3	4
0	0.631^{bc}	0.951^{b}	0.310^{c}	0.188^{h}
10	0.427^{a}	0.671^{a}	0.209^{a}	0.254^{h}
20	0.437^{a}	0.575^{a}	0.280^{b}	0.285^{b}
30	0.524^{ab}	0.910^{b}	0.371^{e}	0.427^{d}
40	1.317^{h}	0.946^{b}	1.012^{m}	0.549^{f}
50	1.053^{f}	1.327^{g}	0.417^{f}	0.641^{h}
60	1.159^{g}	1.302^{g}	0.758^{l}	0.676^{i}
70	0.702^{c}	0.920^{b}	0.314^{c}	0.392^{d}
80	0.707^{c}	0.951^{b}	0.341^{d}	0.432^{e}
90	0.905^{d}	0.976^{c}	0.422^{f}	0.371^{c}
100	1.032^{f}	1.210^{f}	0.524^{i}	0.564^{f}
110	1.068^{f}	1.063^{d}	0.458^{g}	0.529^{e}
120	1.012^{f}	1.114^{e}	0.620^{k}	0.564^{f}
130	0.936^{e}	1.127^{e}	0.585^{j}	0.580^{g}
140	0.931^{e}	0.981^{c}	0.488^{h}	0.529^{e}

[1] n = 2.

[2] TBA values expressed as mg malonaldehyde/kg meat.

[3] Treatment: 1 = link without antioxidants; 2 = link with antioxidants; 3 = pattie without antioxidants; 4 = pattie with antioxidants.

[4] Day: Time in days treatments stored at -18°C.

[a-m] Means in the same column with different superscripts are significantly different at $P<.05$.

differences were found within treatments across time with general trends indicating a gradual increase in TBA values as storage time increased. This point is illustrated in Figure 7. Links again had higher initial TBA values than patties and the links maintained the difference throughout storage. It should be noted that links treated with antioxidants had a higher initial TBA value than links without antioxidants, and generally, the links with antioxidants maintained a higher TBA value throughout storage (Figure 7) which could be the contributing factor to overall significant differences between link products.

Results from color evaluation of frozen products using the Hunterlab Color/Difference Meter are listed in Table 12 by treatment and storage time. Significant differences were found between treatments for all colorimetric values and will be discussed by Hunter color value.

Hunter "L" values were significantly different among all treatments. In general, patties (treatments 3 and 4) had higher ($P<.05$) Hunter "L" values than links. Differences in Hunter "L" values were also found between products treated with antioxidants and products without antioxidants. Links without antioxidants had a higher ($P<.05$) Hunter "L" value than links with antioxidants and patties without antioxidants had a lower ($P<.05$) Hunter "L" value than patties containing antioxidants. This conflicting data indicates that factors

