To the Graduate Council:

I am submitting herewith a dissertation written by Craig D. Bacon entitled "Effect of Cooking Method, Antioxidant and Storage of Sensory Characteristics and Flavor Volatiles of Pre-cooked Sausage Links". I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Technology and Science.

H. O. Jaynes, Major Professor

We have read this dissertation
and recommend its acceptance:

M. James Riemann

Sharon L. Melton

H Dwight Loveday

John P. Hitchcock

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

EFFECT OF COOKING METHOD, ANTIOXIDANT AND STORAGE ON SENSORY CHARACTERISTICS AND FLAVOR VOLATILES OF PRECOOKED SAUSAGE LINKS

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ABSTRACT

Fresh, post-rigor pork was formulated to 42% fat and ground through a 4 mm plate. One half of the treatment was seasoned with a commercial spice mix which contained a mixture of butylated hydroxytoluene and butylated hydroxyanisole (BHT/BHA) at 200 ppm and the other half with rosemary oleoresin (200 ppm). Each treatment was stuffed into a natural casing to produce sausage links (SL). A Wearever Impinger Oven was used to cook one half of the treatments and a multipurpose oven (MPO) was used on the other. After leaving the oven, initial cooking yields and temperatures were recorded. The SL were stored for either 0, 45 or 90 d. Reheating yields, Warner-Bratzler shear, lipid and moisture percentages of reheated SL and thiobarbituric acid values (TBA) were determined for each treatment. A sensory panel which had received 6 h of orientation scored the SL at each storage period on the attributes of tenderness, juiciness, flavor and intensity of warmed over flavor (WOF). Flavor volatiles, collected on 0 and 90 d stored samples using simultaneously distillation extraction, were analyzed qualitatively by gas chromatography (GC)/mass spectroscopy and quantitatively by GC/flame ionization..

The impingement oven had superior initial cooking yields for SL as compared to the MPO SL. This difference appeared to be due to less lipid loss. The greater lipid retention may have been due to dehydration of the natural casing which may have served as a superior barrier and/or less cooking time in the impingement oven. The impingement cooked SL also tended to have higher Warner-Bratzler shear force values and were perceived by panelists as tougher than the MPO SL. Reheating yields were

lower for the impingement SL than the MPO cooked SL. No differences were found for WOF, flavor, juiciness or reheated moisture and lipid percentages between ovens.

The rosemary oleoresin SL were perceived as having a hotter flavor than the BHT/BHA SL. This may be due to a concentration of spices caused by a dispersion problem with the rosemary oleoresin. In addition, panelists made comments about a sage flavor note in rosemary SL. This sensation may have been scored as hotter on the flavor scale. Finally, the difference may have been due only to a formulation error at the production facility. The rosemary oleoresin was as effective as the BHT/BHA SL up to 45 days storage for retarding WOF. As would be expected, no consistent differences were found for tenderness, juiciness, reheated lipid percentages or reheating yields between antioxidants.

As expected, oxidative rancidity was highest at 90 days storage. Moisture percentages of reheated SL were lowest at 90 d storage and the inverse was true for lipid percentages. This is most likely due to a dehydration of the SL during storage. Reheating yields were lower for 90 d SL.

No differences in relative percentages of volatiles were found between SL cooked in the impingement oven compared to those in the MPO. More camphene, α-pinene, ß-pinene and camphor were found in the rosemary oleoresin SL than in the BHT/BHA SL. Hexanal, as been reported in the literature as being associated with WOF development, increased in concentration with increasing storage; however, in contrast to other reported findings T,T-2,4 heptadienal decreased at 90 d.

TABLE OF CONTENTS

CHAPTER **PAGE**

LIST OF TABLES

TABLE **PAGE**

TABLE PAGE

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Food service operations demand an acceptable quality product that is easily and quickly prepared. Cooking method, the use of antioxidants and storage time all influence the quality of pre-cooked, uncured meat products.

The rapid development of rancid flavors during storage is a major problem facing the meat industry as the demand for pre-cooked meat items is expected to increase dramatically over the next decade (National Live Stock and Meat Board, 1988). One example is warmed over flavor (WOF). Warmed over flavor is the rapid onset of rancidity in cooked meats during refrigerated storage (Tims and Watts, 1958). Sato and Hegarty (1971) and Love and Pearson (1971) demonstrated that non-heme iron, not the myoglobin molecule itself, catalyzed lipid oxidation in model meat systems. Igene et. al. (1979a) demonstrated that rapid development of warmed over flavor (WOF) following cooking of fresh meat is due to the release of Fe^{++} ions from the muscle pigments. Apparently, Fe^{++} ions are released from the myoglobin in muscle and serve as the active catalysts for lipid oxidation. Chen et. al. (1984) found that both the final temperature and rate of heating influenced the non-heme iron content of cooked meat. They reported that the optimum temperature for releasing non-heme iron was 63-70°C and that slow heating resulted in the release of more non-heme iron than fast heating. An impingement oven may be useful in reducing Fe^{++} levels because cooking times for beef top loin steaks were reduced 45-55 % as compared to

the Farberware grill (Powell, 1989). In the same study, the impingement oven improved cooking yields ($P < .05$).

Antioxidants can also be used to decrease effects of WOF. Pearson et al. (1983) pointed out that antioxidants which can be added to foods subject to oxidation are of two types, naturally occurring and synthetic. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are three synthetic antioxidants which have been used widely in meat systems and are effective in retarding lipid oxidation. Many spices and herbs have been shown to function as antioxidants to fats and oils in model food systems (Bishov et. al., 1977). Barbut et. al. (1985) demonstrated that rosemary oleoresin, when added to turkey breakfast sausage at the 20 mg/kg level, produced an antioxidant effect comparable to that of a commercial blend of BHA/BHT/citric acid and did not adversely effect overall palatability of the product.

The objectives of this study were to determine the effects of impingement oven cooking, rosemary oleoresin and storage time on the yield and sensory characteristics of pre-cooked, uncured pork sausage links. Flavor volatile compounds responsible for WOF in the links were also identified by gas chromatography/mass spectrometry.

CHAPTER 2

REVIEW OF LITERATURE

DEVELOPMENT OF WARMED OVER FLAVOR

Definition

According to the National Live Stock and Meat Board (1988), the demand for precooked meat items is expected to increase dramatically over the next decade. Because of the increase in precooked meat use, they report that the rapid development of rancid flavors during storage is a major problem facing the meat industry. Warmed over flavor (WOF) is one example of a rancid flavor, which was first defined by Tims and Watts (1958). They reported flavor problems developed with cooking and storage of uncured meat that received preliminary heating. These changes in flavor and odor greatly affect meat's acceptability and contribute to the loss of palatability. Bessert (1956) reported that cooked meats which received high palatability ratings immediately after cooking deteriorated in flavor rapidly after a few hours of refrigerated storage. When tested for oxidative rancidity with thiobarbituric acid (TBA) reagent, this flavor loss paralleled an increase in TBA value. Tims and Watts (1958) defined these rapid changes during storage with such terms as "warmed over", "stale" or "rancid". Gros et al. (1986) found that the deterioration in flavor and aroma

that resulted from the oxidation of lipids during refrigerated storage could be detected by sensory panelists.

Development

Sato and Hegarty (1971) found that uncured meat stored for a relatively short time after cooking developed a WOF. They saw WOF as a large flavor problem due to the increase in fast food service facilities requiring the use of large quantities of precooked or partially cooked meats or meat products. Bailey et al. (1980) reported that rapid lipid oxidation occurred in beef that had been roasted at 163°C to an internal temperature of 71°C and stored at 4°C. Pearson et al. (1977) concluded that the development of WOF in refrigerated cooked meats became apparent within 48 h storage at 4°C.

Lundberg (1962) presented the steps involved in lipid oxidation at a food symposium, entitled, "Lipids and their Oxidation". She concluded that a free radical chain reaction involves three stages which are initiation, propagation and termination. A free radical is formed when a labile hydrogen is extracted from the carbon atom adjacent to the double bond. The free radical can react with oxygen to form a peroxyl radical which may extract a hydrogen from another fatty acid. This in turn propagates the chain reaction. Termination is achieved when two free radicals are joined.

Liu and Watts (1970) reported that both the heme and non-heme iron served as catalysts in lipid oxidation in meats. However, Sato and Hegarty (1971) and Love and Pearson (1971) demonstrated that the non-heme iron, not the myoglobin molecule itself, catalyzed lipid oxidation in model meat

systems. Sato and Hegarty (1971) found that the heme compounds had little effect on the development of WOF. Love and Pearson (1971) evaluated the addition of purified metmyoglobin and ferric iron to a model meat system. They found that ferric iron was a prooxidant in cooked meats, whereas metmyoglobin at concentrations from 1 to 10 mg/g of meat failed to catalyze lipid oxidation. Younathan and Watts (1959) also concluded that the rapid oxidation of lipids in cooked meat is attributed to the irreversible conversion of iron in the porphyrin ring of myoglobin pigments to the ferric form during heating. Igene et al. (1979b) found that cooking released a significant amount of non-heme iron from the bound heme pigment. This non-heme iron accelerated lipid oxidation. Wills (1966) reported that non-heme iron was apparently more important than the heme proteins in catalyzing the oxidation of the lipids within tissue homogenates. He also reported that the non-heme iron was a more active prooxidant at acid pH values, whereas heme proteins were less pH sensitive.

