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SOME FACTORS INFLUENCING THE NON-HEME IRON CONTENT AND LIPID OXIDATION IN MEAT

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A. M. Pearson
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SOME FACTORS INFLUENCING THE NON-HEME IRON CONTENT AND LIPID OXIDATION IN MEAT

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ABSTRACT

SOME FACTORS INFLUENCING THE NON-HEME IRON CONTENT AND LIPID OXIDATION IN MEAT

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Three methods of determining the non-heme iron content of meat pigment extracts were compared. The methods differed in final pH and in application of heat or in using no heating. The pH was shown to have little effect, while heating resulted in higher values for non-heme iron. Both the final temperature and rate of heating influenced release of heme iron from meat pigment extracts, with optimum temperatures for release being 63 - 70°C. Slow heating was more effective than fast heating in releasing heme iron from the extracts. Addition of nitrite stabilized the heme iron in the pigment extracts, apparently by preventing disruption of the porphyrin ring. Addition of salt accelerated lipid oxidation of meat during cooking and subsequent storage at 4°C. Salt coated with α-tocopherol inhibited oxidation during cooking but not during storage. Both BHA-citric acid-propylene glycol coated salt and Tenox 4 (added with salt) were effective in preventing lipid oxidation during cooking and storage of meat.

To my dear parents

Mr. and Mrs. Huan-Huei and Li-Ting Chen

and to my lovely wife Yi-Hwa

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

The term warmed-over flavor (WOF) was first used by Tims and Watts (1958) to describe the rapid development of oxidized flavor in refrigerated uncured cooked meat. The rancid flavor usually becomes apparent within 48 hours at $4^{o}C$. WOF has been recognized by consumers for years as evidenced by their aversion to "warmed-over" steaks, roasts and other "leftover" meat items, yet not until the last 15-20 years have scientists paid any attention to its significance. The need for a good understanding of WOF is generated because changing social patterns and eating habits have greatly increased the demand of precooked quick-frozen meals which frequently are plagued by this problem.

WOF was considered to result from lipid oxidation by Tims and Watts (1958), who showed that the loss of meat flavor was paralleled by an increase in TBA values. Sato and Hegarty (1971) first proposed that non-heme iron played a major role in accelerating lipid oxidation. Love et al. (1974) confirmed the observations of Sato and Hegarty (1971) by showing that TBA values were not accelerated when metmyoglobin was added at level of 1 to 10 mg/g of meat. However, levels of ferrous iron as low as 1 part per million (ppm)

resulted in enhanced lipid oxidation in water-extracted cooked meat. By using a model system, Igene et al (1979) demonstrated that the concentration of free iron in a cooked meat extract was 4.18 μg/g meat compared to 1.80 μg/g meat for the uncooked extract.

Nitrite at a level of 156 ppm was shown to inhibit WOF in a meat model system by Igene et al. (1979) and in meat system by Fooladi et al. (1979). Although several mechanisms have been proposed for the antioxidant activity of nitrite (Sato and Hegarty, 1971; Zipser et al., 1964; Kanner, 1979), none of them have been proven. Therefore, it is the purpose of the present study to investigate the mechanism by which nitrite prevents WOF.

It is also of great importance to test the effects of sodium chloride, and of antioxidants such as α-tocopherol and Tenox 4 on development of WOF in refrigerated cooked meat. Salt has been shown to be a prooxidant in some cases and either have no effect or be an antioxidant in other foods (Lea, 1939). Both prooxidant activity (Ellis et al., 1974; Kimato et al., 1974) and antioxidant activity (Lips, 1957; Witting, 1975) of α-tocopherol has also been found in carcass lipids and meat products. But the effect of antioxidant-coated salt on cooked meat has not yet been studied. Specifically, the objectives of this investigation were:

(1) To determine whether nitrite reacts with myoglobin to inhibit the release of free iron from myoglobin during cooking.

(2) To ascertain the effects of NaCl on the development of WOF in cooked meat;

and (3) To investigate the effects of various antioxidants on development of WOF in cooked meat which contains 2% added salt.

LITERATURE REVIEW

Distribution and Composition of Animal Fats

Lipids in meat, poultry and fish are commonly classified as depot or adipose tissue and as intramuscular or tissue lipid (Watts, 1962; Love and Pearson, 1971). The depot fats are largely localized as fat globules within the individual cells, and consist mainly of triglycerides (Watt, 1962). The tissue lipids contain proportionately larger amounts of phospholipids, which occur largely, if not entirely, in association with proteins as lipoproteins and proteolipids (Watts, 1962). Tissue lipids are an integral part of various cellular structures, such as the cell wall (Kono and Colowick, 1961), the mitochondria (Holman and Widmer, 1969), the sarcoplasmic reticulum (Newbold et al., 1973), and the microsomes (Macfarlane et al., 1960).

Although the amount of tissue lipids in meat is highly variable, ranging from 2.0% to 12.0% in beef (Orme et al., 1958), the level of phospholipids (0.5-1.0%) remains nearly constant when expressed as a percentage of muscle (Dugan, 1971). According to Hornstein et al. (1961), the phospholipids in muscle contain a larger percentage of unsaturated fatty acids than the neutral lipids, with particularly high levels of linoleic and arachidonic acids.

The lipid composition for the beef lean tissue is given in Table 1. Variation in the phospholipid content was found to vary between species by Kaucher et al. (1944) and from location to location within the same species by Gray and Macfarlane (1961). Luddy et al. (1970) found that porcine light muscles contained 20% more lipid and that these lipids contained 20% more triglycerides and 40% less phospholipids than those from dark muscles. They also found the level of fatty acids in the phospholipids from light muscles were higher in monoenes while the dark muscle phospholipids predominated in polyunsaturates. A similar report was established with poultry by Peng and Dugan (1965) and by Acosta et al. (1966). Katz et al. (1966) have shown that dark meat (legs) from the chicken contains only about half as much phospholipids as white meat (breast).

Component phospholipids expressed as a percentage of the total phospholipids are somewhat similar in most animal tissues (Body et al., 1966; 1970). Keller and Kinsella (1973) reported the composition of beef phospholipids as being 53-58% phosphatidyl choline (PC), 23-25% phosphatidyl ethanolamine (PE), 5-7% sphingomyelin (SP), 5-7% phosphatidyl inositol (PI), 1-4% phosphatidyl serine (PS) and 1-6% all others.

Table 1. Lipid composition of lean beef muscle[a]

Lipid Fraction	%
Triglycerides (per cent of tissue)	2-4
Phospholipids (per cent of tissue)	0.8-1.0
Fatty acids with 2 and 3 double bonds	
Per cent of triglycerides	6.1
Per cent of phospholipids	25.2
Fatty acids with 4 or more double bonds	
Per cent of triglycerides	0.1
Per cent of phospholipids	19.2

[a]Hornstein et al. (1961)

Role of Phospholipids in Lipid Oxidation

All of the early work which had been done on rancidity in meats was concerned with the oxidation of adipose tissue (Watt, 1954). Tims and Watts (1958) first noted that rapid deterioration in the flavor of cooked meat was correlated with the amount of phospholipids on using the TBA method to measure the amount of oxidation. They postulated the possible denaturation of protein during heating may free the phospholipids, and thus make them more susceptible to oxidative attack.