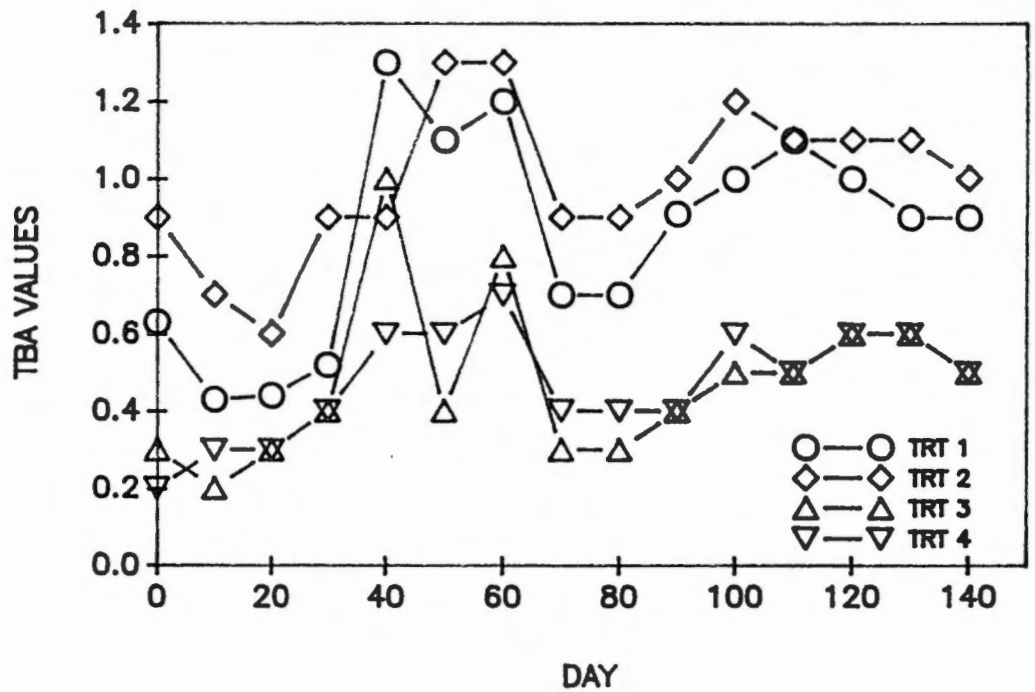

FIGURE 7. Mean TBA Values for Treatments and Storage Times for Pork Sausage Products Stored at -18°C.

TABLE 12. Mean Hunter Color Values by Treatments for Pork Sausage Products Stored up to 140 Days at -18°C

Variable	Treatment[1] 1	2	3	4
Hunter "L" Values[2]	51.7[b]	49.2[a]	57.9[c]	59.8[d]
Hunter "a" Values[3]	10.2[b]	12.9[c]	8.00[a]	7.6[a]
Hunter "b" Values[4]	11.0[a]	12.1[b]	10.9[a]	10.9[a]

[1]Treatments: 1 = link without antioxidants; 2 = link with antioxidants; 3 = patty without antioxidants; 4 = patty with antioxidants.

[2]Hunter "L" values denote lightness or darkness; 100 is white, 0 is black.

[3]Hunter "a" values denote redness/greenness; higher positive numbers indicate greater redness.

[4]Hunter "b" values denote yellowness/blueness; higher positive numbers indicate greater yellowness.

[abcd]Means in the same row with different superscripts are significantly different at $P<.05$.

other than antioxidants were affecting overall mean Hunter "L" values. It should be noted that the difference between treatments (within a product) was not as large as the difference between products. Product differences were probably a result of different fat contents and seasonings as mentioned earlier.

Again, Hunter "L" values were evaluated within treatment across time to determine if antioxidants provided any delay in color degradation. Table 13 lists mean Hunter "L" values by treatment and frozen storage time. Significant differences were found within each treatment across time. General trends (Figure 8) indicate that Hunter "L" values increased slightly over time for both patties and links. There is large variation in means but Figure 8 shows that the Hunter "L" value for products containing antioxidants and products without antioxidants followed the same general line when graphed across time. In addition, the product Hunter "L" values were higher for frozen compared to refrigerated products, and in general, both the Hunter "L" values of links and patties acted similarly during frozen storage.

Significant differences were found in Hunter "a" values (Table 12) between products with links having a higher ($P<.05$) redness value. Also, links containing antioxidants (treatment 2) had a higher ($P<.05$) Hunter "a" value than links without antioxidants (treatment 1). No differences

TABLE 13. Mean[1] Hunter "L" Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at -18°C

Day[4]	Treatment[3] 1	2	3	4
0	50.1^{c}	45.9^{cd}	57.9^{cd}	60.5^{f}
10	50.4^{d}	45.6^{c}	57.1^{b}	60.4^{e}
20	53.3^{g}	51.2^{f}	60.4^{i}	60.5^{f}
30	56.3^{i}	53.0^{h}	60.0^{h}	61.0^{g}
40	51.2^{e}	55.5^{i}	55.9^{a}	59.5^{c}
50	49.5^{c}	49.0^{e}	57.3^{bc}	56.6^{a}
60	49.3^{b}	48.1^{e}	57.4^{bc}	56.9^{a}
70	51.0^{d}	45.0^{b}	58.2^{e}	58.8^{b}
80	52.0^{f}	48.1^{e}	59.0^{f}	60.6^{f}
90	50.9^{d}	44.6^{a}	57.2^{b}	59.0^{b}
100	47.1^{a}	51.3^{f}	56.9^{b}	58.5^{b}
110	51.2^{e}	46.6^{d}	57.7^{cd}	59.8^{d}
120	56.7^{i}	52.6^{h}	60.8^{j}	62.7^{h}
130	54.0^{h}	51.6^{g}	59.3^{g}	62.2^{h}
140	52.9^{g}	50.6^{f}	58.8^{f}	60.5^{f}

[1]n = 4.