There are also chemicals and manufacturing steps that accelerate the development of oxidative rancidity. Love and Pearson (1971) found that low levels of ascorbic acid enhanced the prooxidant activity of ferrous iron. Keeton (1983) found that levels of 1 and 2 % NaCl in pork patties accelerated lipid oxidation during all storage conditions while the control with no salt added remained the least oxidized. Ellis et al. (1968) had found similar results with increased concentrations of NaCl accelerating autoxidation in pork tissue. Sato and Hegarty (1971) concluded that the reduction in particle size by grinding, chopping, flaking or emulsification all have similar effects on WOF, differing only in degree. All of these operations disrupt membranes, leading to incorporation of air or oxygen into

the tissue. This disruption increases tissue susceptibility to oxidation and hastens development of WOF. Pearson and Tauber (1984) similarly concluded that frozen meat products are very susceptible to lipid oxidation due to extensive grinding, fabrication and reforming during manufacture. Chiang et al. (1981) also proposed that ground pork is even more prone to lipid oxidation than beef, due to disruption of muscle membranes and catalysts such as salt.

The degree of unsaturation of the fat plays an important role in the development of oxidative rancidity. Moerck and Ball (1974) found that fatty acids must have three or more double bonds to be very susceptible to oxidation during refrigerated storage of muscle foods and that susceptibility increases with the number of double bonds in the fatty acid. In a study involving the cooking and frozen storage of ground beef, Keller and Kinsella (1973) demonstrated that fatty acid composition of lipids provides an indirect measure of lipid oxidation susceptibility. Igene et al. (1979b) found that cooking increased the proportion of polyunsaturated fatty acids and non-heme iron in meat. They concluded these were the major contributors to increased propensity of cooked meat to have oxidative breakdown and WOF development.

The greatest concentration of polyunsaturated fatty acids is found in the phosphatidyl ethanolamine (PE) fraction which is, therefore, of greatest interest in studies on WOF (Pearson et al., 1977). In addition, the phospholipid fraction is approximately 15 times more unsaturated than the triacylglyceride fraction (Igene and Pearson, 1979). To examine the role of phospholipids in rancidity development of lean meat, chloroform-methanol extractions were completed on cooked, refrigerated pork stored 3 d

(Younathan and Watts, 1959). Phospholipid and lipoproteins were separated from the neutral fat and both phases tested for rancidity by the TBA method. The phospholipid and lipoprotein portions represented only a small proportion of the total lipid weight, yet, they had a TBA value approximately 30 times greater than the neutral fat portion. Igene and Pearson (1979) and Igene et al. (1981) examined phospholipids and triglycerides to more specifically find the components that had an impact on WOF. Igene and Pearson (1979) used model systems from beef and dark and light chicken meat. Triglycerides, total lipids, total phospholipids, phosphatidyl choline (PC) and PE were added back to lipid extracted muscle from each system and WOF development was evaluated by TBA values and sensory panel scores for odor. Total phospholipids, especially PE, was shown to be the major contributor to WOF development in cooked meat. Changes in phospholipid polyunsaturated fatty acids after cooking were shown to be related to WOF development. Most losses were in 18:2, 20:4 and 22:4 demonstrating their susceptibility to cooking and subsequent break down into WOF products. Keller and Kinsella (1973) also reported that cooking hamburger causes a 25 % decrease in the 20:4 content of PE, and an increased TBA number and carbonyl content. Igene et al. (1981) compared fatty acid profiles of triglycerides and phospholipids and the levels of component phospholipids in beef and dark and light chicken meat. Uncooked samples were initially stored (-18°C) for 0, 8 and 13 mo. After cooking, the samples were held at either 4°C or -18°C for 48 h. There was a significant decline in amount of PE and PC during frozen storage, but the decline was much greater upon cooking. Although PE and PC declined more upon cooking, their decrease was still less than the nonpolar lipids. After

cooking, PE and PC were much more stable at a holding temperature of -18°C than 4°C. The increased proportion of PE in cooked meat, coupled with its susceptibility to oxidation, indicated that it played a key role in autoxidation of cooked meats.

Flavor volatiles involved

Although the mechanism of WOF development is becoming better understood, less is known about the volatiles responsible for this problem. Hornstein (1971) concluded that fatty acid oxidation leads to the formation of carbonyl compounds. Furthermore, unsaturated fats produce greater concentrations of carbonyls than saturated fats during oxidation. These carbonyl compounds are present in organoleptically significant amounts in meat and at higher concentrations may produce undesirable off flavors.

The differences in concentration and number of carbonyls among meats may cause the different species flavors and also explain why some meats develop WOF faster than others. Lamb fat which is more saturated than beef or pork fat produces virtually no carbonyls upon heating or oxidation. In comparison pork fat is the most unsaturated and produces carbonyls to the greatest extent. This may account for the observation that fat oxidation produces characteristic beef and pork flavors but is not responsible for lamb flavor. Differences in carbonyl levels may also explain the results of Wilson et al. (1976). They found turkey was the most susceptible to WOF followed by chicken, pork, beef and mutton. The order of increasing unsaturation in the fat and carbonyl production of these species meat is the same (deMan, 1985) as their susceptibility to WOF. Barbut et al.

(1985) also found carbonyls in the volatiles isolated from oxidized sausages without antioxidants but not in volatiles from antioxidant treated sausage.

St. Angelo et al. (1987) examined three treatments; raw, freshly cooked, and stored and recooked beef muscle samples. These samples were assessed by chemical, instrumental and sensory methods for flavor quality with particular emphasis on WOF development. Compounds usually associated with lipid oxidation reactions could be used as marker compounds to follow WOF. Hexanal, 2, 3-octanedione and total volatiles had higher correlations to sensory scores and TBA numbers. Pearson et al. (1983) found that hydroperoxides in muscle foods are the major initial oxidation products, which in turn, decompose into pentanal, hexanal and malonaldehyde. Cross and Ziegler (1985) reported similar oxidation products. They found large quantities of hexanal and pentanal present in uncured ham, but absent in cured ham. They concluded that these compounds were derived by oxidative cleavage of polyunsaturated fatty acid residues.

FACTORS AFFECTING WARMED OVER FLAVOR

Cooking method

Researchers have compared the effects of cooking methods on WOF development. Chen et al. (1984) reported that the final temperature and heating rate influenced the release of non-heme iron from meat pigment extracts. The most non-heme iron was released at a internal temperature of 63-70°C. Slow heating resulted in release of more non-heme iron than fast

heating. Siu and Draper (1978) also found that meat cooked the longest time had the greatest increase in TBA numbers. They reported that roasts cooked for 3 h had up to 10-fold increases in malonaldehyde compared to shorter cooking times. Schricker and Miller (1983) cooked fresh beef round by common household methods (braising, roasting and microwave). These methods resulted in non-heme iron increases that were generally less than 10 %. A linear increase was observed between the non-heme iron level in meat and time of exposure to heat treatment. However, Law et al. (1967) did not find a difference between conventional and microwave cookery for TBA values of top-round steaks. Huang and Greene (1978) studied the conditions that favor retardation of TBA activity in cooked meat. Beef semitendinosus muscle was heated by different home cooking methods (roasting, braising, pressure cooking and electrical slow cooking). Based on TBA numbers, lipid oxidation was at the highest level after 5 d storage. Meat which was subjected to high temperatures and/or short heating periods developed lower TBA numbers than samples subjected to low temperatures for shorter time periods. They concluded that browning products were responsible for the lower TBA values. Gros et al. (1986) compared the microwave, microwave/convection combination and oven broiling in retarding WOF development in ground beef patties. The oven broiling method was found to delay WOF development in refrigerated patties. They suggested that the delayed WOF was due to antioxidative substances produced during browning. Similar conclusions could be made from a study by Yamauchi (1972) when he found heating meat to 70°C for 1 hr caused rapid development of rancidity, but heating to 120°C for 1 hr resulted in virtually no oxidation upon refrigerated storage.

An impingement oven may be a possible solution to reducing WOF. Heat and Control, Inc. (1989) reported that an impingement oven could increase production rate by 25 % while also increasing cooking yields. In an American Institute of Baking Technical Bulletin, Walker (1987) reported that air jet impingement's high heat transfer rates can result in much shorter oven times and greater thermal efficiencies. Powell (1989) also found that the impingement oven decreased cooking times 45 to 55 % as compared to the Farberware grill for beef top loin steaks. By decreasing cooking time, one might expect lower TBA values.

Synthetic antioxidants

Antioxidants can be used to decrease WOF effects. Pearson et al. (1983) pointed out that antioxidants which can be added to foods subject to oxidation are of two types, naturally occurring and synthetic. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are three synthetic antioxidants which have been used widely in meat systems and, in general, are effective in retarding lipid oxidation. Chastain et al. (1982) reported the effects of adding antioxidants on restructured combinations (50:50) of beef-pork steaks. BHA, TBHQ and a combination of BHA and TBHQ were all used at 0.02 % level. Flavor and overall acceptability were superior ($P < .05$) in treated samples as compared to control samples. BHA was more effective in protecting color and TBHQ was more effective in protecting flavor ($P < .05$). All samples had lower TBA values than the control ($P < .05$). The antioxidant combination of BHA and TBHQ appeared to offer the best combined protection against both flavor

and color changes. Chen et al. (1984) demonstrated that Tenox4 (combination of BHA, citric acid and propylene glycol) coated salt was an effective inhibitor of lipid oxidation. These researchers also found that a mixture of BHA and BHT in salt completely inhibited lipid oxidation in cooked meats. Liu and Watts (1970) and Igene et al. (1979a) both examined the effects of ethylenediamine tetra acetic acid (EDTA) on lipid oxidation. Liu and Watts (1970) reported that EDTA inhibited the non-heme iron activity at the natural acidic pH in beef round and fresh pork loins. Also, ascorbic acid or EDTA added separately inhibited malonaldehyde formation. Protection of beef round and fresh pork loins was increased when EDTA or ascorbic acid were added. Igene et al. (1979a) also found that EDTA was effective in chelating the non-heme iron and, thus, significantly reduced lipid oxidation. Chen et al. (1984) found that nitrite prevented the release of non-heme iron, apparently through stabilization of the porphyrin ring. Igene et al. (1979b) reported that 156 ppm of nitrite reduced ($P < .01$) TBA numbers and prevented development of WOF.