On fractionating the total lipids into triglycerides and proteolipids, it was demonstrated by the TBA test that the latter are responsible for the intensive oxidative reaction induced by heating porcine muscle tissue (Younathans and Watts, 1960). Campbell and Turkki (1967) have shown that the phospholipid concentration is higher in cooked meat than in raw meat because the neutral lipids are lost from the meat more readily on heating than the phospholipids. Igene et al. (1981) found that the drippings collected upon cooking contained largely triglycerides, whereas PE was essentially absent, indicating that it was bound to the membranes. The increased proportion of PE in cooked meat coupled with its susceptibility to oxidation indicate that it may play a key role in the autoxidation of cooked meat.

Igene et al. (1981) also found there was a significant decrease during frozen storage in the amount of PE and PC, but the decline was much greater upon cooking and storage. Decreases in the component phospholipids may be due to either autoxidation, hydrolytic decomposition, the lipid-browning reaction of lipid protein copolymerization as outlined by Lea (1957).

Tissue Iron

Monier-Williams (1950) stated that blood contains about 70% of the total iron in the body, while the other 30% is located in the tissues in various forms, partly as heme iron and partly as non-heme iron. Among the heme compounds, they stated that hemoglobin and myoglobin are the major iron-containing porphyrins present in blood and muscle, respectively. Iron is also associated with the flavin nucleotide enzymes, cytochrome oxidase, xanthine oxidase and succinic dehydrogenase (Merkel, 1970). Iron is not only stored in the non-heme compounds as ferritin and hemosiderin, but it is also present in transferrin (Merkel, 1970). According to Thompsett (1934) the non-heme iron,may be present in both states of oxidation. Ferric iron appears to combine with the non-diffusible phosphorus compounds, such as phosphatides and phosphoproteins. Ferrous iron apparently does not form such complexes if the iron becomes reduced, since it is liberated in the ionizable form.

Tompsett (1935) gave values for non-heme iron ranging from 100 to 150 ppm in liver, spleen and bone down to roughly 5 or 10 ppm in other tissues, such as the brain and kidney. The concentration of non-heme iron was reported to be 1 ppm in beef top round steak by Sato and Hegarty (1971), and 1.8 ppm or 8.7% of the total iron of the pigment extract from beef longissimus dorsi muscle by Igene et al. (1979). In contrast, Cook and Monson (1976) reported that the total non-heme iron comprised from 40-50% of the total iron in beef muscle. Similar results of 61.6% for beef muscle were found by Schricker et al. (1982). Table 2 shows the distribution of iron in the human body.

Effect of Heating on Myoglobin

Myoglobin and other heme-containing proteins undergo denaturation during heating. Denaturation of these proteins causes the rapid release of the heme moiety from the globin part of the molecule leaving free heme, which is very sensitive to oxidation (Lawrie, 1966). Cooking, if sufficiently thorough, destroys the porphyrin complex and liberates the available iron (Monier-Williams, 1950). Igene et al. (1979) demonstrated that cooking released a significant amount of non-heme iron from bound heme pigment, which they demonstrated increased the rate of lipid oxidation in cooked meat. Younathan and Watts (1959) have attributed the rapid oxidation of lipids in cooked meat to the conversion of ferrous iron from

Table 2. Iron compounds and their distribution in the adult human body.

Compounds	Iron in grams	Per cent of total iron
Iron porphyrin (heme) compounds		
Blood hemoglobin	3.0	60-70
Myoglobin	0.13	3-5
Heme enzymes		
Mitochondrial cytochromes		
c	0.004	0.1
a_3,a,c_1,b	-	-
Microsomal cytochrome b_5	-	-
Catalase	0.004	0.1
Peroxidase	-	-
Nonheme compounds		
Flavin-Fe enzymes		
Succinic dehydrogenase	-	-
Xanthine oxidase of liver	-	-
$NADH^c$-cytochrome c reductase	-	-
Iron chelate enzyme aconitase	-	-
Transferrin	0.004	0.1
Ferritin	0.4-0.8	7-15
Total available iron stores	1.2-1.5	-
Total iron	4-5	100

[a]Granick (1958).

the porphyrin to the ferric form during heating. Tims and Watts (1958) have suggested that denaturation of protein during heating may free phospholipids, and thus make them more susceptible to oxidative attack.

Effect of pH on Myoglobin

Apomyoglobin, which is myoglobin devoid of its heme group, can be obtained by lowering the pH of a myoglobin solution to pH 3.5 according to Stryer (1972). This author stated that the heme group binds only weakly to the protein at this acidic pH, and thus can be removed by extraction with an organic solvent. Lewis (1954) found less than 10% of the prophyrin was cleaved from MetMb at pH 3.6. In contrast, Snyder (1963) showed that at pH 6.6 40% of the heme present in Mb was extracted with acetone but negligible amounts of hematin were extracted from MetMb.

Heme Compounds as Prooxidants

The catalytic effect of iron prophyrins on the oxidative decomposition of polyunsaturated fatty acids was first described by Robinson (1924), who attributed the catalysis to the iron content of the molecule. According to Greene (1971), the main forms in which myoglobin (Mb) exist in meat are as reduced Mb, as oxymyoglobin (MbO_2) and as nitric oxide ferrohemochrome, in which the iron porphyrin is in the ferrous (Fe^{++}) state. The other forms of myoglobin

include ferrihemochrome and metmyoglobin (MetMb), both of which contain iron in the ferric (Fe^{+3}) state (Kendricks and Watts, 1969; Greene, 1971).

Ferric hemochromogen, the denatured form of this pigment in cooked meat, is postulated to be the active catalytic form of the muscle pigments (Younathan and Watts, 1959; Tappel, 1953). Tarladgis (1961) attributed the catalytic activity of ferric hemoproteins to the paramagnetic character of the prophyrin-bound iron. He suggested that the presence of five unpaired electrons in metmyoglobin produces a strong magnetic field that would favor the initiation of free radical formation.

A number of investigators (Watts, 1954; Tappel, 1952; Younathan and Watts, 1959; Liu and Watts, 1970; Greene, 1975) have also indicated that hematin compounds are involved in lipid oxidation in meat.

