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THE EFFECT OF SALT ON NON-HEME IRON RELEASE AND LIPID OXIDATION IN PORK

by

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ABSTRACT

THE EFFECT OF SALT ON THE NON-HEME IRON RELEASE AND LIPID OXIDATION IN PORK

By

HSING-FENG LIU

Sodium chloride is a well known prooxidant in the meat systems. However, the prooxidant mechanisms of NaCl are unclear. This study specifically focused on the effect of NaCl on the release of non-heme iron in ground pork and water-washed muscle fibers and its relationship to lipid oxidation.

Three non-heme iron quantitation methods were evaluated to better understand why different methods realize different non-heme iron values when quantitating the same sample. Ferrous chloride (0, 3, 6 and 9 ug Fe/g meat) was added to ground pork. The pork was formed into patties. The modified Schricker, Igene, and ferrozine procedures were used to quantitate the non-heme, heme and total iron concentrations. The modified Schricker method gave greater overall recoveries of added iron than the ferrozine and Igene methods. The non-heme and heme iron distributions for each pork sample was different when evaluated using the different analytical methods. Greater non-heme iron and smaller heme iron concentrations were found when using the modified Schricker method.

The effect of NaCl concentration (0, 0.15, 0.30 and 0.45 M) and different salts (NaCl, KCl, NaBr and KBr) on non-heme iron concentrations and lipid oxidation was studied in ground pork. As NaCl concentration increased from 0 to 0.45 M,

Thiobarbituric acid-reactive substances (TBARS) and peroxide values increased ($p<0.05$) in both raw and cooked samples. Non-heme iron concentrations increased in raw samples after 6 days at 4 °C and for cooked samples immediately after cooking (day 0). Treatments containing different salts had higher ($p<0.05$) TBARS and peroxide values than the control, and significantly ($p<0.05$) increased non-heme iron concentration in raw samples after 6 days. At the same molarity, lipid oxidation and non-heme iron concentrations produced by different salts were not significantly different.

The effects of NaCl, KCl, NaBr and KBr on non-heme iron release from hemoglobin, myoglobin and recovery of added iron were studied in raw and cooked water-washed muscle fibers. The addition of various salts to the model system increased TBARS values. However, it had no effect on the release of non-heme iron from hemoglobin and myoglobin. The addition of various salts to cooked washed muscle fibers containing 3 ug Fe/g meat added iron produced greater TBARS values compared to the model system without salt. Salt addition did not significantly increase the non-heme iron concentration in the model system. Sodium chloride promoted lipid oxidation without increasing the non-heme iron fraction in this model system. It is proposed that a major function of NaCl in lipid oxidation is to reduce the integrity of the muscle structure. Therefore, lipids are more accessible for the attack of prooxidants such as non-heme iron.
KEY WORDS: SALT, LIPID OXIDATION, NON-HEME IRON.

DEDICATION

to my parents who always support me
to my young brother who takes the responsibility to take care of my parents so I can pursue what I want
to my wife who always with me
to my little boy and girl

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INTRODUCTION

Sodium chloride (NaCl) is an important additive and is widely used by the food industry. It is added to food for the purpose of flavor, preservation, or to attain proper functional properties such as texture and water holding capacity. It is also an important ingredient in surimi-based products such as kamaboko, chikuwa, and satsumage (Japanese fish gel products). One of the adverse effects of salt in food products is its ability to promote lipid oxidation .

Salt has been reported to act as a prooxidant of lipid oxidation in meat (Ellis et al., 1968; King and Earl, 1988; Kanner et al., 1991b; Arnold et al., 1991; Osinchak et al., 1992; Ahn et al. 1993a, b). Salt increased lipid oxidation in dark meat turkey patties (King and Earl, 1988). Minced turkey tissue with salt was more oxidized than minced turkey tissue alone (Kanner et al., 1991b). Many possible mechanisms have been proposed for the prooxidant effect of salt. One mechanism has linked the prooxidant effect of NaCl to iron ions in meat (Kanner et al.,1991b; Osinchak et al.,1992). Kanner et al. (1991b) proposed that NaCl increased the availability of iron ions during lipid oxidation. They suggested that when free iron ions were added to minced turkey dark muscle, a large part of the added iron interacted with protein macromolecules. This

interaction prevented iron ions from contacting with membranous lipids and acting as a catalyst of lipid oxidation. Addition of NaCl interrupted the interaction between the iron and the protein macromolecules, therefore, more free iron ions were available to interact with the lipid fraction and enhance lipid oxidation.

Osinchak et al. (1992) used a different model system (phosphatidylcholine liposomes) but a similar approach to study the prooxidant effect of NaCl. They confirmed that the prooxidant effect of NaCl involved iron ions. They also observed that when using NaCl and a high molecular weight fraction (>10 kilodalton) of mackerel press juice as a prooxidant in the model system, the amount of lipid oxidation increased 7-8 times compared to using the high molecular weight fraction (>10 kilodalton) of mackerel press juice alone as a prooxidant. They speculated that part of the iron was released from the high molecular iron-containing protein in mackerel muscle press juice when NaCl was present. Therefore, free iron dramatically increased lipid oxidation in this model system.

Iron in high molecular weight iron-containing proteins such as hemoglobin, myoglobin and ferritin accounted for 51.6 to 74.5% of total iron depending on the type of muscle (Hazell, 1982). It was demonstrated that iron-containing proteins could release iron during cooking (Love and Pearson 1974; Oellingrath, 1988) or during storage (Decker and Hultin, 1990). Cooking denatures the heme protein, degrades the heme and subsequently releases iron (Oellingrath, 1988). Salt may have a similar effect as cooking on the denaturation of iron-containing proteins. Salt can alter the stability of meat protein, causing a reduction in denaturation temperature (Kijowski and Mast, 1988). Ahn and Maurer (1989) reported that NaCl will decrease the heat stability of hemoglobin and myoglobin. Because of their high iron concentration and possible destabilization by NaCl,

it is possible that part of the free iron released is from iron-containing proteins in the presence of NaCl.