Natural antioxidants

According to Yamauchi (1972) the heating of meat to 70°C for 1 hr and the subsequent refrigerated storage caused rapid development of rancidity, but heating to 120°C for 1 hr resulted in virtually no oxidation upon refrigerated storage. Huang and Greene (1978) postulated that retardation of oxidation in meats at higher temperature was from antioxidant substances produced by the browning reaction which progresses as meat is heated. Sato et al. (1973) found that reductic acid, maltol and

products of the amino sugar reaction were effective inhibitors of WOF development in cooked ground beef. They concluded heat catalyzed the interaction between amino acids or proteins and carbohydrates resulting in the antioxidant compounds.

Watts (1962) and Bishov et al. (1977) both investigated effects of various natural antioxidants. Watts (1962) found that pepper (pods and seeds), onion extract and potato peelings were effective antioxidants when added to sliced roast beef. These materials reduced WOF as indicated by sensory panel acceptability scores and TBA values. Bishov et al. (1977) evaluated twenty spices, herbs and plant protein hydrolyzates. Of the spices tested, clove, cinnamon, sage, rosemary, mace, oregano, allspice and nutmeg were highly effective antioxidants. They noticed a synergistic effect of these spices when combined with BHA. Barbut et al. (1985) compared rosemary oleoresin (200 ppm), BHA/BHT (200 ppm) and a control with no antioxidant in turkey meat. TBA values on samples stored at 4°C demonstrated that rosemary oleoresin was comparable with a commercial blend of BHA/BHT in suppressing lipid autoxidation. Furthermore, sensory analysis indicated that rosemary oleoresin was comparable to BHA/BHT rancidity scores. MacNeil et al. (1973) compared a rosemary extract at 0.01 % and 0.05 %, polyphosphate and BHA + citric acid. Rosemary extract at .05 % and BHA + citric acid were most effective in control of autoxidation. Houlihan et al. (1985) isolated rosmariquinone from the leaves of rosemary. Based on the peroxide values, the rosmariquinone's antioxidant activity was superior to BHA, but it was slightly less than BHT when compared on steam lard. Wu et al. (1982) isolated two compounds (carnosol and ursolic acid)

from rosemary leaves and found carnosol to be one of the active antioxidant components in rosemary.

Storage

Numerous studies have examined the effect of freezer storage on WOF. Poste et al. (1986) studied the effect of storage on ground pork which had been cooked to an internal temperature of 75°C. The samples were stored for 0, 2, 4, 8 and 16 d at 4°C and evaluated for warmed over aroma by an experienced panel. The warmed over aroma mean scores from 0 to 8 d of storage correlated well with the TBA means (r=.88). However, 16 d panelist scores decreased whereas TBA numbers remained constant. Poste et al. (1986) concluded that there was a rancidity threshold effect. Gros et al. (1986) found that panelists were able to detect changes in flavor as well as aroma of ground beef patties during refrigerated storage for 48 h. There was a significant negative correlation for both aroma and flavor (r=-.66) scores and TBA values over time. Freshly cooked samples received the highest panel scores and lowest TBA values while cooked, refrigerated samples received the lowest panel scores and highest TBA values. Love (1988) reported on trained panelists ability to use descriptive terminology in describing WOF. The majority of the panelist were able to discriminate between samples for flavor descriptors used.

Greene (1969) found that stability of frozen meat was dependent upon the degree of fatty acid unsaturation and that as unsaturation increased so did susceptibility to WOF. Bailey et al. (1980) extracted the volatiles from oxidized beef lipid in a Likens-Nickerson extraction apparatus with diethyl

ether. The compounds that increased most rapidly during frozen storage, as determined by mass spectrometry, were hexanal and 2-pentyl furan. In addition, pentanal increased during the storage period.

Packaging

Packaging meat in a vacuum package with a good oxygen barrier film can reduce autoxidation by limiting available oxygen (National Live Stock and Meat Board, 1988). Keeton (1983) found that pork patties stored in a vacuum pouch for 21 d at 3°C had a similar TBA value as patties stored in a permeable bag for only 7 d. Chiang et al. (1981) also studied the effects of processing and packaging on the quality of uncooked, frozen pork patties. Processed patties were placed in a vacuum package or wrapped in an air permeable package. At each sampling period (2, 4, 8, 16, 24 wk) TBA values, sensory evaluations and cooking losses were determined. Lipid oxidation was enhanced by the seasonings added, but tended to be inhibited by use of vacuum packaging. McDaniel et al. (1984) had similar results with beef roasts. Precooked beef roasts were packaged by either vacuum packaging, 100 % CO_2 or a combination of 15 % CO_2, 30 % O_2 and 55 % N_2. All samples were stored at 4°C for up to 21 days. At the conclusion of the study, vacuum packaged roasts were preferred by panelists.

MEASUREMENT OF WARMED OVER FLAVOR

Thiobarbituric acid test

The most widely used test for measuring the oxidative deterioration in muscle foods is thiobarbituric acid (TBA) and the most popular procedure is the steam distillate method (Gray, 1978). Tarladgis and Watts (1960) described an improved distillation method for quantitative determination of malonaldehyde in foods containing oxidized fats. A high correlation of .89 was found between TBA numbers and cooked meat rancid odor scores as determined by an experienced panel. The TBA number threshold for detecting pork off-odors was approximately 0.5 to 1.0. Greene and Cumuze (1981) were less successful in correlating TBA number and oxidized beef flavor. They had 52 inexperienced sensory panelists rate a series of ground beef samples for the attribute of oxidized flavor intensity. Correlation coefficients between intensity of WOF sensory scores and TBA numbers were significant, but low (.16 to .53, $P < .01$). Other modifications, such as Rhee (1978), have minimized further lipid peroxidation during distillation by the addition of propyl gallate and EDTA. Another problem with the TBA test is that the TBA number does not necessarily continue to increase throughout storage of frozen cooked meat (Tarladgis and Watts, 1960). Igene et al. (1979a) concluded that sensory evaluation was necessary along with TBA tests to accurately measure WOF. This may be due to malonaldehyde being a secondary product of lipid oxidation. Malonaldehyde itself is odorless and does not contribute to meat flavor.

Isolation and identification

One of the principal methods for separating volatiles from food and beverages is steam distillation followed by organic solvent extraction. Likens and Nickerson (1964) first developed a simple and effective means for performing these two operations simultaneously. This method concentrated substances a thousand fold and used a relatively small quantity of solvent, thus reducing artifact introduction. Schultz et al. (1977) described a modification to the Likens and Nickerson apparatus. The advantages of this modification were a larger condensing surface and a mixing chamber for the vapors at the top of the condenser. This new apparatus gave very satisfactory recovery, but the extraction conditions still needed to be optimized according to the type of food material.

After the initial isolation of the volatiles, the sample is often injected into a gas chromatograph for isolation of relatively pure fractions. The gas chromatograph is often connected to a mass spectrometer which according to Issenberg (1969) has become one of the most useful physical methods for characterization and structure determination of these relatively pure fractions. The spectrum observed from a compound under a given set of operating conditions reflects the manner in which the molecules decompose after bombardment with moderate energy electrons. This spectra and retention times can be matched to a known compound injected under similar conditions or to preexisting compounds stored in the computer library.

FACTORS AFFECTING COOKING LOSS

Bowers et al. (1987) compared characteristics of beef longissimus muscle broiled or roasted to seven internal temperatures between 55° and 85°C. Cooking loss percentages were greater ($P < .001$) for broiled steaks than roasted (26.2 % vs 15.7 %, respectively). Losses increased as final internal endpoints increased. Cooking losses increased about 2 % for each 5°C temperature increase, except between 75-85°C where a 7 % increase ($P < .001$) occurred. Flavor and juiciness scores were affected by final endpoint temperature and heating method. Steaks which were roasted had characteristics of steaks broiled at lower temperatures. Furthermore, roasted steaks were more juicy. Davenport and Meyer (1970) compared beef sirloin butts cooked by forced convection at 93.3°C to 148.9°C and found longer cooking times resulted in lower cooking losses ($P < .001$). Berry and Leddy (1984) studied the effects of fat content and cooking method of ground beef patties. Cooking methods were electric broiling, charbroiling, roasting, convection heating, frying and microwave heating. There was an interaction between fat level and cooking method ($P < .05$) for percent yield, cooked patty fat and moisture content. Microwave cooking produced the lowest yield percentages across all fat levels. Berry and Leddy (1984) concluded this may be due to lack of crust formation in microwave cooked patties. In the cooked patties, there was no difference between convection and broiling in percent moisture as formulated fat levels increased. Charbroiling, frying and microwave cooking resulted in decreased moisture percentage as fat increased. Substantial increases in cooked patty fat retention occurred in all ovens, except the microwave, as formulated fat

level increased. In the microwave, patties with 14 % fat increased in fat percentage after cooking, patties with 19 % remained constant and patties with 24 % decreased in cooked fat percentage. Law et al. (1967) examined the storage and cooking effects on loin steak quality. Steaks were stored for 0, 3, 6 and 9 mo frozen storage at 0°C . Loin steaks had increased cooking losses, decreased juiciness scores and decreased percent moisture with each storage period.