Heme Compounds as Antioxidants

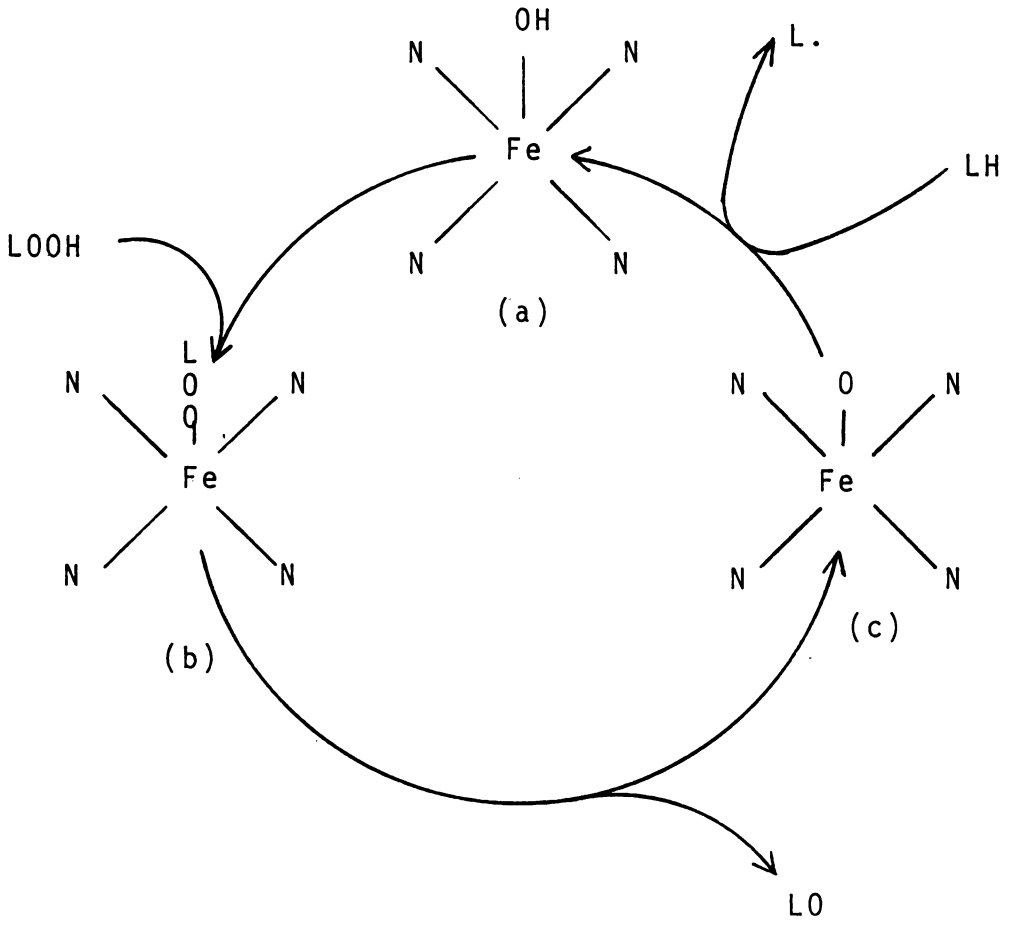
The antioxidant activity of heme compounds was established in fatty acid model systems but there have been no studies on meat products. Kendrick and Watts (1969) reported the linoleate-to-heme ratio for maximum catalysis of lipid oxidation to be 100 for hemin and catalase, 250 for metmyoglobin, 400 for cytochrome c and 500 for methemoglobin. At heme concentrations of two to four times the optimum catalytic amount, they noted that lipid oxidation

did not occur. They theorized that a stable lipid hydroperoxide-heme derivative was formed at inhibitory heme concentrations. At lower heme concentrations, it was postulated that the heme may be unable to contain the lipid radicals, and oxidation results.

Mechanism of Heme Catalysis

Catalysis by iron porphyrins is characterized by rapid initiation and propagation of the lipid oxidation chain reaction according to Tappel (1962). He suggested that hematin involves the formation of a lipid peroxide-hematin compound and its subsequent decomposition into free radicals, which propagate the chain reactions with the concomitant destruction of the catalyst. Tappel (1955) suggested the mechanisms for hematin-catalyzed unsaturated lipid oxidation as shown in Figure 1. The hematin compound (a) and the lipid peroxide (LOOH) are postulated to form an activated compound (b). Subsequent scission of the peroxide bond occurs, resulting in production of a lipid radical (LO·) and a heme radical (c). Abstraction of a hydrogen atom (H) from a lipid molecule (LH) regenerates the hematin and produces a lipid radical (L·). Tappel (1962) also suggested that a direct attack on the lipid by the heme compound could result in generation of lipid radicals according to the following mechanism:

$$LH + \text{hematin} - Fe^{3+} \longrightarrow L\cdot + \text{hematin} - Fe^{2+} + H^{+}$$



$LO^{\cdot} + LH \longrightarrow LOH + L.$

$L. + O_2 \longrightarrow LOO^{\cdot}$

$LOO^{\cdot} + LH \longrightarrow LOOH + L.$

Where:

LH = Linoleic acid

LOOH = Hydroperoxide linoleate

Figure 1. Mechanism for hematin-catalyzed lipid oxidation (Tappel, 1955).

Role of Non-Heme Iron in Lipid Oxidation

Although the catalytic effect of hemoglobin and other porphyrins on lipid oxidation has been a generally accepted phenomen in the past, the importance of non-heme iron on lipid oxidation has been pointed out by scientists in recent years. Kwoh (1971) reported that heme was the dominant catalyst in cooked meat, but that significant lipid oxidation still occurred in cooked meats in which the heme had been destroyed by H_2O_2. Wills (1966) indicated that non-heme iron was a more active prooxidant at acid pH values, whereas, hemoproteins were less pH-sensitive. Sato and Hegarty (1971) reported that beef muscle, which had been thoroughly extracted with water, did not develop warmed-over flavor indicating that the substance(s) responsible for initiating the reaction was/were water soluble. Heme compounds were found to have little effect on the development of warmed-over flavor in this system. The reaction was apparently catalyzed by ferrous iron and ascorbate. They suggested that ascorbic acid functions by keeping at least a portion of the iron in the ferrous state. At higher levels ascorbic acid inhibited the reaction, possibly by upsetting a balance between ferrous and ferric iron.

The observations of Sato and Hegarty (1971) were confirmed by Love and Pearson (1974), who showed that metmyoglobin at concentrations from 1 to 10 mg/g meat did not influence

TBA values of water extracted cooked beef, but that Fe^{2+} was effective as a prooxidant. Love and Pearson (1974) also pointed out that the prooxidant activity was located in the low molecular weight fraction of the extract. Igene et al. (1979) demonstrated that the increased rate of lipid oxidation in cooked meat is due to the release of non-heme iron from the meat pigments during cooking. Addition of 2% EDTA was shown to effectively chelate the non-heme iron and significantly reduced lipid oxidation.

Metallic Ions as Catalysts of Lipid Oxidation

Ingold (1962) pointed out that metals, such as iron, cobalt, and copper which possess two or more valency states with a suitable oxidation-reduction potential between them, are particularly important catalysts. Ingold (1962) also stated that the effect of metals can be reflected in an altered rate of chain initiation, propagation, or termination, as well as by an altered rate of hydroperoxide decomposition.

The basic function of the metal catalyst is to increase the rate of formation of free radicals (Ingold, 1962). Uri (1956) has described a commonly accepted mechanism for metal catalysis involving the oxidation of a metal ion with hydroperoxide decomposition resulting as follows:

$$M^{+n} + ROOH \longrightarrow M^{+(n+1)} + {}^{-}OH + RO\cdot$$

Ferrous iron has been shown to have greater prooxidant activity than ferric iron in a number of experimental systems (Brown et al., 1963; Sato and Hegarty, 1971).

Measurement of WOF in Cooked Meat

WOF was considered to result from lipid oxidation by Tims and Watts (1958). They showed that the loss of meat flavor was paralleled by an increase in TBA value, which measures the amount of malonaldehyde - a product of lipid oxidation. Zipser et al. (1964) found a high correlation between TBA values and oxidative off flavor in cooked meat. Thus, TBA test has been used routinely to measure off flavor development in cooked meat.