Although Kanner et al. (1991b) and Osinchak et al.(1992) suggested that the prooxidant effect of NaCl is to increase the availability of iron ions by either breaking the iron-protein macromolecule or releasing the iron from the high molecular weight fraction (>10 kilodalton) of mackerel muscle press juice. There are few data which illustrate the relationship between NaCl and the release of iron ions in meat. There are also few data available to identify the free iron source in the presence of NaCl. The purposes of these studies are to determine the relationship between NaCl and iron ion release in meat and to define the possible source of free iron in meat using a model system. **These studies are based on the hypothesis that the prooxidant effect of NaCl in meat is due to its ability to enhance the availability of iron ions to promote lipid oxidation. This is done by: either facilitating the release of iron-ions from iron-containing proteins (hemoglobin and myoglobin); and/or by breaking the protein-iron complex in the meat system.**

Four objectives were established to verify this hypothesis:

1. To compare existing iron analysis methodologies and establish an iron analysis method that would provide reproducible data in studies designed to evaluate the role of salt in the oxidation process;
2. To establish the effect of NaCl on the release of non-heme iron and lipid oxidation in ground pork;
3. To study the effect of NaCl on the release of iron from iron-containing proteins in a model system; and

4. To determine the effect of NaCl on the binding of free iron to protein macromolecules in a model system.

REVIEW OF LITERATURE

Introduction

Lipid oxidation is a major deteriorative reaction in meat and meat products during storage. It has an adverse effect on flavor, color, texture and nutritive value of meat. Iron is believed to be the most important catalyst for lipid oxidation in biological systems (Kanner et al., 1987). Different forms or complexes of iron can directly initiate lipid oxidation or facilitate the generation of very reactive species such as the hydroxyl radical which is capable of initiating lipid oxidation.

Sodium chloride has been reported to act as a prooxidant in lipid oxidation of meat. Raw and cooked dark meat turkey patties with 1-2% NaCl oxidized more rapidly than dark meat turkey patties that did not contain salt(King and Earl, 1988). In earlier studies, NaCl was reported to act either as an antioxidant (Chang and Watts, 1950) or as a prooxidant (Ellis et al., 1968). The prooxidant effect of NaCl in the lipid oxidation is further complicated by the fact that salt may contain metal contaminants, which could serve as catalysts of lipid oxidation. The exact mechanism for the prooxidant effect of NaCl is still unclear. This review provides an overview of the lipid oxidation mechanism, how iron catalyzes lipid oxidation in biological systems, the prooxidant effect of various

salts in meat, possible mechanisms involved in the prooxidant of salt and the problems related to non-heme iron measurement methods of analysis.

Lipid Oxidation

Lipid oxidation is a major deteriorative reaction in meat and meat products during storage. It leads to the development of off-flavors, nutrient and shelf life reduction (Ho and Chen, 1994). Lipid oxidation products are implicated in the disruption of biological membranes, the inactivation of enzymes, and damage to proteins. (Frankel, 1984; Kubow, 1993; Marshall and Elswyk, 1995; McCord, 1994).

1. Mechanism of lipid oxidation

Initiation: $LH \rightarrow L\bullet$

propagation: $L\bullet + O_2 \rightarrow LOO\bullet$

$LOO\bullet + LH \rightarrow LOOH + L\bullet$

$LOOH \rightarrow LO\bullet + \bullet OH$

$LO\bullet + LH \rightarrow LOH + L\bullet$

termination $L'OO\bullet + L''OO\bullet \rightarrow L'CO + L''CH_2OH + O_2$

$L'OO\bullet + L'' \rightarrow L'OOL''$ (stable products)

$L'\bullet + L''\bullet \rightarrow L'L''$

Lipid oxidation is a free radical chain reaction (Bolland and Gee, 1946). Generally, it can be divided into three distinct phases: initiation, propagation and termination. Lipid oxidation is initiated by abstracting a hydrogen atom from lipid (LH) and producing a free radical (L•). The resulting carbon-centered radical (L•) can react

with oxygen to form a peroxyl radical (LOO•). The peroxyl radical can abstract hydrogen from adjacent fatty acid side chains and form a hydroperoxide and another free radical which will further propagate the chain reaction of lipid oxidation. Hydroperoxides are unstable and will undergo homolytic and heterolytic cleavage to form various types of free radicals (LO• and •OH). These free radicals will further propagate the chain reaction. During the termination step, free radicals are eliminated by formation of nonradical products with other free radicals.

2. Role of iron in lipid oxidation

The initiation reaction in lipid oxidation is not only endothermic (Evans and Uri, 1949), but also a rate-determining step. The direct reaction of lipids with oxygen is spin-forbidden because the ground state of lipids is of singlet multiplicity whereas that of oxygen is of triplet multiplicity. Therefore, lipid oxidation is unlikely to be a major reaction in meat unless catalysts or other factors are involved. However, this spin-forbidden barrier does not apply to reactions with single electrons, hydrogen atoms or other atoms or molecules containing unpaired electrons such as transition metals or free radicals. Iron is the most important and abundant catalyst in biological systems. Biological oxidation is due almost exclusively to metal promoted reactions (Aisen and Liskowsky, 1980; Harrison and Hoare, 1980; Kanner, 1994). Iron can have two or more valences. Therefore, the iron has a range of accessible oxidation states which enables itself to transfer electrons.

Mechanisms of iron-catalyzed lipid oxidation depend on the presence or absence of preformed lipid hydroperoxides. In the presence of hydroperoxides, iron catalyzes the decomposition of preformed hydroperoxides to form LO• radicals (Fenton reaction). The decomposition of these hydroperoxides increases the rate of chain re-initiation or propagation. This reaction is referred to as LOOH-dependent lipid oxidation (Gardner, 1989). In the absence of preformed LOOH, or when the concentration of LOOH is very low, hydrogen peroxide promotes the Fenton reaction (Fee, 1982)and hydroxyl radical formation (superoxide driven-Fenton reaction). Iron can also promote lipid oxidation either by directly initiating lipid oxidation (Uri, 1961) or by metal autooxidation (Smith and Dunkley, 1962)which produces reactive oxygen species such as $O_2^-/HO_2\bullet$ and H_2O_2. These reactions are summarized below:

LOOH-dependent lipid oxidation;

$$Fe^{2+} + LOOH \longrightarrow Fe^{3+} + LO\bullet + OH^-$$
$$LO\bullet + L \longrightarrow LH + R\bullet$$

superoxide-driven Fenton reaction;

$$O_2^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$
$$2\ O_2^- + 2\ H^+ \longrightarrow 2\ H_2O_2 + O_2$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \bullet OH + OH^-$$

direct initiation;

$$Fe^{3+} + LH \longrightarrow Fe^{2+} + L\bullet + H^+$$

metal iron autooxidation;

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^- \xrightarrow{H+} HO_2\bullet$$
$$O_2^- / HO_2\bullet \longrightarrow H_2O_2 + O_2$$

(L, LH = lipid; LOOH = lipid hydroperoxide; LO• = alkoxy radical; R• = lipid free radical O_2^- = superoxide radical; •OH = hydroxyl radical; Fe^{2+} = ferrous iron; Fe^{3+} = ferric iron).