SUMMARY OF LITERATURE

WOF was first defined by Tims and Watts (1958) as the rapid oxidation of lipids during storage of precooked meats. Most researchers agree that sensory panelists can detect these undesirable flavor and aroma changes and that WOF will become a more serious problem with increased demands for precooked items.

Recent research indicates that non-heme iron is a catalyst for lipid oxidation and that cooking causes an irreversible conversion to the ferric form. In addition, there are chemicals and processing steps that accelerate oxidative rancidity. Particle size reduction, salt and low levels of ascorbic acid enhance WOF development. Researchers agree that disruption of the muscle membranes, which leads to incorporation of oxygen, speeds up WOF development.

Greater unsaturation of the fatty acids plays an important role in oxidative rancidity. Studies agree that meat is more susceptible to oxidative rancidity as the unsaturated fatty acid level increases in the muscle. Furthermore, the literature supports the findings that the

phospholipid, PE, plays a major role in WOF. PE contains the greatest concentration of polyunsaturated fatty acids and has been implicated in increasing the TBA values 30 times greater than the neutral fat portion.

Cooking method also affects the WOF development. The optimum temperature for releasing non-heme iron was 63-70°C and slow heating resulted in the release of more non-heme iron than fast heating. Furthermore, there was a linear increase between non-heme iron and time of heat treatment. The development of browning products with some cooking methods has delayed WOF development.

Antioxidants can be used to decrease production of WOF. Antioxidants are either naturally occurring or synthetic. BHT, BHA, TBHQ and EDTA are examples of synthetic antioxidants and, in general, are very effective in retarding lipid oxidation. Many naturally occurring antioxidants have been studied. Rosemary extracts appear to be one of the most promising. Several studies found that rosemary derivatives were equal to or greater than synthetic antioxidants in retarding oxidative rancidity. Some researchers have also examined the TBA retarding activity of some of the browning reaction products.

Packaging can also reduce lipid oxidation. Researchers have reported that a good oxygen barrier film can significantly reduce or stop oxidative rancidity.

Numerous researchers have examined the effect of freezer storage on WOF. Most agree that TBA values increase as storage time increases and that sensory panelists can detect flavor and aroma differences. In addition, hexanal, pentanal and 2-pentyl furan were found to increase as frozen storage increased.

The TBA test is the most common measure of lipid oxidation. High correlations between TBA numbers and experienced sensory panel scores have been reported by several researchers. Various modifications of the TBA procedure have been proposed. However, the TBA method is not without disadvantages because TBA numbers do not necessarily continue to increase throughout storage as the development of WOF progresses. Therefore, most agree that sensory evaluation is necessary along with the TBA test to accurately evaluate WOF.

The most common method to isolate the WOF compounds is simultaneous distillation extraction. Isolation is usually followed by qualitative analysis on a gas chromatograph/mass spectrometer.

Less is known about the volatiles responsible for WOF. Some research would support that fatty acid oxidation leads to formation of carbonyl compounds. Others have found that hexanal and pentanal specifically are compounds that are responsible for WOF.

Cooking losses of meat are affected by cooking method and endpoint temperature. Generally, cooking method effects were a result of variations in cooking time. Furthermore, as endpoint temperature increased, cooking yields decreased.

CHAPTER 3

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Fresh post-rigor pork was formulated to 42 % fat and then ground through a 4 mm plate. The experiment was an incomplete block design as shown in Table 1 (Sanders, personal communication). One half of the treatment was seasoned with a commercial spice mix which contained BHT and BHA (200 ppm) and the other one-half with the same commercial spice mix with rosemary oleoresin (200 ppm) substituted for BHT and BHA. Each treatment was stuffed at 30-34° C into a natural collagen casing using a commercial Vemag stuffer. A Wearever Impinger Oven (Model 1132) was used to cook one half of treatments and a commercial multi-purpose oven (MPO) used for the other half. The impingement oven forces hot air both to the top and bottom of the product. The multipurpose oven differs in that it has a combination of steam and dry heat located at the center of the oven. The heat then radiates out randomly to each end. All links were cooked to an approximate internal temperature of 65°C. Temperatures were recorded on a random sample (n=100) of cooked links immediately after leaving the oven. After cooking, links were cryogenically chilled at -10°C for 25 min and then at -20°C for 25 min prior to packaging. The links were packaged in a cardboard box that contained a polyvinyl liner and held at 0°C. The links were stored for 0, 45 and 90 d depending upon the treatment.

TABLE 1. INCOMPLETE BLOCK DESIGN[a] FOR CHEMICAL AND SENSORY ANALYSIS OF PORK SAUSAGE

	Conventional						Impingement					
	BHT/BHA			Rosemary			BHT/BHA			Rosemary		
Days	0	45	90	0	45	90	0	45	90	0	45	90
Rep												
1	1				10*						12*	20
2	5*				14		3*				16	
3			17*			18*		11		4		
4		9		2					19*			24*
5				6*		22		15*	23			
6		13*	21				7			8*		

[a] Numbers denote where treatments were completed in the incomplete block design for chemical analysis and sensory analysis.

* Denotes where duplicates were prepared for sensory analysis.

PHYSICAL ANALYSIS

Cooking yields, both pre-cooking and after reheating, were determined. Pre-cooking yields were determined by the weight of 10 replicates of 10 links after cooking divided by their weight after stuffing times 100. The same procedure was followed for reheated cooking yields (weight after microwaving/weight before reheating)(100). The links were reheated for 4 min or to approximately 60°C internal temperature in a White-Westinghouse microwave oven (Model KM540G). Final endpoint temperatures after initial cooking were recorded for each treatment. After reheating, Warner-Bratzler shear was completed for each treatment . Eight links were allowed to cool to room temperature and sheared at the midpoint and approximately 2 cm from the end of the link.

CHEMICAL ANALYSIS

A random sample of approximately 800 g of initially cooked and reheated sausage links from each block were placed into liquid nitrogen and powdered using a Waring blender to assure a uniform sample (Borchert and Briskey, 1965). The powdered sample, stored at 0°C, was used for chemical moisture and lipid analysis. Two grams of powdered sample were enclosed in sample envelopes made from Whatman No. 41 filter paper and dried in the vacuum oven according to AOAC (1984) methods. These samples were then placed into the Soxhlet apparatus for lipid extraction (AOAC, 1984). TBA analysis was completed on the reheated samples. TBA analysis (Rhee, 1978) was performed using a modified distillate method (Appendix A). The equation for the standard curves, absorbance vs malonaldehyde (MA)

concentration, was determined by linear regression. The sample absorbance was converted to moles of MA by linear regression and then converted to mg MA/kg meat, which is the TBA number.

SENSORY ANALYSIS

Fifteen panelists received 6 h of orientation on the attributes of sausage tenderness, juiciness, flavor and intensity of WOF. Anchor terms were developed for a 150 mm line for each attribute (Appendix B). Anchors for the attributes juiciness and tenderness were a fresh Oscar Mayer link (juicy and tender) and a precooked Tennessee Pride link that was overcooked in the microwave oven (dry and tough). Anchors for the flavor attribute were Jimmy Dean's hot sausage (hot) and Rudy's Farm mild sausage (mild). Warmed over flavor anchors were a 2 year old Rudy's Farm brand sausage (rancid) and fresh Oscar Mayer links (fresh).

After the orientation sessions, panelists were presented four samples during each of three additional sessions in sensory booths under white lights. The four samples presented were a freshly cooked sample, a sample reheated in the microwave oven after 3 d storage, a sample reheated in the microwave oven after 5 d storage and a sample that had been frozen for 2 mo, cooked on a grill and reheated in the microwave oven. Nine panelists were selected for their ability to evaluate WOF of the samples.

The nine sensory panelists evaluated tenderness, juiciness, flavor and intensity of WOF for samples from each storage period during three

sessions. Twelve treatments were replicated for sensory analysis to increase the treatments to 36 for panelist evaluations (Table 1).

FLAVOR VOLATILE ANALYSIS

Flavor volatiles were collected from sausage links to determine compounds, if any, responsible for warmed over flavor. Flavor volatiles were collected for 0 and 90 d samples using a modified simultaneous distillation/extraction (SDE) method (Schultz et al., 1977). This method is based on the co-distillation of water and the meat sample along with methylene chloride (MeCl) in a Likens-Nickerson apparatus (Figure 1). All glassware in this method went through a seven step washing procedure to insure the glassware was volatile clean. The glassware was washed with Alconox detergent, distilled water, acetone, distilled water, 4 % acetic acid, distilled water and rinsed with HPLC grade water. A 200 g cooked sausage sample was combined with 700 ml of HPLC grade water at 60-70°C. The water and meat was blended for 5 min using an Osterizer blender set on "grind". The contents were poured into a 1000 ml round bottom flask with a nitrogen inlet tube. The flask was attached to the Likens-Nickerson apparatus. Nitrogen introduced via a side tube in the round bottom flask was bubbled slowly through the sample slurry. On the opposite side of the apparatus, a 125 ml Erlenmeyer collection flask containing 45 ml of HPLC grade MeCl was attached. The round bottom flask was then lowered into a silicone oil bath which was heated to a temperature of 127°C. The collection flask was placed on a Corning PC-35 hot plate that was on a setting of 2. The cold finger of the apparatus was filled with dry ice and