[2]Hunter "L" values denote lightness or darkness; 100 is white, 0 is black.

[3]Treatments: 1 = link without antioxidant; 2 = link with antioxidants; 3 = patty without antioxidants; 4 = patty with antioxidants.

[4]Day represents time in storage at -18°C.

[a-i]Means in the same column with different superscripts are significantly different at $P<.05$.

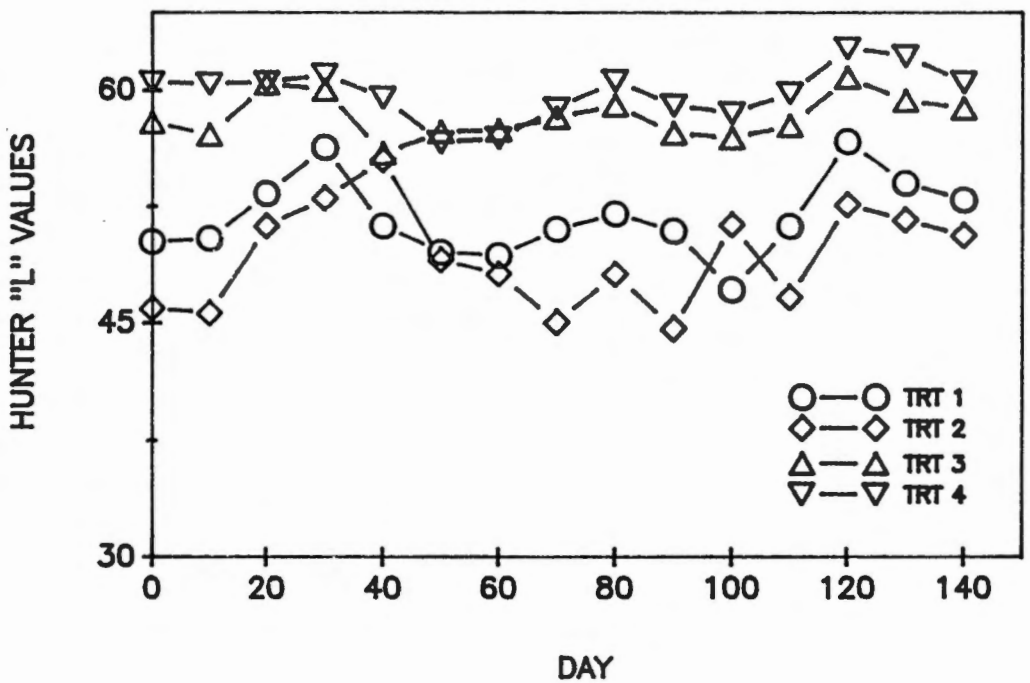

FIGURE 8. Mean Hunter "L" Values for Treatments and Storage Times for Pork Sausage Products Stored at -18°C.

were noted between patties with antioxidants (treatment 4) and patties without antioxidants (treatment 3) (Table 12). The differences between links and patties can be attributed to differences in fat content and seasoning.

Hunter "a" values within each treatment, when evaluated across time, were significantly different (Table 14). When each treatment mean was graphed across time the general trend of Hunter "a" values of both links and patties with and without antioxidants was to decrease as time increased (Figure 9). The Hunter "a" values for links with antioxidants were higher than the Hunter "a" values for links not containing antioxidants (Table 12). This difference was maintained throughout storage. In general, antioxidants did not delay or slow color degradation as measured by Hunter "a" values.

Significant differences were also evident with regard to Hunter "b" values (Table 12). Treatment 2 (links treated with antioxidants) had a significantly higher mean "b" value than treatments 1, 3 and 4 (links and patties without antioxidants and patties with antioxidants, respectively). Table 15 indicates significant differences existed across time within treatments. In general, as time increased Hunter "b" values decreased slightly (Figure 10). This statement holds true for both links and patties and products with and without antioxidants.