Younathan and Watts (1959) concluded that the difference in flavor between nitrite-cured and non-nitrite cured meat soon after cooking is due to the development of warmed-over flavor caused by the rapid oxidation of unsaturated fatty acids in the uncured meat. On the other hand, nitrite protected the meat against oxidation.

Action of Antioxidants on Food Lipids

Antioxidants may interfere with or delay the onset of oxidative breakdown of fats and fatty foods (Blanck, 1955). Primary or phenolic antioxidants (such as tocopherols, butylated hydroxyanisole or butylated hydroxytoluene) function by breaking the oxidative reaction chains

(Shelton, 1959). In support of this viewpoint, Cort (1974) reported that the phenolic antioxidants act as electron or hydrogen donors to quench electron mobility with subsequent interruption of free-radical chain reactions.

According to Uri (1961), the mechanism of antioxidant action is as follows:

$$ROO^{\cdot} + AH \text{ (antioxidants)} \longrightarrow ROOH + A^{\cdot}$$

The radical may be stabilized by recombination in either of two ways:

$$A^{\cdot} + A^{\cdot} \longrightarrow A_2$$

$$ROO^{\cdot} + A^{\cdot} \longrightarrow ROOA$$

This means that during autoxidation, the antioxidants are converted into dimers and other products (Uri, 1961). It is also possible that the antioxidant is oxidized directly by oxygen. This is the case for tocopherol, which is partly oxidized to tocoquinone in fats (Tappel, 1962). At the end of the induction period, the antioxidants disappear with little being known as to their exact fate (Cort, 1974).

α-Tocopherol as a Lipid Antioxidant

The tocopherols are products of synthesis by plants, and may be deposited in animal tissues in the non-saponifiable portion of the lipid fraction, usually together with sterols, vitamin A, vitamin K and other naturally occurring antioxidants (Mervyn and Morton, 1959).

The *in vitro* antioxidant activity of the four known tocopherols increases in order of alpha, beta, gamma and delta, while the activity *in vivo* increases in the opposite order (Parkhurst *et al.*, 1968). According to Aruand and Woods (1977), vitamin E (tocopherols) is not destroyed by acid, alkali, the process of hydrogenation, or by high temperature, but is oxidized slowly by air and rapidly in the presence of rancid fats.

The chemical basis of the antioxidant action of vitamin E is its combination with free radical intermediates of lipid oxidation and lipid peroxides, thus inhibiting further lipid peroxidation (Tappel, 1962). A number of studies have demonstrated the beneficial antioxidant activity of α-tocopherol in improving the stability of carcass lipids of poultry (Marusich *et al.*, 1975), of beef (Kimoto *et al.*, 1974) and of pork (Astrup, 1973). Nevertheless, some workers regard α-tocopherol as a poor antioxidant, particularly in products containing highly unsaturated fatty acids (Lips, 1957; Witting, 1975).

At low concentrations, α-tocopherol functions as an antioxidant, but at high concentrations may become a pro-oxidant (Chipault, 1961; Labuza, 1971). Witting (1975) suggested that an increase in tocopherol concentration results in increased peroxide formation through free-radical initiation, an increased rate of autocatalysis and an increased rate of destruction.

Effect of NaCl on Oxidation of Meat

Sodium chloride, a common meat additive, has a puzzling effect on oxidative changes in meat. The role of NaCl in initiating color and flavor changes in meat is well known but poorly understood. Some of the studies on salt are complicated by the fact that salt may contain metal contaminants, which may serve as catalysts of lipid oxidation. Rancidity may still develop in the fat of dry cured hams, even though a low metal containing salt (0.1 ppm copper and 0.4 ppm iron) is used (Olson and Rust, 1973).

An increase in the concentration of NaCl from 2 to 6% has been reported to result in a rapid rate of monocarbonyl formation during freezer-storage of pork tissue by Ellis et al. (1968).

Lea (1937) suggested that NaCl influences lipid oxidation by promoting the activity of lipoxidase in meat. Later work by Banks (1961) and Tappel (1952) showed that meat does not contain lipoxidase.

Chang and Watts (1950) attributed the prooxidant activity of salt to the possibility that salt may affect the physical state of meat in such a way that the hemoglobin would be brought into closer contact with the fat.

Nitrite as an Antioxidant in Cooked Meat

Nitrite has been shown to be an effective antioxidant in cooked meat by many researchers. Younathan and Watts (1959) reported that nitrite and sodium chloride acted synergistically to retard oxidation of lipids in cooked meat stored at refrigerated temperatures. Sato and Hegarty (1971) showed WOF in cooked ground beef was eliminated by nitrite at a level of 220 ppm and partially inhibited at 50 ppm.

Swain (1972) found the TBA values of nitrite-treated hams were initially lower than comparable samples without nitrite and remained lower during storage up to 2 weeks. Fooladi *et al.* (1979) and Igene *et al.* (1979) demonstrated that the addition of nitrite at a level of 156 ppm protected against oxidative changes during the storage of cooked meat.

Several mechanisms by which WOF is inhibited have been proposed. Zipser and Watts (1967) reported that the lower level of oxidation in stored, cured meat results from the conversion of the pigments to the catalytically inactive ferrous nitric oxide hemochromogen. Nitrite was also

suggested to inhibit WOF by stabilizing lipids in all membranes which are normally disrupted and exposed to oxygen by cooking or grinding (Sato and Hegarty, 1971; Pearson et al., 1977).

Kanner (1979) demonstrated a potent antioxidant, s-nitrosocysteine, was generated during curing of meat with nitrite, and thus serves as an inhibitor of WOF in cured meat.

EXPERIMENTAL

Materials

Source of Meat

Beef was obtained from the Michigan State University Meat Laboratory. Portions of semitendinosus muscle were excised from carcasses of 9 month old cattle immediately after slaughter. The meat was placed in a 4°C cold room until 24 hours postmortem, then it was wrapped, frozen and stored at -20°C for future use.

Solvent and Chemicals

Experiment A - All chemicals and reagents were of analytical grade except acetone which was of reagent grade.

Experiment B - All chemicals were of reagent grade unless otherwise specified. The sodium chloride was of analytical grade. Tenox 4 (Eastern Chemical Products, Inc., Kingston, TN, 37662) consists of 20% butylated hydroxyanisole (BHA), 20% butylated hydroxytoluene (BHT) and 60% corn oil. The α-tocopherol-coated salt was obtained from Diamond Crystal Salt Company, St. Clair, Michigan. It contained 3.25% α-tocopherol coated on 96.75% salt. The other antioxidants used in this experiment included BHA-citric acid-propylene glycol-coated salt, which contained 3% BHA, 1.6% citric acid and 2.2%

propylene glycol coated on 93.2% salt.

Methods

Experiment A

Preparation of Meat Model System

A meat sample weighing approximately 500 grams was thawed at room temperature. After removing all visible fat and connective tissue, the lean tissue was homogenized with one volume of deionized water in a Waring blender. The homogenate was saved and the pigments were extracted by adding two additional volumes of deionized water and stirring constantly with a magnetic stirrer at $4^{o}C$ for 24 hours. The extract was then separated from the meat residue by filtering through cheese cloth. Extraction with three volumes of water was repeated four times. At this point residue was practically colorless. The extracted meat pigments were concentrated in a Virtis II freezer-drier, until each milliliter of the extract represented 2 grams of raw meat sample.