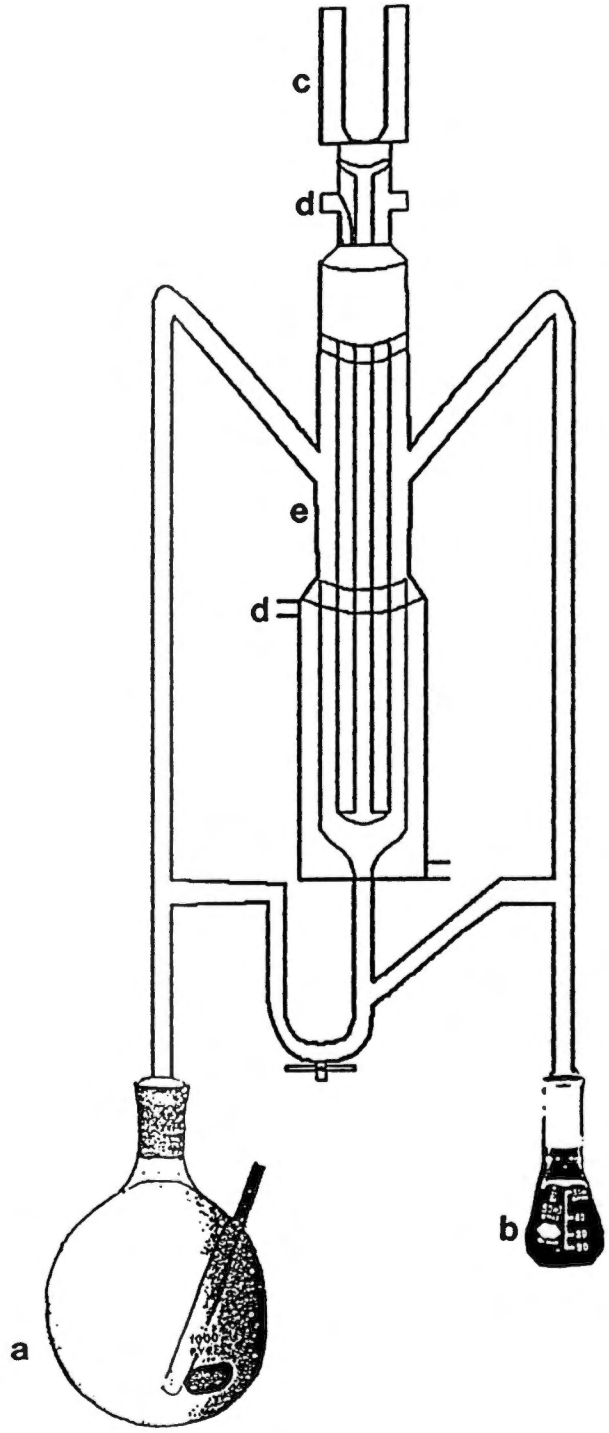

FIGURE 1. Likens-Nickerson apparatus used to isolate volatiles from pork sausage.

a. 1000 ml round bottom flask containing HPLC water and sample.
b. 125 ml Erlenmeyer collection flask containing MeCL.
c. Cold finger trap.
d. Inlets for water condensor.
e. Chamber for volatile and MeCl mixture.

ethanol. The volatiles were collected for 2.5 h with the continual replenishment of dry ice to the cold finger. At the end of the collection, the apparatus was allowed to cool and 1 ml of pyrogallol (0.1 mg/ml in MeOH) was added to the volatile extract in MeCl to help prevent oxidative changes. The sample was then concentrated to 1 ml using a T-bar with a positive nitrogen flow over the sample. The sample vial was placed into an ice bath and the "U" in the T-bar was placed into liquid nitrogen. The outlet was attached to a vacuum. The 45 ml sample was concentrated by removing 10 ml increments of solvent until only 1 ml remained.

Two µl of each sample was then injected into the Shimadzu Gas Chromatograph model 9 AM interfaced with a Shimadzu Mass Spectrometer, model QP 1000 (GCMS). This mass spectrometer was a low resolution instrument, which gave mass weights of ions only to the nearest single unit. The gas chromatograph (GC) was equipped with a 30 m x 0.25 mm fused silica SP-2350 capilliary column (Supelco Inc., Bellafonta, PA) which was held at an initial temperature of 25°C for 2.5 min and then increased 2.0°C per min to a final temperature of 220°C where it remained for 30 min A chromatogram and mass spectrum for each peak in the chromatogram were obtained for each sample.

Positive identification of the compound for each peak was made by matching sample peak spectrum and retention time with corresponding spectrum and retention time of known standards analyzed under identical conditions. Tentative identification of a volatile compound was based on matching it's mass spectrum with that of a known compound from the GCMS computerized library of flavor volatiles with a similarity index of 60 or

greater. Unknown volatiles had similarity indexes of less than 60 or could not be matched to any of the standards injected.

After each sample was analyzed by GCMS, the SP2350 column was removed from the mass spectrometer and connected to a flame ionization detector (FID). Each sample was then analyzed under identical conditions as the GCMS analysis. The area of each volatile peak was determined by a Shimadzu Model CR601 data processor. The concentration of each volatile in a sample was determined as the relative percentage of the total area (RPA) of all volatile peaks in the GC-FID sample chromatogram.

STATISTICAL ANALYSIS

The experiment used an incomplete block design as shown in Table 1. The General Linear Models procedure according to SAS (1985) was used to analyze the data with the independent variables, antioxidant treatments, oven type and storage time. Least square means and the probability of difference procedure of SAS (1985) were used when there was a significant effect due to an independent variable on Warner-Bratzler shear, chemical lipid and moisture concentrations, TBA values, cooking yields and flavor volatile percentages. Sensory data was analyzed independently for each storage period.

CHAPTER 4

RESULTS AND DISCUSSION

OVEN TYPE

Least squares mean temperatures, initial cooking yields and initial moisture and lipid percentages of sausage links cooked by impingement or multipurpose oven (MPO) are presented in Table 2. The mean temperatures for the two ovens were not different ($P > .05$). Therefore, the endpoint temperature is not a factor when making comparisons between links cooked in the two oven types. The impingement oven had a superior ($P = .01$) cooking yield of 88.3 % vs 83.1 % for the MPO. A 5.2 % improvement in cooking yield could make a substantial difference in profit and loss for a precooked sausage link operation. Heat and Control Inc. (1989) claimed the impingement oven increases cooking yields with meat products. Powell (1989, personal communication) also found that cooking in the impingement oven resulted in greater cooking yield for top loin steaks. Initial cooked moisture percentage of links was not different ($P = .72$) between oven types. However, lipid percentages of cooked links tended ($P = .06$) to be higher for the impingement links than for the links cooked in the MPO. This is probably due to the difference in dwell times between ovens. The impingement links spent less time in the oven than the MPO links.

TABLE 2. LEAST SQUARES MEAN[a] INITIAL COOKING YIELDS, TEMPERATURES AND COMPOSITION OF SAUSAGE LINKS COOKED IN TWO DIFFERENT OVENS

Characteristic	Oven type		Significance level
	Impingement	Multipurpose	
Yield[b]	88.3 ± 1.5	83.1 ± 1.4	.01
Temperature, °C	73.7 ± 0.9	75.5 ± 1.3	.23
Moisture, initial cooked %	47.1 ± 1.0	47.7 ± 1.0	.72
Lipid, initial cooked %	38.3 ± 1.2	35.0 ± 1.2	.06

[a] The ± values represent the SE of least squares means.

[b] Yields were calculated as: (cooked weight/raw weight)(100).

Reheating yield (Table 3) was greater ($P < .01$) for the MPO links than for the impingement oven links (91.2 vs 88.2 %, respectively). This was opposite to the effect found for initial yields. The greater lipid retention of the impingement links may have been due to dehydration of the natural casing which may have served as a superior barrier, holding in more moisture and fat during initial cooking. Upon reheating in the microwave oven, the casings of the links cooked by the impingement did not allow for expansion and therefore had greater losses than the MPO links.

There was a trend ($P = .06$) for the impingement links to be tougher than the MPO links as indicated by Warner-Bratzler shear values. The TBA (thiobarbituric acid) numbers, used as an indication of oxidation, were not different ($P = .37$) between oven types. The faster cooking time of the links in the impingement oven did not appear to reduce oxidation. Oven type did not affect moisture percentage ($P = .47$) or lipid percentage ($P = .17$) of the cooked links. A hypothesis would be that the difference in initial and reheating yields were offsetting, resulting in no net difference between ovens.

Nine sensory panelists, who were selected on their ability to discern differences in sausage links, evaluated tenderness, juiciness, flavor and intensity of warmed over flavor of sausage links during three sessions for each storage period of 0, 45 and 90 d. Sensory characteristics were evaluated on a 150 mm line. Anchors for lines at 0 and 150 mm, respectively, were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor). Least squares means for initial (0 d storage) sensory characteristics of sausage links are

TABLE 3. LEAST SQUARES MEANS[a] FOR PHYSICAL AND CHEMICAL CHARACTERISTICS OF SAUSAGE LINKS COOKED IN TWO DIFFERENT OVENS

Characteristic	Oven type		Significance level
	Impingement	Multipurpose	
Reheating yield, %	88.2 ± 0.9	91.2 ± 0.9	< .01
Warner Bratzler shear, kg	1.4 ± 0.1	1.3 ± 0.1	.06
TBA[b]	4.5 ± 0.3	4.1 ± 0.3	.37
Moisture, reheated %	46.3 ± 0.8	45.4 ± 0.8	.47
Lipid, reheated %	30.5 ± 0.8	32.9 ± 0.8	.17

[a] The ± values represent the SE of least squares means.

[b] Thiobarbituric acid test was an indicator of oxidative rancidity.

TABLE 4. LEAST SQUARES MEANS[a] FOR SENSORY CHARACTERISTICS OF SAUSAGE LINKS COOKED IN TWO DIFFERENT OVENS AFTER 0 DAYS STORAGE

Sensory characteristic[b]	Oven type		Significance level
	Impingement	Multipurpose	
Tenderness	70.3 ± 5.1	50.2 ± 5.1	< .01
Juiciness	86.8 ± 4.8	94.5 ± 4.8	.23
Flavor	76.4 ± 4.8	81.8 ± 4.8	.43
Warmed over flavor	51.1 ± 5.1	66.0 ± 5.1	.04

[a] The ± values represent the SE of least squares means.