TABLE 14. Mean[1] Hunter "a" Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at -18°C

Day[4]	Treatment[3] 1	2	3	4
0	12.1^{g}	15.8^{h}	9.6^{h}	9.3^{f}
10	11.9^{f}	16.2^{h}	9.6^{h}	9.2^{f}
20	11.8^{f}	14.3^{g}	10.6^{i}	11.1^{g}
30	11.4^{f}	13.9^{g}	10.5^{i}	11.0^{g}
40	10.7^{e}	14.0^{g}	8.8^{g}	7.7^{e}
50	9.6^{c}	12.1^{c}	7.6^{f}	7.7^{e}
60	10.5^{e}	12.8^{e}	7.3^{f}	7.0^{d}
70	9.5^{c}	13.3^{f}	7.3^{f}	6.1^{a}
80	10.3^{d}	12.4^{d}	7.1^{d}	6.5^{c}
90	9.1^{b}	13.5^{fg}	6.8^{d}	6.5^{c}
100	12.7^{h}	10.3^{a}	6.6^{b}	6.6^{c}
110	9.5^{c}	12.8^{e}	7.2^{e}	7.0^{d}
120	8.9^{b}	11.4^{b}	7.3^{f}	6.7^{cd}
130	8.5^{a}	10.4^{a}	6.7^{c}	6.4^{ab}
140	8.6^{a}	10.5^{a}	6.2^{a}	6.2^{ab}

[1] n=4.

[2] Hunter "a" values denotes redness/greenness; higher numbers indicate greater redness.

[3] Treatments: 1 = link without antioxidants; 2 = link with antioxidants; 3 = pattie without antioxidants; 4 = pattie with antioxidants.

[4] Day represents time in storage at -18°C.

[a-i] Means in the same column with different superscripts are significantly different at $P<.05$.

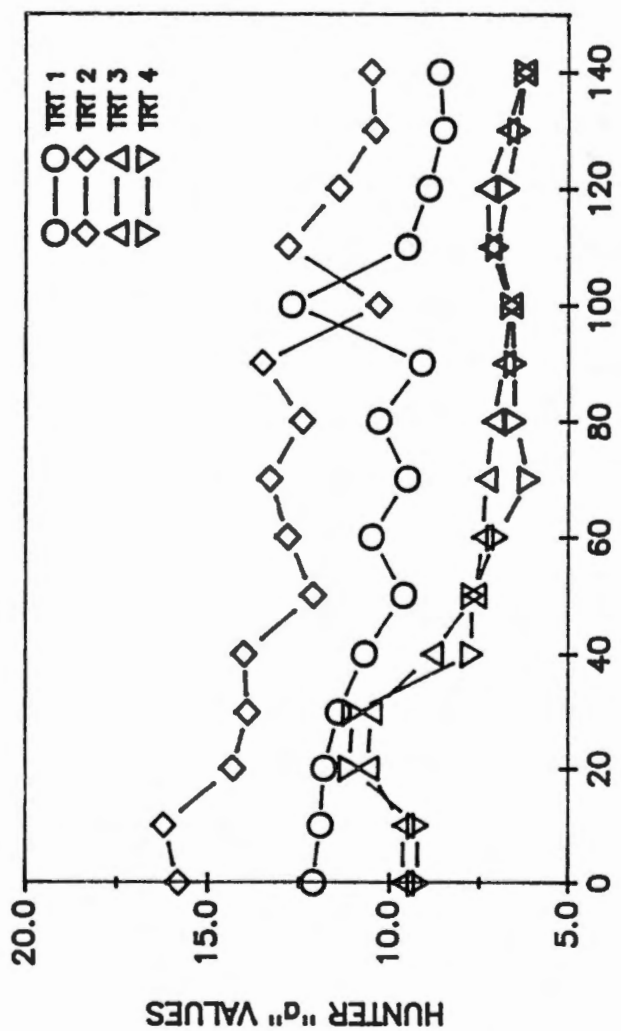

FIGURE 9. Mean Hunter "a" Values for Treatments and Storage Times for Pork Sausage Products Stored at -18°C.