One particular reason why iron is so important in lipid oxidation is that it acts as a catalyst in hydroxyl radical production (the superoxide driven-Fenton reaction). The hydroxyl radical is believed to be necessary for the initiation of lipid oxidation (Fong et al., 1973; Girotti and Thomas, 1984). The hydroxyl radical is the most potent oxidant that can be formed from oxygen (E° =1.6 V). It is capable of oxidizing lipids and any other biological molecules. However, some researchers (Samokyszyn et al., 1989; Minotti and Aust, 1989; Gutteridge and Halliwell, 1990) doubt that the hydroxyl radical is a primary initiator of lipid oxidation because it is a short-lived radical with diffusion-limited activity. As such, the hydroxyl radical will not diffuse into the hydrophobic interior of a phospholipid membrane to initiate lipid oxidation. Evidence such as the lack of correlation between hydroxyl radical production and the rate of lipid oxidation (Minotti and Aust, 1987b; Braughler et al., 1986) indicates that the hydroxyl radical is not a primary initiator of lipid oxidation. The addition of hydroxyl radical scavengers such as catalase (to remove hydrogen peroxide and block hydroxyl radical formation) also rarely inhibits lipid oxidation (Minotti and Aust, 1987a). However, supporters of the hydroxyl radical theory argue that the generation of the hydroxyl radical could be site specific, such that the damaging radical species would be generated in the proximity of the polyunsaturated fatty acids (Schaich and Borg, 1988; Fukuzawa and Fujii, 1992). Schaich and Borg (1988) have found that iron and hydrogen peroxide can partition from water to lipid. Therefore, the Fenton reaction may take place in the lipid.

Other forms or complexes of iron have been suggested as catalysts of lipid oxidation. These include iron-oxygen complexes, hypervalent iron (ferryl, perferryl iron, porphyrin cation radical) and iron-containing proteins such as heme protein or

lipoxygenase (Kanner et al., 1987; Minotti and Aust, 1992; Kanner, 1994). The form of iron is involved in lipid oxidation has not been fully determined. However, it is generally agreed that iron plays a vital role in the initiation of lipid oxidation. Some of the more commonly proposed mechanisms will be discussed.

Aust and colleagues (Samokyszyn et al., 1989; Minotti and Aust, 1989) proposed that the iron-oxygen complexes (Fe^{2+}-O_2-Fe^{3+}) are responsible for the initiation of lipid oxidation. They studied the effect of different ratios of Fe^{2+} and Fe^{3+} on lipid oxidation in liposomes (Minotti and Aust, 1987c). The rate of lipid oxidation was greatest when approximately 50% of Fe^{2+} had been oxidized to Fe^{3+}. Lipid oxidation did not occur when all the iron remained in the reduced form or when all the Fe^{2+} had oxidized to Fe^{3+}. They observed that maximum lipid oxidation occurs when Fe^{2+} and Fe^{3+} are present in a 1:1 ratio and postulated that the function of the superoxide radical and hydrogen peroxide is not to form hydroxyl radicals but rather to promote Fe^{3+} reduction or Fe^{2+} oxidation from which the appropriate Fe^{2+}/ Fe^{3+} initiating species originated.

Another active form of iron is hypervalent iron (iron valence of +4 to +6). These hypervalent iron complexes include ferryl $(FeO)^{2+}$, perferryl $(FeO)^{3+}$ and porphyrin cation radicals (heme-associated ferryl species). The interaction of hydrogen peroxide with metmyoglobin or methemoglobin leads to the generation of active forms of heme proteins (porphyrin cation radical)(Rhee, 1988). It was reported that porphyrin cation radical can initiate membrane lipid oxidation (Kanner et al., 1991a; Kanner and Harel, 1985). The proposed mechanism is as follows:

$$P^{+}\text{-}Fe^{+4}{=}O + LH \rightarrow P\text{-}Fe^{+4}{=}O + L\bullet + H^{+}$$

$$L\bullet + O_2 \rightarrow LOO\bullet$$

$LOO\bullet + LH \rightarrow LOOH + L\bullet$

$P\text{-}Fe^{+4}{=}O + LOOH \rightarrow P\text{-}Fe^{+3}\text{-}LOOH \rightarrow P^{+}\text{-}Fe^{+4}{=}O + LOH$

Although ferryl or perferryl iron complexes also contain hypervalent iron, they may not be as significant as the porphyrin cation radicals in lipid oxidation. This is because of the fact that hypervalent iron complexes require macromolecular complexes or a very high pH for formation and stabilization. Thus, hypervalent iron should not be a significant reactant since, in complex biological systems, decay of hypervalent states to Fe^{3+} would be instantaneous, eliminating any enhanced catalytic capability.

Iron-containing proteins such as heme protein or lipoxygenase also play an important role in lipid oxidation. Watts and Peng (1947) examined the prooxidant effect of hog muscle extract and concluded that myoglobin and hemoglobin were the catalysts responsible for fat oxidation. Tappel (1952) reported heme compounds such as hemoglobin, myoglobin and cytochromes were the dominant catalysts of lipid oxidation in meat systems. He proposed that heme iron catalyzed the decomposition of lipid hydroperoxides into free radicals which propagated the free radical chain reaction.

$LOOH + Heme\text{-}Fe^{2+} \rightarrow Heme\text{-}Fe^{2+} + LO\bullet + \bullet OH$

It has also been suggested that heme catalysis may involve an indirect mechanism in which the hydroxyl radical is produced and subsequently initiates lipid oxidation reactions. The autooxidation of ferrous heme iron produced the superoxide radical which was reduced to hydrogen peroxide in the meat system (Wallace et al., 1982; Sadrzadeh et al., 1984). The hydrogen peroxide was then decomposed by iron to hydroxyl radicals and subsequently promoted lipid oxidation. It is also possible that in systems which contain

hydrogen peroxide, the hydroxyl radical attacks hemoglobin and releases iron which then catalyzes lipid oxidation as non-heme iron (Puppo and Halliwell, 1988).

Lipoxygenase is widely distributed in many plant, animal and fish tissues. It catalyzes the insertion of oxygen into polyunsaturated fatty acids to generate position specific hydroperoxides. The hydroperoxides can be further broken-down into several secondary oxidation products which may promote lipid oxidation. It has been suggested that lipoxygenase can initiate lipid oxidation in fish microsomal lipids (German and Kinsella, 1985). Grossman et al. (1988) suggested that lipoxygenase may contribute to lipid oxidation in chicken during frozen storage.