[b] Sensory characteristics were evaluated on a 150 mm line. Anchors for lines were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor).

given in Table 4. Panelists perceived the links cooked in the impingement oven as being tougher ($P < .01$) than the links cooked in the MPO. Panelists found no differences in the characteristics of juiciness and flavor between oven types ($P = .23$ and .43, respectively). The links cooked in the MPO were rated as more rancid than the links from the impingement oven ($P = .04$).

Least squares mean values for sensory characteristics of sausage links stored 45 d are shown in Table 5. As with the 0 d storage, panelists indicated that 45 d links were tougher ($P < .01$) when cooked in the impingement oven. They also scored the links cooked in the MPO as being juicier ($P = .05$). No differences were found for flavor or warmed over flavor intensity ($P = .87$ and .31, respectively) between the oven types.

Sensory characteristics of precooked sausage links stored for 90 d are presented in Table 6. Significant ($P < .01$) differences were found between links for the characteristics of tenderness and juiciness for the two oven types. The MPO links were more tender and juicy than the links cooked in the impingement oven. No differences were found for the sensory characteristics of flavor and warmed over flavor between oven types ($P= .60$ and .99, respectively).

At all three storage periods, panelists perceived the impingement oven links to be tougher than the MPO links. The trend was the same for the Warner-Bratzler shear values. Although panelists found no differences in juiciness initially (0 d), they found 45 d and 90 d storage MPO prepared links more juicy than the impingement oven links. Cooked moisture and lipid percentage do not support the panelists findings. No consistent differences were found for flavor or warmed over flavor among storage periods. TBA values are in agreement with the panelists findings in warmed over flavor,

TABLE 5. LEAST SQUARES MEANS[a] FOR SENSORY CHARACTERISTICS OF SAUSAGE LINKS COOKED IN TWO DIFFERENT OVENS AFTER 45 DAYS STORAGE

Sensory characteristic[b]	Oven type		Significance level
	Impingement	Multipurpose	
Tenderness	72.0 ± 4.6	48.7 ± 4.5	< .01
Juiciness	82.9 ± 4.7	95.5 ± 4.6	.05
Flavor	70.4 ± 5.3	69.2 ± 5.2	.87
Warmed over flavor	61.7 ± 5.1	69.0 ± 5.0	.31

[a] The ± values represent the SE of least squares means.

[b] Sensory characteristics were evaluated on a 150 mm line. Anchors for lines were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor).

TABLE 6. LEAST SQUARES MEANS[a] FOR SENSORY CHARACTERISTICS OF SAUSAGE LINKS COOKED IN TWO DIFFERENT OVENS AFTER 90 DAYS STORAGE

Sensory characteristic[b]	Oven type		Significance level
	Impingement	Multipurpose	
Tenderness	93.2 ± 4.3	68.7 ± 4.3	< .01
Juiciness	61.4 ± 5.0	83.0 ± 5.0	< .01
Flavor	68.2 ± 4.6	64.8 ± 4.6	.60
Warmed over flavor	60.0 ± 5.0	60.1 ± 5.0	.99

[a] The ± values represent the SE of least squares means.

[b] Sensory characteristics were evaluated on a 150 mm line. Anchors for lines were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor).

as no difference was found between oven types for oxidation of the sausages.

ANTIOXIDANT

Least squares means for physical and chemical characteristics of sausage links with two different antioxidants are given in Table 7. Neither reheating yield percentages or Warner-Bratzler shear values were different between antioxidants (P = .15 and .51, respectively). Sausages made with the natural antioxidant, rosemary, did have higher (P = .05) TBA numbers than those containing the combination of BHT and BHA (4.8 vs 3.9). This finding disagrees with Barbut et al. (1985) who reported that rosemary oleoresin was comparable to a commercial blend of BHA and BHT in suppressing lipid autoxidation. There was no difference in lipid percentage of reheated links between antioxidants (P = .32). The BHT/BHA links did have a higher (P = .05) moisture percentage than the rosemary links. This difference could not be explained.

Initial sensory characteristics of sausage links with two different antioxidants are shown in Table 8. There were no differences (P = .82 and .43, respectively) for the sausage attributes of tenderness or juiciness between the two antioxidants. There was a difference (P < .01) in flavor, with the sausage containing the rosemary antioxidant being perceived as hotter than the synthetic antioxidant blend. There was also a trend (P = .08) for the rosemary links to be rated as more rancid.

TABLE 7. LEAST SQUARES MEANS[a] FOR PHYSICAL AND CHEMICAL CHARACTERISTICS OF SAUSAGE LINKS USING TWO DIFFERENT ANTIOXIDANTS

Characteristic	Antioxidants[b] BHA/BHT	Rosemary	Significance level
Reheating yield, %	89.2 ± 0.9	90.2 ± 0.9	.15
Warner Bratzler shear, kg	3.0± 0.1	3.0 ± 0.1	.51
TBA[b]	3.9 ± 0.3	4.8 ± 0.3	.05
Moisture, reheated %	47.1 ± 0.5	44.5 ± 0.5	.05
Lipid, reheated %	30.9 ± 1.2	32.6 ± 1.2	.32

[a] The ± values represent the SE of least squares means.

[b] The antioxidants were added at a level of 200 ppm.

[c] Thiobarbituric acid test was an indicator of oxidative rancidity.

TABLE 8. LEAST SQUARES MEANS[a] FOR SENSORY CHARACTERISTICS OF SAUSAGE LINKS USING TWO DIFFERENT ANTIOXIDANTS AFTER 0 DAYS STORAGE

Sensory characteristic[b]	Antioxidants[c] BHA/BHT	Rosemary	Significance level
Tenderness	59.4 ± 5.2	61.1 ± 5.1	.82
Juiciness	93.6 ± 4.8	88.2 ± 4.7	.43
Flavor	60.5 ± 4.8	97.7 ± 4.7	< .01
Warmed over flavor	52.1 ± 5.2	65.1 ± 5.1	.08

[a] The ± values represent the SE of least squares means.

[b] Sensory characteristics were evaluated on a 150 mm line. Anchors for lines were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor).

[c] The antioxidants were added at a level of 200 ppm.

Least squares means for sensory characteristics of sausage links prepared with synthetic and rosemary antioxidants and stored for 45 d are presented in Table 9. The attributes of tenderness and juiciness were not affected by the antioxidants (.45 and .17, respectively). However, a difference ($P < .01$) was found in flavor, with the rosemary links being perceived as hotter than the synthetic links. There also was a trend ($P = .10$) for the rosemary links to be scored as more rancid.

After 90 d of frozen storage (Table 10), panelists rated the rosemary links more tender than the synthetic antioxidant links ($P = .05$). In addition, panelists perceived the synthetic antioxidant links as being more juicy than the rosemary links ($P = .05$). Both of these differences are difficult to explain, and may be only random differences, as each had a P value of only .05. However, flavor and warmed over flavor differences were highly significant ($P < .01$) between antioxidants. Panelists rated the rosemary link as hotter and more rancid than the synthetic antioxidant links.

Over all three storage periods, panelists perceived the rosemary oleoresin links as hotter than the synthetic antioxidant links. This may be due only to a formulation error, since a hotter flavor note from use of rosemary oleoresin has never been reported. Another observation is that several panelists placed comments on score sheets that the rosemary links had a sage-like flavor. Due to the nature of the anchors for flavor (mild to hot), they may have scored those links as hotter. Finally, upon observing the rosemary link, dark spots were noted, indicating a possible spice dispersion problem. The rosemary oleoresin may have concentrated the spices within the link. There was a trend for the rosemary links to be scored as more rancid at 0 and 45 d. In addition, panelists rated the 90 d rosemary links

TABLE 9. LEAST SQUARES MEANS[a] FOR SENSORY CHARACTERISTICS OF SAUSAGE LINKS USING TWO DIFFERENT ANTIOXIDANTS AFTER 45 DAYS STORAGE

Sensory characteristic[b]	Antioxidants[c]		Significance level
	BHA/BHT	Rosemary	
Tenderness	58.0 ± 4.5	62.7 ± 4.6	.45
Juiciness	93.6 ± 4.6	84.9 ± 4.7	.17
Flavor	55.2 ± 5.2	84.4 ± 5.3	< .01
Warmed over flavor	59.5 ± 5.0	71.2 ± 5.1	.10

[a] The ± values represent the SE of least squares means.

[b] Sensory characteristics were evaluated on a 150 mm line. Anchors for lines were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor).

[b] The antioxidants were added at a level of 200 ppm.

TABLE 10. LEAST SQUARES MEANS[a] FOR SENSORY CHARACTERISTICS OF SAUSAGE LINKS USING TWO DIFFERENT ANTIOXIDANTS AFTER 90 DAYS STORAGE

Sensory characteristic[b]	Antioxidants[c]		Significance level
	BHA/BHT	Rosemary	
Tenderness	74.9 ± 4.3	87.0 ± 4.3	.05
Juiciness	79.1 ± 5.0	65.3 ± 5.0	.05
Flavor	46.7 ± 4.6	86.3 ± 4.6	< .01
Warmed over flavor	48.3 ± 5.0	71.8 ± 5.0	< .01

[a] The ± values represent the SE of least squares means.

[b] Sensory characteristics were evaluated on a 150 mm line. Anchors for lines were: tender to tough (tenderness), dry to juicy (juiciness), mild to hot (flavor) and fresh to rancid (warmed over flavor).