TABLE 15. Mean[1] Hunter "b" Values[2] for Treatments and Storage Times for Pork Sausage Products Stored at -18°C

Day[4]	Treatment[3]			
	1	2	3	4
0	11.2^{f}	12.3^{e}	12.1^{g}	11.7^{f}
10	11.2^{f}	12.2^{e}	12.3^{g}	11.7^{f}
20	9.0^{a}	9.7^{a}	10.3^{c}	11.4^{e}
30	9.8^{b}	10.1^{b}	10.6^{d}	11.1^{d}
40	13.3^{j}	14.6^{f}	12.4^{h}	10.8^{c}
50	13.1^{i}	14.4^{f}	12.5^{h}	11.7^{f}
60	11.7^{g}	14.4^{f}	11.9^{f}	11.7^{f}
70	12.4^{h}	15.6^{g}	11.9^{f}	11.5^{e}
80	12.7^{i}	14.4^{f}	11.6^{e}	10.6^{c}
90	9.8^{b}	10.9^{c}	9.6^{b}	10.6^{c}
100	10.9^{e}	11.0^{d}	9.8^{b}	9.4^{a}
110	10.2^{c}	11.2^{d}	8.9^{a}	10.3^{b}
120	10.5^{d}	10.9^{d}	10.8^{d}	10.3^{b}
130	10.2^{b}	11.3^{d}	10.3^{d}	10.2^{b}
140	10.4^{c}	9.4^{a}	9.4^{b}	11.1^{d}

[1] n=4.

[2] Hunter "b" values denote yellowness/blueness; higher numbers indicate greater yellowness.

[3] Treatments: 1 = link without antioxidants; 2 = link with antioxidants; 3 = pattie without antioxidants; 4 = pattie with antioxidants.

[4] Day: Time in days treatments stored at -18°C.

[a-j] Means in the same column with different superscripts are significantly different at $P<.05$.

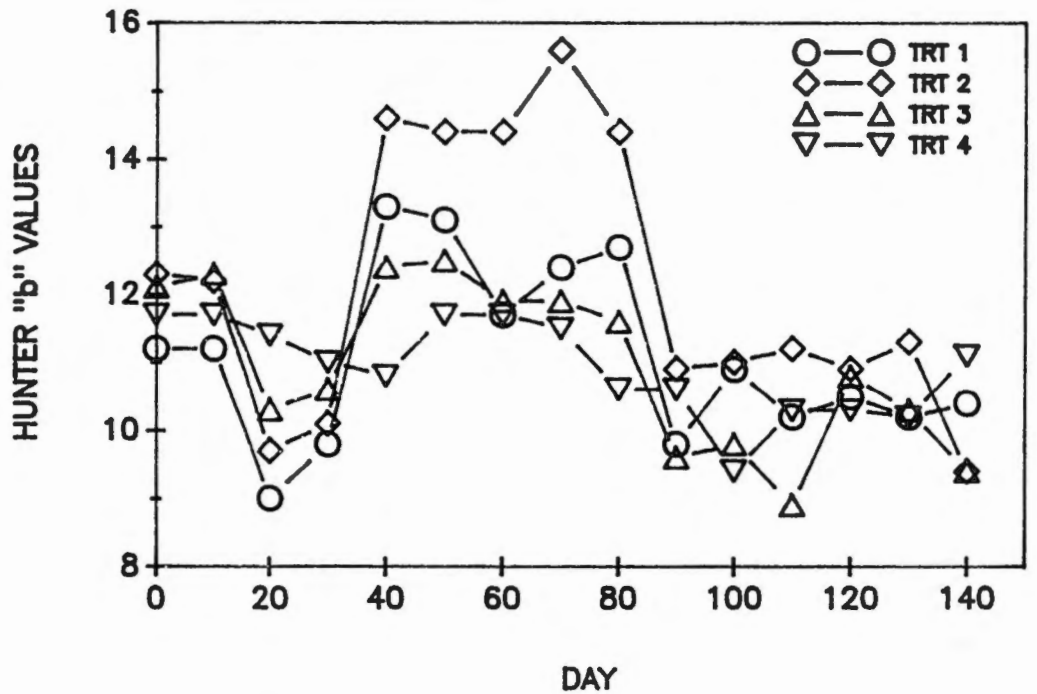

FIGURE 10. Mean Hunter "b" Values for Treatments and Storage Times for Pork Sausage Products Stored at -18°C.