The Prooxidant Effect of Salt on Lipid Oxidation in Meat

Sodium chloride is a well recognized prooxidant in meat. In early studies, NaCl was reported to act either as an antioxidant (Chang and Watts, 1950) or as a prooxidant (Ellis et al., 1968). One possible cause of the dual role is the presence of impurities in the salt. Impurities such as trace metals (e.g., iron and copper) or sodium nitrate may act as prooxidants and antioxidants, respectively (Salih, 1986). Salih (1986) compared the prooxidant effect of rock salt and calcium-magnesium free salt in turkey. He reported that turkey treated with rock salt was more oxidized than turkey treated with calcium-magnesium salt. He suggested that the impurities and the salt itself both could be active prooxidants in ground turkey. However, Olson and Rust (1973) compared the effect of low prooxidant salt (salt containing a low amounts of heavy metals: copper 0.1 ug/g salt and iron 0.4 ug/g salt) and regular flake salt (copper 1.5 ug/g salt and iron 1.5 ug/g salt) on oxidative rancidity development in dry cured hams. They reported no differences in

oxidative rancidity between low prooxidant salt and regular fake salt in dry cured ham. Subsequent studies by King and Earl (1988), Kanner et al. (1991b) and Ahn et al. (1993a) using reagent grade NaCl demonstrated that salt itself was the prooxidant effect in ground turkey. The prooxidant effect was also concentration-dependent. For example, minced turkey or ground beef treated with increasing NaCl concentrations have higher lipid oxidation than the meat treated with low NaCl concentrations. (Kanner et al., 1991b; Torres et al., 1988).

It has also been suggested that NaCl itself is not a prooxidant but that it promotes the activity of unsaturated fat oxidases (lipoxygenase) present in meat and fish. Lea (1937) observed that pork fat when treated with muscle juice and NaCl had higher peroxide values than pork fat treated with muscle juice alone. He suggested that pork muscle juice contains an enzyme (lipoxygenase) which accelerates the oxidation of pork fat, an effect which can be enhanced by adding NaCl.

Salts other than NaCl, such as KCl and $CaCl_2$, can also promote lipid oxidation (Rhee et al., 1983; King and Bosch, 1990; Ahn et al., 1993a). The prooxidant effect varied among different salts (Rhee et al., 1983; King and Bosch, 1990). The consensus in the literature has indicated that significant differences exist in the prooxidant effect of different salts. Potassium chloride had almost no effect on rancidity development in raw ground pork during frozen storage (Chang and Watts, 1949, 1950; Watts and Peng, 1947). Rhee et al. (1983) reported that KCl promoted lipid oxidation in raw ground pork during refrigerated and frozen storage but had no prooxidant effect on cooked ground pork. King and Bosch (1990) reported that KCl promoted lipid oxidation in dark turkey meat. Under different storage temperatures (refrigerated and frozen storage), the order of

the prooxidant effect was different for $MgCl_2$ and NaCl. For example, the prooxidant effect of $MgCl_2$ was higher than NaCl in cooked ground pork stored for 4 days at 4 °C. However, the prooxidant effect of $MgCl_2$ was lower than NaCl during 2 months frozen storage at -20 °C (Rhee et al., 1983).

There are two factors which may attribute to the lack of consensus on the prooxidant effects of various salts on lipid oxidation. First, in most studies, the prooxidant effects were compared at similar concentrations (w/w). However, in some studies, they were compared at similar molarities. Because of the differences in molecular weights of the salts, the same weight percentage will have different molarities. For example, for 0.15 M NaCl, KCl, NaBr and KBr, the respective weight percentages of the salts are 0.87%, 1.12%, 1.54 % and 1.79%, respectively. The second reason is that the data in the literature have been very difficult to compare. Experiments have been performed under different conditions, and it is difficult to extrapolate data from one set of studies to other conditions as we discussed above. Different salts, salt concentrations, meat species and processing/storage conditions were used, thus making comparisons difficult.

Possible Mechanisms for the Prooxidant Effect of NaCl

It has been suggested that the prooxidant effect of NaCl may be related to: 1. the release of iron from meat (Kanner et al., 1991b; Osinchak et al., 1992); 2. heme pigment oxidation (Wallace et al.,1982); 3. water activity (Chang and Watts, 1950); 4. the physical state of the meat (Shomer et al., 1987) and 5. enzyme activity (Lee et al., 1996). It is not known whether the prooxidant effect of NaCl involves more than one mechanism or combination of mechanisms which are dependent on each other.

1. Sodium chloride and free iron ion

Although NaCl is a well known prooxidant in meat, it does not promote lipid oxidation in model systems such as emulsions of methyl linoleate and linoleic acid (Mabrouk and Dugan, 1968) or water-washed muscle fibers (Kanner et al., 1991b). Mabrouk and Dugan (1968) reported that lipid oxidation in aqueous emulsions of methyl linoleate and linoleic acid did not occur regardless of the concentration of the dissolved NaCl. Kanner et al. (1991b) reported similar results in a model system containing water-washed muscle fibers. Osinchak et al. (1992) reported that lipid oxidation of phosphatidylcholine liposomes were suppressed by increasing the concentration of dissolved NaCl in the liposome system. Sodium chloride also inhibits reactions which lead to lipid oxidation. Harel (1994) indicated that NaCl inhibited ascorbic acid oxidation and the reactions that produce superoxide radicals, hydrogen peroxide and the hydroxyl radical, species that are reported to be initiators of lipid oxidation. It is not clear why NaCl influences lipid oxidation differently in meat and model systems.

Ellis et al. (1968) suggested that NaCl may activate a component in lean meat which results in a change of the oxidative characteristics of adipose tissue. It is also possible that other compounds which are present in meat systems but not in model systems may react with NaCl and promote lipid oxidation. Recently, Kanner et al. (1991b) and Osinchak et al. (1992) indicated that the prooxidant effect of NaCl in meat may be related to its ability to release free iron. Kanner et al. (1991b) proposed that the prooxidant effect of NaCl is derived from its ability to displace iron ions from binding to protein macromolecules. They proposed that free iron ions are major catalysts in meat. Most of the free iron ions (chelatable iron) are in the cytosol fraction and are probably chelated by

proteins. When NaCl is added to the meat, the protein-iron interaction is disrupted. More iron ions will be released to promote lipid oxidation. Kanner et al. (1991b) found that the addition of iron salts to minced turkey only slightly increased the rate of lipid oxidation. However, in the presence of NaCl, lipid oxidation increased approximately 3-5 times compared to the control (turkey + iron ions). Kanner et al. (1991b) suggested that a large portion of the added iron ions interacted with proteins in the meat. This interaction prevented iron ions from affecting membranous lipids and acting as catalysts of lipid oxidation. Sodium chloride can disrupt the interaction between the iron ions and proteins. Therefore, more free iron is available to interact with the lipid fraction and to enhance lipid oxidation. This hypothesis is further supported by the observation that a NaCl solution will extract more free iron ions than water during the preparation of water-washed muscle fibers (Kanner et al., 1991b). Sodium chloride may break the protein-iron interaction. Thus, more iron ions will be released and fewer free iron ions will remain in the water-washed muscle fibers.