[c] The antioxidants were added at a level of 200 ppm.

significantly more rancid. This difference at 90 d mirrors the differences found in TBA numbers. One might conclude that the rosemary oleoresin was quite effective under normal storage times (45 d), but after 90 d the BHT/BHA combination was superior. As would be expected, no consistent differences were found among storage periods for tenderness and juiciness due to antioxidant type.

Storage

Means for physical and chemical characteristics of sausage links as affected by days of frozen storage are presented in Table 11. Reheating yields were lower at 90 d storage ($P < .05$) than at 0 and 45 d. The links were observed to have more frost on them at 90 d due to their storage in a polyvinyl lined cardboard box. The loss of this moisture frost during reheating in the microwave oven could explain the lower yields. Warner-Bratzler shear values were higher ($P < .05$) for 90 d (1.7, kg) than at 0 and 45 d (1.2, kg). Furthermore, TBA values were higher ($P < .05$) at 90 d (10.8) than at 0 and 45 d (.7 and 1.5, respectively). This is in agreement with Poste et al. (1986), Greene (1969), and Bailey et al. (1980) who found that TBA values increased with either cooler or frozen storage. Moisture percentage of cooked links was lowest at 90 d ($P < .05$) storage. Lipid percentage of cooked links was greatest at 90 d ($P < .05$) storage. These differences in lipid and moisture percentages could be explained by the dehydration of the links during storage. Figure 2 illustrates the relationship between storage and TBA numbers for the two antioxidants. Both antioxidants had similar TBA numbers at 0 d. At 45 d of frozen

TABLE 11. LEAST SQUARES MEANS[a] FOR PHYSICAL AND CHEMICAL CHARACTERISTICS OF SAUSAGE LINKS AS AFFECTED BY DAYS OF FROZEN STORAGE[b]

	Days of storage		
Characteristic	0	45	90
Reheating yield, %	95.0[c] ± 1.0	95.1[c] ± 1.0	79.0[d] ± 0.8
Warner Bratzler shear, kg	1.2[c] ± 0.1	1.2[c] ± 0.1	1.7[d] ± 0.1
TBA[e]	.7[c] ± 0.4	1.5[c] ± 0.4	10.8[d] ± 0.4
Moisture, reheated %	48.8[c] ± 1.0	47.1[c] ± 1.0	41.6[d] ± 1.1
Lipid, reheated %	29.9[c] ± 1.5	30.5[c] ± 1.5	34.8[d] ± 1.5

[a] The ± values represent the SE of least squares means.

[b] Samples stored at 0°C.

[cd] Means in a row with a different superscript differ ($P < .05$).

[e] Thiobarbituric acid test was an indicator of oxidative rancidity.

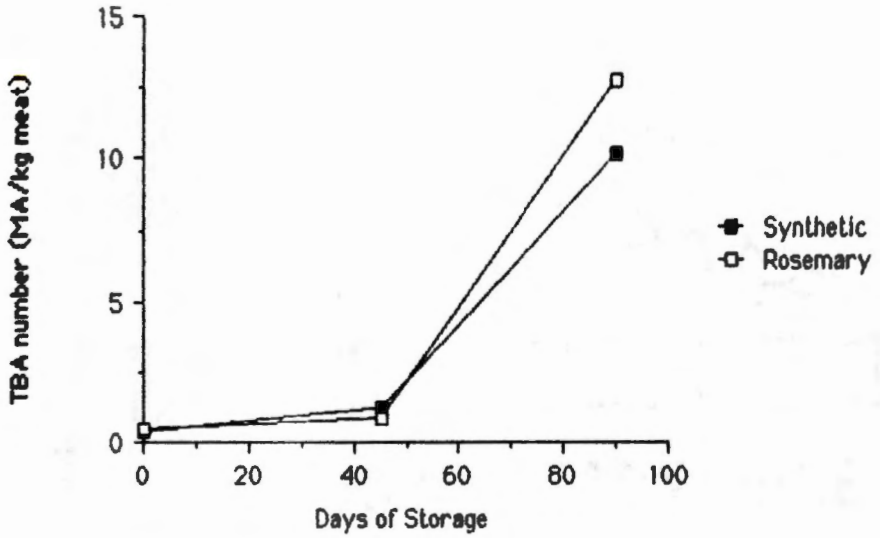

FIGURE 2. STORAGE BY ANTIOXIDANT INTERACTION.

storage the BHT/BHA links had higher TBA numbers than the rosemary oleoresin links. At 90 d storage, the rosemary links had higher TBA numbers (more oxidative rancidity) than the BHT and BHA combination links. From this, one could conclude that the rosemary antioxidant was not as effective as the BHT/BHA after 45 d frozen storage.

FLAVOR VOLATILES

Eighteen volatile compounds were found in sausage samples extracted by modified simultaneous distillation/extraction (SDE) method (Table 12). Least squares mean relative percentages (RPA) and mean retention times for volatile compounds in sausage cooked in two different ovens are presented in Table 12. No differences ($P < .05$) were found for volatile RPA values between the impingement oven and the MPO. This would be expected as there was no difference in oxidative rancidity between ovens as measured by TBA values.

Least squares mean volatile compound RPA values and mean retention times of compounds isolated from sausages containing two different antioxidants are presented in Table 13. There was an increase ($P < .05$) in several volatiles associated with spices. Camphene, a-pinene, ß-pinene, and camphor all had higher volatile percentages in the sausage with rosemary than in the BHT/BHA sausage. According to Heath (1981); pinene, camphene, camphor and borneol have all been reported in rosemary. Our study found that all of these volatiles were higher, except borneol in the rosemary sausage than in the BHT/BHA sausage. The 9,12,15-octadecatrienal (tentative identification) was also present in a higher

TABLE 12. LEAST SQUARES MEAN VOLATILE COMPOUND PERCENTAGES[a] ISOLATED FROM PORK SAUSAGE COOKED BY TWO DIFFERENT OVENS

Compound	Mean retention time[b]	Oven	
		Impingement	Multipurpose
a-pinene	6.2	2.32	1.74
Camphene	7.7	5.41	4.60
Hexanal	11.6	2.82	2.99
ß-pinene	12.5	2.62	2.44
2-methyl-2-hepten-6-one[t]	16.4	10.80	11.01
P-cymene	18.5	0.74	0.83
Unknown 1	28.1	0.39	0.36
Unknown 2	29.0	0.34	0.35
T,T-2-4-heptadienal	32.4	22.11	23.01
Unknown 3	33.2	8.54	9.40
Linalool	34.4	0.61	0.61
9,12,15-octadecatrienal[t]	35.5	1.06	1.07
Camphor	38.7	33.94	33.16
Ocimene[t]	39.6	2.29	2.21
Borneol	43.1	3.75	3.37
Butylated hydroxytoluene	53.9	1.60	2.20
Unknown 4	60.3	0.41	0.42
Unknown 5	64.0	0.26	0.23

[a] Means are expressed as relative percentage of the sum of the compound peak areas.

[b] Mean retention times are in min.

[t] Tentative identification of compound based upon library similarity index.

TABLE 13. LEAST SQUARES MEAN VOLATILE COMPOUND PERCENTAGES[a] ISOLATED FROM PORK SAUSAGE USING TWO DIFFERENT ANTIOXIDANTS

Compound	Mean retention time[b]	Antioxidant[c]	
		BHA/BHT	Rosemary
a-pinene	6.2	1.31[d]	2.75[e]
Camphene	7.7	3.61[d]	6.40[e]
Hexanal	11.6	2.64	3.16
ß-pinene	12.5	2.05[d]	3.01[e]
2-methyl-2-hepten-6-one[t]	16.4	11.01	10.80
P-cymene	18.5	0.73	0.84
Unknown 1	28.1	0.35	0.39
Unknown 2	29.0	0.33	0.36
T,T-2-4-heptadienal	32.4	23.83[d]	21.29[e]
Unknown 3	33.2	11.05[d]	6.89[e]
Linalool	34.4	0.59	0.63
9,12,15-octadecatrienal[t]	35.5	0.89[d]	1.23[e]
Camphor	38.7	31.78[d]	35.32[e]
Ocimene[t]	39.6	2.06	2.43
Borneol	43.1	3.69	3.43
Butylated hydroxytoluene	53.9	3.32[d]	0.49[e]
Unknown 4	60.3	0.46	0.36
Unknown 5	64.0	0.29	0.21

[a] Means are expressed as relative percentage of the sum of the compound peak areas.

[b] Mean retention times are in min.

[c] Antioxidants were added at a level of 200 ppm.

[de] Means in a row with a different superscript differ ($P < .05$).

[t] Tentative identification of compound based upon library similarity index.

percentage in the rosemary treated sausage. There was less ($P < .05$) T,T-2-4-heptadienal, unknown 3 and BHT in the rosemary treated sausage. Based upon retention times and corresponding spectra, BHT was found in all treatments. There was more BHT in the BHT/BHA treated sausage than in the rosemary treated sausage. Two possible explanations for BHT in the rosemary treated sausage are a contamination with BHT in the mixer during batching or spice preparation. In either instance, only a small amount was detected in the rosemary treated sausage. BHA was not detected in either type of sausage, mainly because it decomposes at 250°C, the temperature of the GC injector during analysis.