The cured pigment was produced by adding nitrite to the meat at 156 ppm level. A period of 24 hours was allowed to ensure the curing reaction. The cured pigment was then extracted using the same procedure as for the raw meat pigments.

Analysis of Total Iron in Muscle Tissue

The concentration of total iron and non-heme iron in the sample were measured by using an atomic absorption spectrophotometer (Instrumentation Laboratory, Inc., Lexington, MA).

To measure the concentration of total iron, nitric acid and perchloric acid digestion was carried out on the entire muscle sample. Five grams of meat were placed in acid-washed 150 ml Erlenmeyer flasks with 20 ml of concentrated nitric acid and 5 ml of perchloric acid. The mixture was heated on a hot plate until the digest appeared clear. The samples were then diluted with glass-distilled water to appropriate volumes so that the mineral concentration fell within the linear range using the atomic absorption spectrophotometer.

If 5 ml of muscle pigment extract was used as sample, 5 ml of concentrated nitric acid and 1 ml of perchloric acid would be needed for digestion of sample.

Analysis of Non-Heme Iron by Different Methods

Method 1 - Direct Acid Digestion. This procedure was a modification of the method of Schricker et al. (1982) who used direct acid digestion of the meat samples. Muscle pigment extracts of 2 ml were incubated in the presence of 2 ml of an acid mixture containing equal volumes of 6 N HCl and 40% trichloroacetic acid (TCA) in a 65^{o}C water bath for 20 hours in loosely stoppered 15 ml centrifuge tubes. After centrifugation at 3000 rpm for 15 minutes, the clear supernatants were transferred to small tubes and directly analyzed using an AA spectrophotometer.

Method 2 - Chelation with EDTA and Precipitation with TCA. This method utilized the procedure described by Igene et al. (1979). To 2 ml of pigment extract, 1 ml of a

12% ethylenediaminetetraacetic acid (EDTA) solution was added to chelate any non-heme iron in the sample. Then the heme iron was removed by precipitating heme compounds with 1 ml of 40% TCA. After centrifugation at 3000 rpm for 15 minutes, the supernatants were transferred and analyzed by AA spectrophotometry.

Method 3 - Chelation with EDTA and Precipitation with Acetone. This procedure was a modification of the method of Igene et al. (1979) with the TCA being replaced by acetone. Two ml of the pigment extract were treated with 1 ml of 12% EDTA to chelate the non-heme iron. Then 1 ml of acetone was added to precipitate the heme iron (myoglobin).

For all three methods, the amount of heme iron was obtained by the difference between total iron and non-heme iron.

Heating Treatments of Muscle Pigment Extracts

The pigment extract was placed in 50 ml test tubes. To show the effect of incomplete cooking, the uncooked extract was heated for time periods of 5, 10 and 20 minutes in an 85-87^{o}C water bath. Temperature change of each sample was monitored by thermocouples in the center of the tubes.

Cleaning of Glassware

All of the glassware was cleaned by soaking in dilute HCL (1:3, v/v) for more than 30 minutes then rinsed with deionized water to eliminate iron contamination. Caution

was also taken to avoid contamination of the sample with air.

Experiment B

Experimental System

This part of the study was designed to test the effects of salt and antioxidants on the development of WOF. A sample weighing 1300 grams was thawed and ground through a 3/8-inch plate then through a 3/16-inch plate. The ground meat was divided into five different groups and assigned to one of the five different treatments as shown on Table 3. One third of the samples in each treatment group was used as raw meat while the remaining samples were cooked. After cooking, half of the cooked samples were refrigerated at $4^{o}C$ for 48 hours, while the other half were immediately used for the TBA test. The TBA test was carried out on the raw meat immediately after addition of the antioxidants and on the cooked meat at 0 and 48 hours refrigeration. Since the amount of antioxidants added to the samples was based on the fat content, it was necessary to determine the content of fat and moisture before adding the antioxidants to the samples.

Addition of Antioxidants

According to FDA regulations, when a single antioxidant is added, it may not exceed 0.01% based on the fat content of the food. When more than one antioxidant is added, the combined total may not exceed 0.02%, of which no one antioxidant may exceed 0.01%. When antioxidants

Table 3. Design of experiment for testing effect of antioxidant-coated salt on cooked meat.

Sample Number	Treatment
1	Control - no added salt or antioxidants
2	2% NaCl[a] w/o antioxidants
3	2% NaCl[a] coated with 0.01% α-tocopherol[b,c]
4	2% NaCl[a] coated with 0.01% BHA[b], 0.007% citric acid[b,c] and 0.005% propylene glycol[b,c]
5	2% NaCl[a] with 0.01% BHA[b,c] and 0.01% BHT[b,c]

[a]% salt based total weight of sample.

[b]% antioxidant based on fat content of sample.

[c]Chemicals coated on the salt.

and synergists are added, the combined total may not exceed 0.025%, with no one antioxidant exceeding 0.01%.

During the preparation of sample, additional salt was added to the original formula of antioxidant-coated salt to end up with 2% salt in the sample. The reason for choosing 2% as the level of salt to be tested is because 2% salt is commonly used for many commercial cured meat products.

Moisture Analysis

A variation of the A.O.A.C. (1975) procedure (24,003) was used for moisture analysis. Aluminum sample dishes were dried at least one hour before use, cooled and handled with tongs to avoid any fat and/or moisture contamination from the hands of the analyst. A sample of approximately 4 g was weighed out into the previously tared, dried dishes. The sample were dried in an air convection oven at 100^{o}C for 18 hours, then cooled in desiccator. The percentage weight loss of the sample was calculated and used as the moisture content.

Fat Analysis

A variation of A.O.A.C. (1975) procedure (24,005) was used for fat analysis with extraction by the Goldfisch apparatus. The Goldfisch beakers were dried in an air convection oven for at least one hour at 100^{o}C then cooled in a desiccator. The dried samples after moisture analysis were used for determining the fat content. The dried samples were rolled together and then placed in the white

porous thimble. Care was taken to avoid spilling the dried material out of the dishes. Then 30 ml of anhydrous diethyl ether was poured into each of the tared and dried beakers. The thimble containing the dried sample was put in a monel holder and clipped on the apparatus with the beaker being attached firmly to the condenser. The hot plate was raised to touch the bottom of the beaker and the heat was applied. Heating was set for about three hours to complete the extraction of fat. By replacing the monel holder with a reclaiming tube, nearly all the ether in the beaker was driven off and collected. The remaining ether was removed by placing the beaker in the drying racks of the apparatus. When no odor of ether remained, the beakers were dried in an air convection oven for one hour then cooled in a desiccator. The weight gain of the beaker was taken as weight of fat in the sample, and was used to calculate the percentage of fat.

Cooking Treatments

Meat samples were put in retortable pouches with the bags left open at one end, and cooked in boiling water until the internal temperature of the samples reached 70^{o}C. They were then cooled by tap water. Drippings were collected and added back to sample before further testing.