Kanner et al. (1991b) reported that ethylenediaminetetraacetic acid (EDTA) and ceruloplasmin (ferroxidase) can suppress NaCl-promoted oxidation in minced turkey meat. EDTA, a metal chelator, will chelate metal ions and reduce the free metal ion concentrations in meat. Ceruloplamin is an enzyme which works specifically on the oxidation of ferrous iron to ferric iron. Because the functions of EDTA and ceruloplasmin are closely related with iron ions, it was postulated that iron ions are involved in NaCl-promoted lipid oxidation (Kanner et al., 1991b).

Osinchak et al. (1992) used a different model system (phosphatidylcholine liposomes and mackerel press juice) with a similar approach to study the prooxidant effect

of NaCl. They confirmed the results of Kanner et al. (1991b) that the prooxidant effect of NaCl involved iron ions. They further suggested that part of the iron may be released from the high molecular weight fraction (>10 kilokalton) of the mackerel muscle press juice. They reported that using NaCl and the high molecular weight fraction (>10 kilodalton) of mackerel press juice as a prooxidant increased the amount of lipid oxidation by 7-8 times when compared to that generated by the high molecular weight fraction (>10 kilodalton) of mackerel press juice alone. They suggested that this was due to iron ions being released from the high molecular weight fraction (>10 kilodalton) of the mackerel press juice. The effectiveness of NaCl to displace iron may be linked to the high stabilization constant of chlorine (Kanner et al., 1991b). Although Kanner et al. (1991b) and Osinchak et al. (1992) proposed that the mechanism for the prooxidant effect of NaCl is to increase the availability of non-heme iron in meat, no data have been presented to prove NaCl directly increases the non-heme iron concentrations in meat.

2. Anion-promoted autooxidation

In addition to lipid oxidation, a problem encountered on adding NaCl to muscle is heme pigment discoloration (Andersen and Skibsted, 1991; Andersen et al., 1990; Asghar et al., 1990). Lipid and heme pigment oxidation are closely related. The possible chemical coupling mechanism between heme pigment oxidation and lipid oxidation in meat is not completely understood. It has been suggested that pigment oxidation will produce superoxide radicals and initiate lipid oxidation (Andersen et al., 1990; Akamittath et al., 1990). On the other hand, lipid oxidation also causes pigment discoloration. O'Grady et al. (1996) reported that stimulation of lipid oxidation with $FeCl_3$/ascorbate led to an

increase in both lipid and oxymyoglobin oxidation in a microsome-enriched muscle fraction model system. Oxymyoglobin oxidation did not occur when the lipid was removed from the microsome-enriched muscle fraction containing the prooxidant ($FeCl_3$/ascorbate).

Wallace et al. (1982) indicated that certain anions (Cl^-, CN^-, N_3^-) either increase the rate of oxymyoglobin oxidation (anion-promoted autooxidation) by binding to heme iron and changing its redox potential in favor of oxidation of Fe^{2+} to Fe^{3+}, or by preferentially stabilizing the Fe^{3+} oxidation state relative to the Fe^{2+} state of heme iron. This anion-promoted autooxidation process not only leads to the formation of metmyoglobin but also to the formation of superoxide free radicals (Wallace et al., 1982), which is subsequently converted to hydrogen peroxide. These species are highly reactive and are capable of reacting directly either with lipid compounds in muscle, or with metmyoglobin to produce an activated molecule, activated metmyoglobin (MMb-H_2O_2) that can initiate lipid oxidation (Rhee, 1988; Harel and Kanner, 1985).

3. Water activity

The effect of water activity (a_w) on the rate of lipid oxidation is well documented (Berends, 1993; Gopala-krishna and Prabhakar, 1992). As a_w increases from 0 to about 0.3-0.4, lipid oxidation decreases. When a_w increases from 0.3-0.4 to 0.75-0.85, lipid oxidation increases. Lipid oxidation decreases again when a_w is over 0.75 (Cheah and Ledward, 1995; Karel and Yong, 1981). At low a_w, lipid free radicals are soluble in the oil fraction which allows them to diffuse longer distances and spread the reaction (Kanner, 1994). When a_w increases from 0 to 0.3-0.4, water molecules lower the

effectiveness of metal catalysts such as copper and iron, form hydrogen bonds with hydroperoxides and retard hydroperoxide decomposition. Water reduces oxygen diffusion by forming a barrier for oxygen over the lipid surface and lipid oxidation decreases. When a_w increases from 0.4 to 0.75-0.85, the possible liberation of transition metals cause lipid oxidation. When a_w is over 0.85, the reactants are diluted and lipid oxidation decreases.

The effect of NaCl on lipid oxidation also depends on the amount of moisture present in the system (Chang and Watts, 1950). Salt itself is probably not a prooxidant but rather it shifts the a_w of the food to an intermediate level where lipid oxidation occurs at a more rapid rate.

4. Physical state of meat

Addition of NaCl to meat causes extraction of the myofibrillar proteins and results in a less intact muscle microstructure (Ofstad et al., 1995; Velinov et al., 1990). Kanner et al. (1991b) reported that increasing the concentration of NaCl enhanced lipid oxidation in raw minced muscle, especially after a freeze-thaw cycle. They suggested that NaCl and freeze thaw cycles may cause fusion of the intracellular compounds and the destruction of the cell structure which further enhances lipid oxidation (Shomer et al., 1987). "Sodium chloride may affect the physical state of meat in such a way that hemoglobin or other catalysts would be brought into closer contact with the fat" (Chang and Watts, 1950). Ahn et al. (1993b) reported that turkey breast patties which contained hemoglobin and NaCl together were more oxidized than patties which contained only hemoglobin or NaCl alone. Sodium chloride may have caused a physical change in the liposome structure due to osmotic pressure differences between the inside and outside of the bilayer (Arnold et

al., 1991). The hydrophobic interior of a phospholipid membrane may be more accessible to prooxidants such as non-heme iron. Thus, more lipid is exposed to the oxidation process.