Flavor of the sausage products stored under refrigerated conditions was evaluated by a consumer panel (n=57, 18 to approximately 70 years old and about 50% male and 50% female) that consisted of students, staff and faculty of the University of Tennessee Agriculture Campus. All forms of the products (links, patties, antioxidants, and no antioxidants) were evaluated after 0, 4, 8 and 12 days of storage. The mean flavor score was 5.8 for sausage products with antioxidants and 5.9 for sausage products without antioxidants. A flavor score of 5 on the Sausage Scorecard was described as "Like Slightly" and a score of 6 was "Like Moderately." The statistical analysis of the data (Table 16) revealed no differences ($P>.05$) in flavor between patties and links (product), between products with antioxidants and no antioxidants (treatment), or among storage times. When panelist was used as an independent variable in the statistical model, there was a significant difference among panelists. In addition, there was a significant ($P<.05$) panelist-product interaction. No other interactions were significant. When recruiting panelists, no attempt was made to determine panelists' preference for pork sausage or panelists' preference for pork sausage links vs. patties, so it is reasonable to expect a significant difference among panelists and a significant panelist-product interaction. This combination of results from the statistical analysis could suggest some

TABLE 16. Analysis of Variance of Consumer Panel Flavor Scores for Pork Sausage Products Stored at 4°C

Source	df	Sum of Squares	F Value	PR>F
Product	1	0.06	0.04	0.8507
Treatment	1	5.21	3.26	0.0715
Storage	3	12.01	2.50	0.0581
Panelist	56	240.83	2.69	0.0001
Error	826	1321.16		

panelists liked pork sausage while other panelists did not like pork sausage, and the interaction simply means some panelists preferred pork sausage patties and other panelists preferred pork sausage links. These significant results (panelist and panelist-product interaction) were considered irrelevant because the statistical analysis showed no differences in flavor due to addition of antioxidants to the sausage, or to refrigerated storage time, or to patties vs. links. In addition, the significant results were considered irrelevent to the study because a major objective of the study was to determine the effect antioxidants would have on flavor stability of pork sausage products, rather than panelists' preferences for pork sausage or patties vs. links.

Therefore, the panelist-product interaction was included in the error term of the statistical model.

CHAPTER V

CONCLUSION

The results of this study indicate that antioxidants are not effective in prolonging the onset of oxidation or improving the color stability of prerigor pork sausage through refrigerated and frozen storage.

Pork sausage patties and links were made from prerigor sow carcasses to study the effect of antioxidants on the stability of flavor and color of the sausage products. The treated sausage patties and links contained .003% of an antioxidant mixture of equal proportions of BHA, BHT and citric acid. Storage conditions of 4°C (refrigerated) up to 12 days and -18°C (frozen) up to 140 days were used. Products held under refrigeration were evaluated for oxidation (TBA), color, flavor, and microbial growth. Products held in the freezer were evaluated for oxidation and color.

Antioxidants did not reduce oxidation in refrigerated (4°C) pork sausage links or in pork sausage patties but the TBA values for links (with antioxidants and without antioxidants) were higher ($P<.05$) than TBA values for patties with and without antioxidants. In the frozen (-18°C) products, antioxidants had no effect between the two kinds (with or without antioxidants) of patties but may have promoted oxidation in the pork sausage links because TBA values were

higher ($P<.05$) for links with antioxidants than for links without antioxidants. The links with no antioxidants had lower TBA values at the beginning and maintained that difference throughout the study. Antioxidants did not seem to offer any benefit when evaluating prolongation of the initiation phase of the oxidation process. When evaluated across time, all treatments seemed to follow the same general trend with no delay in the propagation phase of the oxidation process.