Measurement of Lipid Oxidation by the TBA Test

The distillation method of Tarladgis *et al*. (1960) was used for measuring TBA numbers. The distillation

apparatus consisted of a 250 ml round bottom flask, which was attached to a Friedrich Condensor with a three-way connecting tube. It was then placed in an electric heating mantle. A 10 g sample of meat was homogenized with 50 ml of distilled deionized water for 2 minutes in a Virtis homogenizer at low speed. The homogenate was transferred quantitatively into a 250 ml round bottom flask by washing with 47.5 ml of distilled, deionized water. The pH of the meat slurry was adjusted to 1.5 by the addition of 2.5 ml of 4 N HCl. Boiling chips were added and a small amount of Dow antifoam was sprayed into the flask to prevent foaming. The slurry was steam distilled using the highest setting on a power stat until 50 ml of the distillate were collected. The distillate was mixed and 5 ml were transferred to a 50 ml test tube. Then, 5 ml of TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) were added. The tubes were stoppered and the contents mixed. The tubes were heated in a boiling water bath for 35 minutes. After cooling in cold water for 10 minutes, absorbance was read on a Beckman DU spectrophotometer at 538 nm against a blank containing distilled deionized water and TBA reagent. Absorbance readings were multiplied by a factor of 7.8 (Tarladgis et al., 1960). TBA values are expressed as mg malonaldehyde per 1,000 g of sample.

RESULTS AND DISCUSSION

Experiment A

The first part of this study was designed: (1) to determine the influence of various methods of analysis on the amount of non-heme iron in meat pigment extracts; (2) to investigate the effects of heating time on the non-heme iron in the pigment extract; and (3) to ascertain the effects of nitrite on the amount of free iron released during the heating of the pigment extract.

Contents of Pigment Extract

Most of the analyses done in the study were based on the water extract from raw meat samples. The extract of muscle tissue would be expected to contain sarcoplasmic proteins, inorganic constituents, some carbohydrates and their metabolic intermediates. According to Forrest et al. (1975) the sarcoplasmic proteins, which are readily extractable in water, include myoglobin, hemoglobin, and the enzymes associated with glycolysis, the citric acid cycle and the electron transport chain. Although the enzymes of the citric acid cycle and the electron transport chain are contained within the mitochondria, they are readily extracted along with those found directly in the sarcoplasm. As

shown in Table 2, the enzymes and other compounds containing iron are found in the pigment extract.

Analysis for total iron showed that water extraction removed 86.3% of the total iron, or 21.22 µg/g out of 24.57 µg of iron/g of meat sample. Similarly, Sato and Hegarty (1971) reported that the hemin content of the water extracted tissue was reduced to about 25 ppm from a total of 240 ppm found in a 10 g unextracted muscle. This indicates that 89.6% of the total iron was water extractable. This value is comparable to the values obtained in the present study.

Influence of Various Methods for Analysis of Non-heme Iron

Three different methods were used in this study to compare the effects of different pH values and temperatures on the concentration of non-heme iron in the pigment extract. The results are shown in Table 4. The final pH values varied for the different methods, with pH values of 1.21, 1.57 and 5.43 for Methods 1, 2 and 3, respectively. The effects of pH value on the non-heme iron content can be seen by comparing methods 2 and 3. If the pH values were to exert a significant effect upon the value for non-heme iron, the results for the two methods (2 and 3) should be different. However, the values are in good agreement (1.20 and 1.15 µg/g meat, respectively). Although the porphyrin ring binds weakly to globin at pH 3.5 according to Stryer (1972), the differences in pH value did not have any measurable effects on the concentration of non-heme iron. EDTA

Table 4. Mean pH values and concentrations of non-heme iron of muscle pigment extract analyzed by three different methods showing standard deviations.[3]

Method	Reagent	pH Value	Total Iron µg/g meat[1]	Non-Heme Iron µg/g meat[2]	Percentage Non-Heme Iron in %	Heme Iron µg/g meat
Method 1 Schricker et al. (1982)	6N HCl 40% TCA	1.21±0.04	19.16±1.59	1.50[a]±0.06	7.8	17.66
Method 2 Igene et al. (1979)	12% EDTA 40% TCA	1.57±0.03	19.16±1.59	1.20[b]±0.06	6.3	17.96
Method 3 (unpublished)	12% EDTA Acetone	5.43±0.04	19.16±1.59	1.15[b]±0.03	6.0	18.01

[1]Using muscle pigment extract for sample.

[2]Values for non-heme iron followed by different superscripts are statistically significant at $P < 0.05$.

[3]Each value was replicated five times.

was used in both methods 2 and 3 to tie up the free non-heme iron before any other protein-bound iron was precipitated by TCA or acetone.

The effects of temperature and heating time upon the values for non-heme iron were also investigated using Method 1, which was a modification of the procedure of Schricker et al. (1982). Using this method, samples were incubated with acid in a 65^{o}C water bath for 20 hours. This gave a value of 1.50 µg/g meat for the non-heme iron concentration of the pigment extract. This value is considerably higher than that of the other two methods, with Method 2 and 3 giving values of 1.20 and 1.15 µg/g, respectively. The low pH value and long-time heating for Method 1 may release more heme iron from the globin moiety than other two methods.

Concentration of Non-heme Iron in Muscle Tissue

The amount of non-heme iron in raw muscle tissue varies widely between different studies as shown in Table 5. Sato and Hegarty (1971) reported that there was about 1.0 µg/g of non-heme iron in muscle. Igene et al. (1979) reported the percentage of non-heme iron to be 8.7% , or 1.80 µg/g meat out of a total iron content of 20.64 µg/g meat. In the present study, it was found that non-heme iron was 1.31 µg/g meat, which amounted to 6.2% of the total iron (21.22 µg/g meat). These results agree closely with values

Table 5. Concentration of total iron and non-heme iron in muscle tissue.

	Total Iron	Non-Heme Iron	% Non-Heme Iron
Sato and Hegarty (1971)	24.00[a]	1.00[c]	4.2
Igene et al. (1979)	20.64[b]	1.80[b]	8.7
Present study (method of Igene et al. 1979)	21.22[b]	1.31[b]	6.2
Schricker et al. (1982)	23.40[a]	8.40[a]	35.9

[a]Value expressed as μg/g meat, using raw meat for sample.

[b]Value expressed as μg/g meat, using extract for sample.

[c]Sample information unavailable. Approximate value reported by authors with rounding off by the author.

reported by Sato et al. (1971) and Igene et al. (1979). Schricker et al. (1982), however, obtained a much higher value, which translated to 35.9 % of the total iron as being non-heme iron. The high value is probably due to the influence of an acidic environment and long-time heating of the sample. It is pointed out that Schricker et al. (1982) used intact meat samples, whereas pigment extracts were utilized in the current study. The difference observed using the method of Schricker et al. (1982) is no doubt due to the difference in the original samples.

Effect of Fast and Slow Heating on the Stability of Heme Iron

During the early part of this study, it was found that short-time heating did not release the heme iron from the pigment extract, or more specifically, did not increase the concentration of non-heme iron. This was true even though the extract was placed in a 15 ml test tube in a boiling water bath for 50 seconds. The time needed to bring the temperature of the extract up to 70°C was later shown to be approximately one minute.

In order to obtain a slow increase in the temperature of the sample, a test tube containing the extract was placed in a 500 ml beaker containing 450 ml cold water, which was heated to boiling. It required about 9 minutes to bring the temperature of the sample to 70°C. The effects of fast and slow heating on stability of heme iron are shown in Table 6.