5. Enzyme activity

There are many enzymes which exist in meat that will ease the oxidative stress by converting the superoxide radical and hydrogen peroxide into less active initiating species. It has been suggested that changing the activity of these enzymes will affect lipid oxidation. Lee et al. (1996) demonstrated that NaCl could accelerate oxidation by altering the activity of catalase, glutathione peroxidase and superoxide dismutase antioxidant enzymes. Thus, more superoxide radicals or hydrogen peroxide are present in meat which may interact with meat components and form active species such as the porphyrin cation radical. On the other hand, there are some enzymes existing in meat such as lipoxygenase which will increase the oxidative stress by converting polyunsaturated fatty acids into lipid hydroperoxides. Hamberg and Gerwick (1993) reported that the enzyme activity of 12-lipoxygenase isolated from red marine algae by gel filtration was weak (about 3 % the amounts of total 12-lipoxygenase products formed from arachidonic acid) ; however, addition of 0.8-1 M NaCl to a desalted enzymes preparation increased the activity 20-fold (about 68% 12-lipoxygenase products). Addition of NaCl in all fractions containing 12-lipoxygenase resulted in a pronounced stimulation of 12-lipoxygenase activity.

Non-heme Iron Measurement

Accurate determination of heme and non-heme iron concentrations in foods is important not only for the assessment of iron bioavailability (Monsen, 1988; Davis et al., 1992) but also for determining the role of iron in storage stability of foods (Miller et al., 1994 a,b; Han et al., 1995). Igene et al. (1979) reported that the major prooxidant in cooked meat was not myoglobin but free iron which was released from heme pigments during heating. Kanner et al. (1991b) and Osinchak et al. (1992) suggested that the prooxidant effect of NaCl may be related to free iron ions in meat. To further study the role of iron and the prooxidant effects of NaCl, methods accurately determining iron in meats are necessary.

The Schricker (Schricker et al., 1982), modified Schricker (Rhee and Ziprin, 1987), Igene (Igene et al., 1979), and the ferrozine method (Carter, 1971) are commonly used assays to measure non-heme iron concentrations in meat. In general, these methods consist of sample preparation and iron quantitation steps. In the sample preparation step, non-heme iron is usually extracted either by an aqueous solution of a metal chelator such as ethylenediaminetetraacetic acid (Igene et al., 1979), citrate-phosphate (Carter, 1971) and pyrophosphate-trichloroacetic acid (TCA) (Foy et al., 1967) or by solubilization in a strong acid solution (hydrochloric-trichloroacetic acid, Rhee and Ziprin, 1987). The extracts are then separated into soluble (non-heme) and insoluble (heme iron) fractions by the addition of a trichloroacetic acid solution. In the sample quantitation procedure, iron is either directly quantitated by atomic absorption spectroscopy (Igene et al., 1979) or reacted with a metal chelator such as ferrozine (Carter, 1971) or dipyridyl (Awt, 1970; Narasinga Rao and Prabhavathi, 1978) to produce a color chromogen, which is then

quantitated by measuring its absorption. Although these methods have been used to measure the non-heme iron concentration in meat, they were originally used for quantitating total iron concentrations. There are no differences within the quantitation procedures for non-heme and total iron within each method. The only differences are in the preparation steps for extracting non-heme and total iron fractions.

These non-heme iron measurement methods have been evaluated by various researchers (Chen, et al., 1984; Rhee and Ziprin,1987; Ahn et al., 1993c; Carpenter and Clark, 1995). However, some issues need to be further addressed which include the lack of consensus in terminology needed to describe the form of the iron and the variation in iron values from the same sample obtained using non-heme iron measurement methods.

1. Consensus in terminology

Non-heme iron (Igene et al., 1979; Schricker et al., 1982; Chen et al., 1984; Rhee and Ziprin, 1987; Ahn et al., 1993c; Carpenter and Clark, 1995), free iron ions, chelatable iron ions (Kanner et al., 1991b), ionic iron, complex iron, soluble iron (Lee and Clydesdale, 1979), and diffusate iron or low molecular iron (Hazell, 1982; Love and Pearson, 1974) are commonly used to describe the formsof iron in meat system. These terms are often used interchangeably in papers and different terms have been used from paper to paper by the same researchers. Because of the inconsistencies in the terminology used (Han et al., 1995; Rhee and Ziprin, 1987), it is not clear whether "non-heme iron", "free iron" or "chelatable iron ion" describe the same iron form. For example, researchers (Rhee and Ziprin, 1987; Ahn et al., 1993c) classified total iron ion into heme and non-heme iron. Hazell (1982) classified total iron into water-insoluble and water-soluble iron.

The water-soluble iron was further separated into ferritin, hemoglobin, myoglobin, low molecule weight iron (<12 kilodalton) or diffusate iron. Lee and Clydesdale (1979) divided total iron into elemental iron, insoluble iron, complexed and ionic iron.

On the other hand, it is unclear whether the methods used to measure non-heme iron are capable of differentiating between the various forms of iron. Schricker et al. (1982) defined non-heme iron as "all of the iron ions not in the heme form which includes the storage iron ions such as ferritin, transferrin and others ". The Schricker and the modified Schricker methods both are capable of recovering added ferritin (Ahn et al., 1993c). The ferrozine method can recover most of the ferritin present (Ahn et al., 1993c). However, the ability of the Igene (Igene et al.,1979) method to extract iron from ferritin has not yet been examined. Rhee and Ziprin (1987) suggested that the method of Igene (Igene et al.,1979) may underestimate the non-heme iron concentration because of incomplete extraction/recovery of non-heme iron from storage proteins (ferritin, transferrin and others).

2. Differences in iron values from the same sample between non-heme iron measurement methods

Research groups have reported differences in iron values when analyzing the same sample using different methods. For example, Ahn et al. (1993c) reported 3.6, 6.9 and 6.7 ug Fe/g of non-heme iron in turkey leg muscle using the ferrozine (Carter, 1971), Schricker (Schricker et al., 1982) and modified Schricker assays (Rhee and Ziprin, 1987), respectively. Rhee and Ziprin (1987) reported that the modified Schricker's method gave

higher non-heme iron values compared to Igene (Igene et al.,1979) methodology. It is not clear why different methods give different non-heme iron values for the same sample. Chen et al. (1984) and Ahn et al. (1993c) suggested that the Schricker or modified Schricker method overestimates the actual non-heme iron because iron may be released from the heme pigments during the strong acidic extraction conditions and during heating.