Color of the sausage patties did not change because of the presence or absence of antioxidants. Pork sausage links were redder (higher Hunter "a" values) than sausage patties but it is believed that difference was the result of links containing 10% less fat than patties. The sausage links without antioxidants had higher Hunter "a" values than the links with antioxidants throughout the study. Both of the pork sausage products (links and patties) with antioxidants were lighter colored (higher $P<.05$ Hunter "L" values) than the products without the antioxidants. The greater fat content is credited for the higher Hunter "L" values of patties over links in both the refrigerated and frozen storage conditions. Refrigerated pork sausage links with antioxidants had higher Hunter "b" values than the refrigerated and frozen sausage patties with and without antioxidants. Generally, color differences were noted when comparing links to patties,

rather than within products, but the differences may be influenced significantly by the difference in fat content of the two products.

Sensory evaluation of the refrigerated sausage links and patties by consumer panelists found no differences in flavor that could be attributed to presence or absence of antioxidants, type of product or refrigerated storage time.

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APPENDIX

ANALYSES OF VARIANCE FROM COMPUTER PRINT OUT.

General Linear Models Procedure

Dependent Variable: TBA-REFRIGERATED STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	2.4989737	0.0531697	64.07	0.0001
Error	48	0.0398345	0.0008299		
Corrected Total	95	2.5388082			

R-Square	C.V.	Root MSE	TBA Mean
0.984310	8.513814	0.0288	0.3384

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	0.0020813	0.0020813	2.51	0.1198
PROD	1	1.4798183	1.4798183	1783.16	0.0001
DAY	11	0.6647246	0.0604295	72.82	0.0001
TREAT*PROD	1	0.0159393	0.0159393	19.21	0.0001
TREAT*DAY	11	0.0662765	0.0060251	7.26	0.0001
PROD*DAY	11	0.2512350	0.0228395	27.52	0.0001
TREAT*PROD*DAY	11	0.0188986	0.0017181	2.07	0.0415

General Linear Models Procedure

Dependent Variable: COLORL-REFRIGERATED STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	2497.0600	53.1289	115.17	0.0001
Error	144	66.4300	0.4613		
Corrected Total	191	2563.4900			

R-Square	C.V.	Root MSE	COLORL Mean
0.974086	1.281217	0.6792	53.012

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	98.9002	98.9002	214.39	0.0001
PROD	1	2020.2075	2020.2075	4379.19	0.0001
DAY	11	215.7838	19.6167	42.52	0.0001
TREAT*PROD	1	2.3852	2.3852	5.17	0.0245
TREAT*DAY	11	45.6810	4.1528	9.00	0.0001
PROD*DAY	11	102.0863	9.2806	20.12	0.0001
TREAT*PROD*DAY	11	12.0160	1.0924	2.37	0.0102

General Linear Models Procedure

Dependent Variable: COLORA-REFRIGERATED STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	1640.6920	34.9083	184.32	0.0001
Error	144	27.2725	0.1894		
Corrected Total	191	1667.9645			

R-Square	C.V.	Root MSE	COLORA Mean
0.983649	3.649890	0.4352	11.923

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	9.7651	9.7651	51.56	0.0001
PROD	1	1471.3138	1471.3138	7768.60	0.0001
DAY	11	103.3589	9.3963	49.61	0.0001
TREAT*PROD	1	22.0730	22.0730	116.55	0.0001
TREAT*DAY	11	6.9618	0.6329	3.34	0.0004
PROD*DAY	11	21.6731	1.9703	10.40	0.0001
TREAT*PROD*DAY	11	5.5464	0.5042	2.66	0.0039

General Linear Models Procedure

Dependent Variable: COLORB-FROZEN STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	488.53079	8.28018	27.21	0.0001
Error	180	54.77417	0.30430		
Corrected Total	239	543.30496			

R-Square	C.V.	Root MSE	COLORB Mean
0.899183	4.879744	0.5516	11.305

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	22.26504	22.26504	73.17	0.0001
PROD	1	33.54840	33.54840	110.25	0.0001
DAY	14	283.85966	20.27569	66.63	0.0001
TREAT*DAY	14	13.42953	0.95925	3.15	0.0002
TREAT*PROD	1	15.87682	15.87682	52.17	0.0001
PROD*DAY	14	88.54088	6.32435	20.78	0.0001
TREAT*PROD*DAY	14	31.01046	2.21503	7.28	0.0001