Least squares mean percentages of volatile compounds isolated from pork sausage at 0 and 90 d storage are presented in Table 14. Hexanal, Unknown 1, Unknown 2, borneol and 9, 12, 15 octadecatrienal (tentative identification) all increased from 0 to 90 d storage. T,T-2-4-heptadienal and 2-methyl-2-hepten-6-one (tentative identification) both decreased at 90 d storage. Cross and Ziegler (1985) found that large quantities of hexanal and valeraldehyde were present in uncured ham, but absent in cured ham. They concluded that these were derived by oxidative cleavage of unsaturated fatty acid resides from linoleate. St. Angelo et al. (1987) reported high correlations with malonaldehyde, sensory panels and hexanal and 2,3-octanedione. St. Angelo et al. (1987) also reported that 2,4-heptadienal and pentanal had the highest ratio of concentration in warmed over beef as compared to fresh beef. Taste panels rejected samples of stored poultry skins in which high levels of carbonyls, particularly the 2-enals and 2,4-dienals were present (MacNeil and Dimick, 1970). They

TABLE 14. LEAST SQUARES MEAN VOLATILE COMPOUND PERCENTAGES[a] ISOLATED FROM PORK SAUSAGE AT TWO STORAGE PERIODS

Compound	Mean retention time[b]	Storage[c] 0	Storage[c] 90
a-pinene	6.2	1.98	2.09
Camphene	7.7	4.92	5.09
Hexanal	11.6	1.66[d]	4.15[e]
ß-pinene	12.5	2.73	2.34
2-methyl-2-hepten-6-one[t]	16.4	11.87[d]	9.94[e]
P-cymene	18.5	0.83	0.74
Unknown 1	28.1	0.22[d]	0.52[e]
Unknown 2	29.0	0.23[d]	0.46[e]
T,T-2-4-heptadienal	32.4	23.70[d]	21.42[e]
Unknown 3	33.2	9.44	8.50
Linalool	34.4	0.55	0.67
9,12,15-octadecatrienal[t]	35.5	0.90[d]	1.22[e]
Camphor	38.7	33.33	33.78
Ocimene[t]	39.6	1.92	2.58
Borneol	43.1	3.18[d]	3.95[e]
Butylated hydroxytoluene	53.9	2.01	1.79
Unknown 4	60.3	0.37	0.45
Unknown 5	64.0	0.18	0.32

[a] Means are expressed as relative percentage of the sum of the compound peak areas.

[b] Mean retention times are in min.

[c] Pork sausage links were stored at 0°C.

[de] Means in a row with a different superscript differ ($P < .05$).

[t] Tentative identification of compound based upon library similarity index.

suggested that these were the result of oxidation of linoleic and linolenic acid residues. In summary, our findings that hexanal increased at 90 d was in agreement with others' findings and this increase was likely due to cleavage of the unsaturated fatty acid linoleate.

Unlike St. Angelo et al. (1987), a decrease was found in T,T-2,4-heptadienal with storage. This may be due to its binding to the protein. The reason for the increase in borneol is unclear; however, it may be compensatory increase due to the decrease of 4.38 in the RPA's of hexanal plus 2-methyl-2-hepten-6-one which is only partially offset by the increase of 2.81 in the RPA's of T, T-2,4-heptadienal plus 9, 12, 15-octadecatrienal.

CHAPTER 5

CONCLUSION

The objectives of this study were to determine the effects of impingement oven cooking, rosemary oleoresin and storage time on the yield and sensory characteristics of pre-cooked, uncured pork sausage links (SL). In addition, flavor volatile compounds responsible for WOF in SL were studied. Much of this research is difficult to compare to other findings due to the fact that few research reports are available on either the impingement oven or the effects of rosemary oleoresin.

The impingement oven did have superior initial cooking yields for SL as compared to the MPO cooked SL. The greater lipid retention may have been due to dehydration of the natural casing which may have served as a superior barrier and/or less cooking time in the impingement oven. However, the impingement cooked SL did exhibit disadvantages for degree of tenderness and yields upon reheating. The impingement link tended to have higher Warner-Bratzler shear force values and was perceived by panelists as tougher than the MPO cooked SL. Reheating yields were lower for the SL cooked by impingement than by the MPO. No differences were found for WOF, flavor, juiciness or moisture and lipid percentages of recooked SL between ovens.

An unexpected difference was found for flavor when comparing SL with rosemary oleoresin to SL with BHT/BHA. The rosemary oleoresin SL were perceived as having hotter flavor than the BHT/BHA SL. This may be

due to a concentration of spices caused by a dispersion problem with the rosemary oleoresin. In addition, panelists made comments about a sage flavor note in rosemary SL. This sensation may have been scored as hotter on the flavor scale. Finally, the difference may have only been due to a formulation error at the production facility. The rosemary oleoresin was as effective as the BHT/BHA SL up to 45 d storage for retarding WOF, but this effectiveness decreased at 90 d. As would be expected no consistent differences were found for tenderness, juiciness, reheated lipid percentages or reheating yields between antioxidants.

Oxidative rancidity and reheated moisture and lipid percentage results were as expected over storage days. Oxidative rancidity was highest at 90 d storage. Moisture percentages of reheated SL was lowest at 90 d and the inverse was true for lipid percentages. This is most likely due to a dehydration of the SL during frozen storage. Reheating yields were lower for 90 d SL.

No differences in percentages of volatiles were found between SL cooked in the impingement oven compared to those in the MPO. More camphene, α-pinene, ß-pinene and camphor were found in the SL with rosemary oleoresin than with BHT/BHA. All of these compounds have been reported previously in rosemary. Hexanal increased as storage increased and has been reported in the literature as being associated with WOF development. T,T 2,4 heptadienal decreased at 90 d, which is in contrast to other findings.

The yield advantages for the impingement oven need to be studied further. The reasons for the improvement in the yields were not reviewed in the project. Furthermore, the tenderness effect needs to be carefully

examined and consumer panels need to be utilized to determine what effect this would have on acceptability of the product. The possible flavor effect and dispersion characteristics of rosemary also need to be examined. Finally, more research is needed on the volatiles responsible for WOF.

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LITERATURE CITED

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APPENDIXES

APPENDIX A

TBA ANALYSIS

TBA analysis was performed using a modified distillate method as used by Rhee (1978). Ten grams of sample, 5 ml of 0.5% propylgallate (PG), 5 ml of 0.5% ethylenediamine tetra-acetic acid (EDTA) and 40 ml of deionized water were blended for 2 minutes in a Virtis homogenizer at a setting of 30. After blending 2.5 ml of 4N hydrochloric acid (HCL) was added to the homogenate. The homogenate was then placed into a 800 ml Kjeldahl flask and the container is rinsed with 47.5 ml of deionized water. Boiling beads and 1 ml of antifoaming agent were added to the Kjeldahl flask. Sample were completed in duplicate.

Standard curves for malonaldehyde (MA) content were prepared by mixing 1*10-4 M tetraethoxypropane (TEP) with 2.5 ml of 4N HCL and 92.5 ml of deionized water in a Kjeldahl flask. Fifty ml of distillate were collected for both samples and standard. Solutions containing 1*10-5, 2*10-5, 3*10-5, 4*10-5 and 5*10-5 moles MA/10 ml solution were prepared by pipetting 1, 2, 3, 4 and 5 ml of distillate from TEP, respectively, into separate test tubes. Enough deionized water was added to each test tube to bring the total volume to 5 ml. To each tube was then added 5 ml of 0.02 M TBA solution and all tubes were heated 30 to 35 minutes in a water bath for MA-TBA complex formation. The absorbance was determined at 532 nm. The equation of the standard curves as absorbance versus MA concentration was determined by linear regression. Each treatment distillate also had 5 ml of 0.02 M TBA solution added to the

test tube. In like manner the sample was analyzed at 532 nm. The absorbance of the sample was converted to mole of MA by linear regression and then converted to mg MA/kg meat which is the TBA number.

Reagents

1. 0.5 % PG (antioxidant) in alcohol, add 0.5 g propylgallate to 100 ml ethanol.

2. 0.5 % Na_2EDTA (chelating agent), add 0.5 g Na_2EDTA to 100 ml deionized water.

3. 0.02 M 2-thiobarbituric acid (TBA) (speeds up color reaction), add 0.5766 g TBA to 200 ml solvent. Solvent mix, 198 ml glacial acetic acid and 22 ml deionized water.

4. 4 N HCL (prevents MA from attaching to proteins). 1 part concentrated HCL (12 N) to 2 parts water.

5. TEP (gives off MA as a breakdown product during distillation), dissolve 0.2203 g TEP in 100 ml deionized water (10^{-3} TEP), Dilute 1 ml 10^{-3} TEP with 100 ml deionized water (10^{-4} TEP).

APPENDIX B

SENSORY EVALUATION SHEET

NAME _____ SESSION 1 2 3

Product: SAUSAGE

You will be given 4 samples. Please evaluate them as presented. Mark the scorecard by placing a check on the line to represent the degree to which the characteristic is present in the sample.. Please rinse and eat a cracker between samples.

SAMPLE _____

TENDERNESS

tender _____ tough

JUICENESS

dry _____ juicy

FLAVOR

mild _____ spicy

WARMED OVER FLAVOR

fresh _____ rancid

ADDITIONAL COMMENTS REGARDING FLAVOR

Note: On originals, lines were 150 mm in length.

VITA

Craig D. Bacon was born July 30, 1962 in Fair Grove, Missouri, the son Mr. Kenneth W. and Mrs. Betty J. Bacon. He graduated May, 1980 from Fair Grove High School and enrolled that fall at The University of Missouri, Columbia. He received a bachelor of science degree in Agriculture Education in May, 1984. In the summer of 1984, he accepted a research assistantship in Food Technology and Science at the University of Tennessee and began study toward a Master's degree. This degree was awarded in March, 1987. The author then started on a PhD in Food Technology and Science at University of Tennessee. This degree will be awarded in May, 1990.

The author is a honarary member of Farmhouse Fraternity, Gamma Sigma Delta-Honor Society of Agriculture, American Meat Science Association, American Society of Animal Scientists and Institute of Food Technologists.