The Schricker method was modified by Rhee and Ziprin (1987) because of iron release from the heme pigments during extraction (Rhee and Ziprin, 1987; Ahn et al., 1993c; Chen et al., 1984) and nonenzymatic browning. In the modified Schricker method, sodium nitrite was added to prevent the release of iron from heme pigments. A second blank was also applied to correct for higher iron values because of nonenzymatic browning reactions. However, the modified Schricker method still gave higher iron values than the ferrozine and Igene methods. It is not clear whether other factors exist which inflate non-heme iron values in the modified Schricker method, or whether the method extracts more non-heme iron from the sample when compared to the other non-heme iron methods.

To verify whether the different iron values obtained using the non-heme iron measurement methods are due to the extraction procedures or some factors interfering with the non-heme iron quantitation method, it is necessary to measure the total, non-heme and heme iron in the same sample and compare values obtained using each method. However, this has not been done. Regarding the mechanisms involved for the prooxidant effect of NaCl, it is difficult to provide precise data which relates iron release after subsequent NaCl addition to lipid oxidation until differences in the commonly utilized methods can be elucidated. Based on the information available, it cannot be determined which explanation is valid. In many studies, only non-heme iron is measured, or one of

the values (non-heme or heme iron) is calculated by taking the difference from total iron values (Ahn et al., 1993c, Rhee and Ziprin, 1987). Each fraction should be measured using a precise analytical method.

References

Ahn, D.U., Wolfe, F.H. and Sim, J.S. 1993a. Prevention of lipid oxidation in pre-cooked turkey meat patties with hot packaging and antioxidant combinations. J. Food Sci. 58:283.

Ahn, D.U., Ajuyah, A., Wolfe, F.H. and Sim, J.S. 1993b. Oxygen availability affects prooxidant catalyzed lipid oxidation of cooked turkey patties. J. Food Sci. 58:278.

Ahn, D.U., Wolfe, F.H. and Sim, J.S. 1993c. Three methods for determining nonheme iron in turkey meat. J. Food Sci. 58:288.

Ahn, D.U. and Maurer, A.J. 1989. Effects of sodium chloride, phosphate, and dextrose on the heat stability of purified myoglobin, hemoglobin, and cytochrome c. Poultry Sci. 68:1218.

Aisen, P. and Liskowsky, I. 1980. Iron transport and storage proteins. Ann. Rev. Biochem. 49:357.

Akamittath, J.G., Brekke, C.J. and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513.

Andersen, H.J., Bertelsen, G. and Skibsted, L.H. 1990. Color and color stability of hot processed frozen minced beef. Results from chemical model experiments tested under storage conditions. Meat Sci. 28:87.

Andersen, H.J. and Skibsted, L.H. 1991. Oxidative stability of frozen pork patties. Effect of light and added salt. J. Food Sci. 56:1182.

Arnold, A.R., Bascetta, E. and Gunstone, F.D. 1991. Effect of sodium chloride on prooxidant activity of copper (II) in peroxidation of phospholipid liposomes. J. Food Sci. 56:571.

Asghar, A., Torres, E., Gray, J.I. and Pearson, A.M. 1990. Effect of salt on myoglobin derivatives in the sarcoplasmic extract from pre-and post-rigor beef in the presence or absence of mitochondria and microsomes. Meat Sci. 27:197.

Awt, R.G. 1970. Determination of iron in beer. J. Inst. Brewing. 76:299.

Berends, C.L. 1993. Water activity and lipid oxidation on the rate of lipid oxidation at constant oxygen concentration. M.S. Thesis Michigan State Univ., E. Lansing. MI.

Bolland, J.L. and Gee, G. 1946. Kinetic studies in the chemistry of rubber and related materials: III thermochemistry and mechanisms of olefin oxidation. Trans. Faraday Soc. 42:236.

Borg, D.C. and Schaich, K.M. 1988. Fenton reactions in lipid phases. Lipids 23:570.

Braughler, J.M., Duncan, L.A. and Chase, R.L. 1986. The involvement of iron in lipid peroxidation: importance of ferric to ferrous ratios in initiation, J. Biol. Chem. 261:10282.

Carpenter, C.E. and Clark, E. 1995. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. J. Agric. Food Chem. 43:1824.

Carter, P. 1971. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal. Biochem. 40:450.

Chang, I. and Watts, B.M. 1949. Antioxidants in the hemoglobin catalyzed oxidation of unsaturated fats. Food Technol. 3:332.

Chang, I. and Watts, B.M. 1950. Some effects of salt and moisture on rancidity of fat. Food Res. 15:313.

Cheah, P.B. and Ledward, D.A. 1995. High pressure effects in lipid oxidation. J. Am. Oil Chem. 72:1059.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H. and Ku, P.K. 1984. Some factors influencing the nonheme iron content of meat and its implications in oxidation. J. Food Sci. 49:581.

Davis, C.D., Malecki, E.A. and Greger, J.L. 1992. Interactions among dietary manganese, heme iron, and nonheme iron in women. Am. J. Clin. Nutr. 56:926.

Decker, E.A. and Hultin, H.O. 1990. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. J. Food Sci. 55:947.

Ellis, R., Currie, G.T., Thornton, F.E., Bollinger, N.C. and Gaddis, A.M. 1968. Carbonyls in oxidizing fat. II. The effect of the pro-oxidant activity of sodium chloride on pork tissue. J. Food Sci. 33:555.

Evans, M.G. and Uri, N. 1949. The dissociation constant of hydrogen peroxide and the electron affinity of the HO_2 radical. Trans. Faraday Soc. 45:224.

Fee, J.A 1982. In "Oxidase and Related Systems", King, T.E., Mason, H.S. and Morrison, M (Eds.), Pergamon Press, New York, p101.

Fong, K.L., McCay, P.B., Poyer,J.L., Keele, B.B. and Misra, H. 1973. Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. J. Biol. Chem. 248:7792.

Foy, L.A., Williams, H.L., Cortell, S. and Conrad, M.E. 1967. A modified procedure for the determination of nonheme iron in tissue. Anal. Biochem. 18:559.

Frankel, E.N. 1984. Lipid oxidation: Mechanisms, products and biological significances. J. Am. Oil Chem. Soc. 61:1908.

Fukuzawa, K. and Fujii, T. 1992. Peroxide dependent and independent lipid peroxidation: Site-specific mechanisms of initiation by chelated iron and inhibition by α-tocopherol. Lipids 27:227.

Gardner, H.W. 1989. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radical Biol. Med. 7:65.

German, J. B. and Kinsella, J.E. 1985. Lipid oxidation in fish tissue. Enzymatic initiation via lipoxygenase. J. Agric. Food Chem. 33:680.

Girotti, A.W. and Thomas, J.P. 1984. Damaging effects of oxygen radicals on resealed erythrocyte ghosts. J. Biol. Chem. 259:1744.