Table 6. Average concentrations of non-heme iron in slow and fast heated muscle pigment extracts showing standard deviations (±).[3]

Concentration	Unheated extract	Heated extract	
		Fast heating[1]	Slow heating[2]
µg/g meat	1.31±0.50	1.38 ±0.13	2.25 ±0.16
Percentage increase	-	5.34%	71.75%

[1] Final temperature 70°C. Heating time 50 seconds.

[2] Final temperature 70°C. Heating time 8 minutes 50 seconds. Differences in the non-heme urea content between slow and fast heated samples were significant at $P < 0.05$.

[3] Each value was replicated five times.

The concentration of non-heme iron in the heated extract increased from 1.31 µg/g meat in the unheated extract to 2.25 µg/g meat in the slowly heated extract. This was probably due to the release of heme iron from the porphyrin ring, while that of the rapidly heated extract was much lower, being only 1.38 µg/g meat. It may be that the rapid increase of temperature in the extract induced coagulation of the myoglobin molecule before the heme iron had an opportunity to be cleaved from the globin, thus giving a low value.

Short time heating may explain why hemoglobin, myoglobin and metmyoglobin have little or no effect on the TBA numbers in heated meat model systems. Sato and Hegarty (1971) utilized 10 g of water-extracted beef, to which they added hemoglobin and myoglobin at levels up to 25 and 5 mg/g meat, respectively. They then heated the sample until the temperature reached 70°C. Love et al. (1974) followed essentially the same procedure using 10 mg of metmyoglobin/g of meat and obtained approximately the same results. It seems probable that only one or two minutes would be required to bring the temperature of the sample up to 70°C in a boiling water bath. Thus, the concentration of non-heme iron in these samples would not be significantly elevated and the TBA numbers would not be materially influenced.

Effect of Nitrite on the Stability of Heme Iron

Zipser et al. (1961) first proposed that nitrite forms a stable complex with the iron porphyrin of heat denatured meat, thus inhibiting development of WOF in nitrite-treated meat. Since non-heme iron has been shown to be the major lipid prooxidant in heated meat model systems (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979) and nitrite has been shown to effectively inhibit WOF development in cooked meat, the mechanism by which nitrite inhibits WOF needs to be investigated. Thus, the present study was designed to determine whether nitrite serves as a stabilizer of muscle pigments or in some other way protects the lipids from oxidation.

The effect of heating on the non-heme iron content of a fresh and nitrite-treated meat pigment extract is shown in Table 7. The concentration of non-heme iron in the unheated fresh meat pigment extract was 1.31 μg/g meat. After 5 minutes heating, the non-heme iron content increased to 1.78 μg/g meat, which is a 35.9% increase. This suggests that heating time or rate of heating instead of temperature is to be the major factor responsible for the increase in the non-heme iron content. This is true since the temperature of the sample heated for 5 minutes was only 63°C. The above observation may also explain why the method of Schricker et al. (1982) gave a higher value for non-heme iron than other two methods, since the sample was incubated in a 65°C

Table 7. Average concentrations of non-heme iron of fresh and nitrite-treated meat pigment extracts at varying heating times.[a] Also shows standard deviations (±).[e]

Heating time, min.	Non-Heme iron µg/g meat			
	Fresh Pigment		Nitrite-Treated Pigment	
	non-heme iron µg/g meat	Increase in %	non-heme iron µg/g meat	Increase in %
0	1.31±0.05	-	1.29±0.07	-
5[b]	1.78±0.22	35.9	-	-
10[c]	2.25±0.16	71.8	1.14±0.09	-11.6
20[d]	2.34±0.10	78.6	1.14±0.04	-11.6

[a] 90°C water bath.

[b] Final temperature 62°C. Non-heme iron was not determined in nitrite-treated sample during 5 minutes heating.

[c] Final temperature 73°C. Took 9 min. to reach 70°C.

[d] Final temperature 88°C. Took 8 min. 50 sec. to reach 70°C.

[e] Each value was replicated five times.

water bath for 20 hours.

In the present study, a gradual increase in non-heme iron in the extract heated for 10 and 20 minutes occurred with values of 2.25 and 2.34 μg/g meat, respectively. These values represented an increase of 71.8 and 78.6%, respectively, over the original non-heme iron content.

The increase of non-heme iron between 5 and 10 minutes was much greater than that between 10 and 20 minutes heating. Since the final sample temperatures were 62, 73 and 88°C for 5, 10 and 20 minutes heating, respectively, results suggest that the optimum temperature is between 62 and 73°C. At this temperature, the release of heme iron apparently occurred from the myoglobin moiety.

On the other hand, the non-heme iron content of the nitrite-treated pigment extract heated for 10 and 20 minutes was the same for both heating time, being only 1.14 μg/g meat. This amounted to a decrease of 11.6%, apparently resulting from stabilization of the heme iron by addition of nitrite.

Early in the study, it was not possible to tell whether the increase of non-heme iron in the heated pigment extract was due to the cleavage of iron from the porphyrin ring or due to the cleavage of heme (porphyrin ring with iron) from the globin portion of myoglobin. If the heme was cleaved from the globin moiety, the heme iron would be measured by atomic absorption spectrophotometry, irregardless of whether

the heme iron was combined with nitrite or not. Results showed that there was no increase of non-heme iron in the heated nitrite-treated samples. This suggests that the increase of non-heme iron in the heated pigment extracts come from the release of iron from porphyrin ring. On the other hand, nitrite appears to stabilize the muscle pigments by preventing release of heme iron from the cooked sample.

Experiment B

The second part of this study was designed to investigate the effects of salt and some antioxidant-coated salt preparations on the TBA numbers of cooked meat. Antioxidants that were not coated on salt were also added directly in combination with the same level of added salt.

When inspecting the TBA numbers for each treatment group, it is important to consider the TBA number of the raw meat with antioxidants added because the TBA tests were run at different times due to the limitations in equipment. The TBA numbers of the raw meat sample reflect not only the oxidation that occurred in the sample before it was cooked, but also any possible interaction between the additives and the meat system during analysis.

Effect of Heating on TBA Number of Muscle Tissue

The TBA numbers of each treatment group are given in Table 8 for the raw samples, for the samples immediately

Table 8. TBA numbers of raw meat and heated meat with and without antioxidants and salt.[d]

Treatment group	Raw Meat[a]	Cooked Meat	
		0 day	2 days[b]
Control	0.53	1.54	4.43
2% NaCl	0.54	2.25	8.25
2% NaCl with 0.02% α-tocopherol coated on salt	0.54	1.40	6.23
2% NaCl with 0.01% BHA, 0.014% citric acid 0.01% propylene glycol coated on salt	0.56	0.52	0.42
2% NaCl plus 0.02% BHA 0.02% BHT	0.56	0.52	0.41

[a]Raw meat was tested right after addition of additives.

[b]Cooked meat was stored at 4°C for 2 days.

[c]The fat content of meat sample was 2.26%.

[d]Each value was replicated five times.

after cooking and again following 48 hours storage at 4°C. The effect of cooking on lipid oxidation can be seen by comparing the TBA numbers of the raw meat and cooked meat at zero time storage. Since the meat samples were cooked right after the salt was added to the meat, the time interval was limited to 15 minutes and was considered not to influence the TBA numbers. Cooking induced some lipid oxidation in meat samples analyzed without any storage. This is true for all samples which were not protected by an effective antioxidant. This is illustrated by the increase in TBA numbers in the control samples (no added salt or antioxidants), which increased from 0.53 before cooking to 1.54 immediately after cooking.