General Linear Models Procedure

Dependent Variable: COLORA-FROZEN STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	1649.1323	27.9514	51.25	0.0001
Error	180	98.1777	0.5454		
Corrected Total	239	1747.3100			

R-Square	C.V.	Root MSE	COLORA Mean
0.943812	7.628329	0.7385	9.6815

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	67.78751	67.78751	124.28	0.0001
PROD	1	906.52855	906.52855	1662.04	0.0001
DAY	14	389.11315	27.79380	50.96	0.0001
TREAT*DAY	14	34.94516	2.49608	4.58	0.0001
TREAT*PROD	1	123.71047	123.71047	226.81	0.0001
PROD*DAY	14	67.01577	4.78684	8.78	0.0001
TREAT*PROD*DAY	14	60.03167	4.28798	7.86	0.0001

General Linear Models Procedure

Dependent Variable: COLORL-FROZEN STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	5802.1107	98.3409	102.55	0.0001
Error	180	172.6092	0.9589		
Corrected Total	239	5974.7198			

R-Square	C.V.	Root MSE	COLORL Mean
0.971110	1.787804	0.9793	54.774

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	13.3482	13.3482	13.92	0.0003
PROD	1	4313.6460	4313.6460	4498.35	0.0001
DAY	14	719.9915	51.4280	53.63	0.0001
TREAT*DAY	14	161.7917	11.5565	12.05	0.0001
TREAT*PROD	1	212.9985	212.9985	222.12	0.0001
PROD*DAY	14	225.4177	16.1013	16.79	0.0001
TREAT*PROD*DAY	14	154.9172	11.0655	11.54	0.0001

General Linear Models Procedure

Dependent Variable: TBA-FROZEN STORAGE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	11.612312	0.196819	135.86	0.0001
Error	60	0.086919	0.001449		
Corrected Total	119	11.699231			

R-Square	C.V.	Root MSE	TBA Mean
0.992571	5.453195	0.0381	0.6980

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	0.1413847	0.1413847	97.60	0.0001
PROD	1	6.2869674	6.2869674	4339.91	0.0001
DAY	14	3.8745357	0.2767525	191.04	0.0001
TREAT*PROD	1	0.1741170	0.1741170	120.19	0.0001
TREAT*DAY	14	0.6319032	0.0451359	31.16	0.0001
PROD*DAY	14	0.3910525	0.0279323	19.28	0.0001
TREAT*PROD*DAY	14	0.1123519	0.0080251	5.54	0.0001

General Linear Models Procedure

Dependent Variable: COLORB-REFRFIGERATED STUDY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	40.717500	0.866330	4.20	0.0001
Error	144	29.715000	0.206354		
Corrected Total	191	70.432500			

R-Square	C.V.	Root MSE	COLORB Mean
0.578107	3.771767	0.4543	12.044

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	1	5.535208	5.535208	26.82	0.0001
PROD	1	14.520000	14.520000	70.36	0.0001
DAY	11	9.257500	0.841591	4.08	0.0001
TREAT*PROD	1	1.171875	1.171875	5.68	0.0185
TREAT*DAY	11	2.344792	0.213163	1.03	0.4206
PROD*DAY	11	6.200000	0.563636	2.73	0.0031
TREAT*PROD*DAY	11	1.688125	0.153466	0.74	0.6951

VITA

Roger Dean Edens was born August 15, 1963 to Mr. and Mrs. Lora G. Edens. He graduated from Cherokee Comprehensive High School in May of 1981. In the Fall of 1981, he enrolled in The University of Tennessee, Knoxville in Animal Science. He received his Bachelor of Science Degree in Agriculture in June 1985. In August 1985, he enrolled in the Graduate School of The University of Tennessee, Knoxville, majoring in Meat Science. He held the position of Graduate Research Assistant and later took the position of Research and Development Project Manager with Rudy's Farm Company while working towards a Master of Science Degree in the Department of Food Technology and Science. He is a member of the Institute of Food Technologist, American Meat Science Association, and Alpha Zeta National Honorary Agriculture Fraternity.