Effects of Salt and Antioxidants on the TBA Number of Cooked Meat

The presence of 2% salt enhanced the oxidative effect of cooking on the meat samples. This is shown by the TBA numbers, which increased from 0.54 in the raw meat to 2.25 for the sample with added salt and analyzed immediately after cooking. This value is considerably higher than the TBA number of control group (no added salt), which was 1.54 immediately after cooking. The prooxidant activity of added salt on the cooked meat continued to increase even more when storage was continued up to 48 hours. In this case, the TBA number increased to 8.25.

The salt used in this study was labelled as containing 2 ppm or less of iron, which could contribute up to 0.02 ppm iron to each gram of meat sample. This level is believed to be negligible and would have little or no effect on lipid oxidation in comparison to the non-heme iron content, which amounted to 1.31 μg/g meat.

α-Tocoperhol (at the 0.01% level based on the lipid content of the sample) did not show any antioxidant activity when coated on the salt that was added at a level of 2%. The advantage of coating salt with α-tocopherol and other antioxidants is that it supposedly brings the antioxidant into closer contact with the lipids. In this study, however, the salt coated with α-tocopherol had more prooxidant activity on the cooked meat than on the control samples held for 48 hours storage. Previous work by Chippault (1961) and Labuza (1971) has demonstrated that α-tocopherol has an antioxidant effect at low concentrations but has a prooxidant effect at high concentrations, which may account for the higher TBA numbers of the meat samples treated with α-tocopherol coated salt in this study.

The α-tocopherol initially seemed to have some antioxidant effect on lipid oxidation, with the TBA number at zero time storage in the cooked meat being 1.40. This was close to the value of 1.54 for the control group, but considerably lower than the value of the 2.25 for the 2% salt treatment. However, as the storage period was extended,

α-tocopherol failed to protect the cooked meat from further oxidation. The TBA number of the α-tocopherol treatment was 6.23, which is markedly higher than the value of 4.43 for the control group. However, it was lower than the value of 8.26 for 2% salt treatment after storage for 48 hours.

Although α-tocopherol may be present in animal tissue, the amount is usually very small and is much lower than the amount added in this study. Igene et al. (1976) reported the amount of α-tocopherol in the Longissimus dorsi muscle of veal calves to be from 3 to 5 μg/g tissue. Since α-tocopherol is heat resistant, even at high temperatures, cooking at 70°C would not be expected to destroy the α-tocopherol in the sample.

Both Tenox 4 with 2% salt and BHA-citric acid-propylene glycol-coated salt exhibited effective antioxidant activity on cooked refrigerated meat during storage at 4°C for 48 hours. Both of these treatments were equally effective in inhibiting oxidation, with TBA numbers of 0.41 and 0.42 after 48 hours storage at 4°C.

According to Dugan (1976), addition of certain acidic compounds to fat effectively complexes trace metal prooxidants and frequently provides good keeping qualities. Citric acid is usually used since it is particularly effective in sequestering iron and it generally poses no safety problems in foods. Since non-heme iron has been shown to be the major lipid prooxidant in uncured, cooked

meat, citric acid may function as an effective antioxidant and probably contributes greatly to the antioxidant effect of the BHA-citric acid-propylene glycol-coated salt.

Dugan (1976) stated that both BHA and BHT are phenolic antioxidants, that is they inhibit lipid oxidation by donating an electron or a hydrogen directly to the unsaturated fatty acids and subsequently interrupt free radical chain formation. They are known as "carry through antioxidants" since they survive the thermal stress and pH effects of processing to give longer shelf life to lard and to other foods to which lard is added (Dugan, 1976). BHA has a synergestic effect with BHT and with propyl gallate (PG), as well as with several other antioxidants (Dugan, 1976).

The synergestic effect of BHT and BHA probably contributes to the satisfactory antioxidant activity of Tenox 4, which inhibited oxidation of cooked meat during 48 hours storage at $4^{o}C$, giving a TBA number of only 0.41 as compared to the value of 0.56 for the raw meat. Similar results were obtained by using BHA-citric acid-propylene glycol-coated salt, which gave TBA numbers of 0.56, 0.52 and 0.41 to raw meat and to cooked meat stored at $4^{o}C$ for either 0 and 2 days, respectively. Propylene glycol, in this study, was used as a carrier of BHA and citric acid to be coated on salt.

SUMMARY AND CONCLUSIONS

Three different procedures for determining non-heme iron in a meat pigment extract were compared. Method 1 utilized direct acid digestion with heating in a 65^{o}C water bath for 20 hours to give a final pH of 1.21. Method 2 chelated the non-heme iron with EDTA followed by precipitation of the heme proteins with TCA, which gave a final pH of 1.57. Method 3 also used chelation of non-heme iron with EDTA but precipitation was by acetone, which resulted in a final pH of 5.43. The percentage of non-heme iron for Methods 1, 2 and 3 amounted to 7.8 , 6.3 and 6.0%, respectively. Thus, Methods 2 and 3 gave fairly comparable results, but Method 1 was higher. Results indicated that pH had little effect upon the non-heme iron contents. However, heating (Method 1) released more heme iron from the globin moiety, giving a high value for non-heme iron.

It was shown that both the final temperature and the rate of heating influenced the release of heme iron from the pigment extract. There was a 35.9% increase in non-heme iron concentration when the temperature of the pigment extract reached 62-70^{o}C, which was shown to be the optimum temperature for release of heme iron. Further heating resulted in release of lesser amounts of heme iron. Rapid

heating (reached 70°C in 1 minute) resulted in release of very little heme iron, whereas slow heating (reached 70°C in 9 minutes) resulted in a large increase in the amount of nonheme iron with increases of 5.3 and 71.8%, respectively. It is postulated that the rapid increase in the temperature of the extract induces coagulation of the myoglobin molecule so that the heme iron can not be readily cleaved from the globin moiety, thus giving a low value.

The mechanism by which nitrite inhibits WOF development was also investigated. Heating of the fresh (no added nitrite) pigment extract resulted in a 71.8% increase in non-heme iron during 10 minutes of heating. On adding nitrite, there was a decline of 11.5% in the amount of non-heme iron during 10 minutes of heating. Results suggest that nitrite stabilizes the porphyrin ring, thus preventing the release of heme iron.

The effects of salt alone (2%) or in combination with α-tocopherol (coated on the salt) or BHA-citric acid-propylene glycol (coated on the salt) and Tenox 4 (added directly to the salt-not coated) on the TBA numbers of raw and cooked meat after 0 and 48 hours storage at 4°C were determined. Salt was shown to accelerate lipid oxidation both during cooking and subsequent storage. The α-tocopherol-coated salt also accelerated lipid oxidation, but only during subsequent storage for 48 hours at 4°C. On the other hand, both BHA-citric acid-propylene glycol coated

salt and Tenox 4 completely inhibited lipid oxidation, both immediately after cooking and during subsequent storage. Results suggest that the latter two treatments are effective in preventing WOF, while α-tocopherol-coated salt was not effective during subsequent storage.

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