Grossman, S., Bergman, M. and Sklan, D. 1988. Lipoxygenase in chicken muscle. J. Agric. Food Chem. 36:1268.

Gopala-krishna, A.G. and Prabhakar, J.V. 1992. Effect of water activity on secondary products formation in autoxidizing methyl linoleate. J. Am. Oil Chem. 69:178.

Gutteridge, J.M.C. and Halliwell, B. 1990. The measurement and mechanism of lipid peroxidation in biological systems. TIBS 15:129.

Hamberg, M. and Gerwick, W.H. 1993. Biosynthesis of vicinal dihydroxy fatty acids in the red alga *Gracilariopsis lemaneiformis*: Identification of a sodium-dependent 12-lipoxygenase and a hydroperoxide isomerase. Arch. Biochem. Biophys. 305:115.

Han, D., McMillin, K.W., Godber, J.S., Bidner, T.D., Younathan, M.T. and Hart, L.T. 1995. Lipid stability of beef model systems with heating and iron fractions. J. Food Sci. 60:599.

Harel, S. 1994. Oxidation of ascorbic acid and metal ions as affected by NaCl. J. Agric. Food Chem. 42:2402.

Harel, S. and Kanner, J. 1985. Muscle membranal lipid peroxidation initiated by H_2O_2-activated metmyoglobin. J. Agric. Food Chem. 33:1188.

Harrison, P.M. and Hoare, R.J. 1980. Metals in Biochemistry, Chapman & Hall. London, England. p 1.

Hazell, T. 1982. Iron and zinc compounds in the muscle of meats of beef, lamb, pork and chicken. J. Sci. Food Agric. 33:1049.

Ho, C.T. and Chen, Q. 1994. Lipids in food flavors. In "Lipids in Food Flavors", C.T. Ho and T.G. Hartman (Eds.), ACS Symposium Series 558, ACS, Washington, D.C., p. 2-14.

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27:838.

Kanner, J. 1994. Oxidative processes in meat and meat products: Quality implications. Meat Sci. 36:169.

Kanner, J. and Harel, S. 1985. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. Arch. Biochem. Biophys. 237:314.

Kanner, J., Harel, S. and Granit, R. 1991a. Nitric oxide as an antioxidant. Arch. Biochem. Biophys. 289:130.

Kanner, J. Harel, S. and Jaffe. R. 1991b. Lipid peroxidation of muscle food as affected by NaCl. J. Agic. Food Chem. 39:101.

Kanner, J., German, J.B. and Kinsella, J.E. 1987. Initiation of lipid peroxidation in biological systems. CRC Critical Reviews in Food Science and Nutrition. 25:317.

Karel, M. and Yong, S. 1981. "Autoxidation-initiated reaction in food, in "Water Activity: Influences on Food Quality." L.B. Rockland and G.F. Stewart (Ed.), Academic Press, New York, NY. p511.

Kijowski, J.M. and Mast, M.G. 1988. Effect of sodium chloride and phosphates on the thermal properties of chicken meat proteins. J. Food Sci. 53:367.

King, A.J. and Bosch, N. 1990. Effect of NaCl and KCl on rancidity of dark turkey meat heated by microwave. J. Food Sci. 55:1549.

King, A.J. and Earl, L.A. 1988. Effect of selected sodium and potassium salts on TBA values of dark meat turkey patties. J. Food Sci. 53:726.

Kubow, S. 1993. Lipid oxidation products in food and atherogenesis. Nutr. Rev. 51:33.

Lea, C.H. 1937. Influence of tissue oxidases on rancidity. Oxidation of the fat of bacon. J. Soc. Chem. Ind. 56:376T.

Lee, S.K., Mei, L. and Decker, E. 1996. Effect of NaCl on antioxidant enzyme activity and lipid oxidation in ground pork. 1996 IFT Abstract. poster 68E-10. Inst. Food Tech. (New Orleans, LA).

Lee, K. and Clydesdale, F.M. 1979. Quantitative determination of the elemental, ferrous, ferric, soluble, and complexed iron in foods. J. Food Sci. 44:549.

Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and non-heme iron as prooxidants in cooked meat. J. Agric. Food Chem. 22:1032.

Mabrouk, A.F. and Dugan Jr, L.R. 1968. A kinetic study of the autoxidation of methyl linoleate and linoleic acid emulsions in the presence of sodium chloride. J. Am. Oil Chem. Soc. 37:486.

Marshall, A. and Elswyk, M.V. 1995. Dietary polyunsaturated fat. Is it beneficial? Nutr. Today. 30:207.

McCord, J.M. 1994. Free radicals and prooxidants in health and nutrition. Food Technol. 48:106.

Miller, D.K., Smith, V.L., Kanner, J., Miller, D.D. and Lawless, H.T.1994a. Lipid oxidation and warmed-over aroma in cooked ground pork from swine fed increasing levels of iron. J. Food Sci. 59:751.

Miller, D.K., Gomez-Basauri, J.V., Smith, V.L., Kanner, J. and Miller, D.D. 1994b. Dietary iron in swine rations affects nonheme iron and TBARS in pork skeletal muscles. J. Food Sci. 59:747.

Minotti, G. and Aust, S.D. 1987a. The role of iron in the initiation of lipid peroxidation. Chem. Phys. Lipids 44:191.

Minotti, G. and Aust, S.D., 1987b. An investigation into themechanism of citrate-Fe^{+2}-dependent lipid peroxidation. Free Rad. Biol. Med. 3:279.

Minotti, G. and Aust, S.D., 1987c. The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. J. Biol. Chem. 262:1098.

Minotti, G. and Aust, S.D. 1989. The role of iron in oxygen radical mediated lipid peroxidation. Chem. Biol. Interactions 71:1.

Minotti, G. and Aust, S.D. 1992. Redox cycling of iron and lipid peroxidation. Lipids 27:219.

Monsen, E.R. 1988. Iron nutrition and absorption:dietary factors which impact iron bioavailability. J. Amer. Diet. Assoc. 88:786.

Narasinga Rao, B.S. and Prabhavathi, T. 1978. An *in vitro* method for predicting the bioavailability of iron from foods. Am. J. Clin. Nutr. 31:169.

Oellingrath, I.M. 1988. Heat degradation of heme in methemoglobin and metmyoglobin model systems measured by reversed-phase ion-pair high performance liquid chromatography. J. Food Sci. 53:40.

Ofstad, R., Kidman, S., Myklebust, R., Olsen, R.L. and Hermansson, A.M. 1995. Liquid-holding capacity and structural changes in comminuted salmon (Salmo Salar) muscle as influenced by pH, salt and temperature. Lebens WissenTechnol. 28:329.

O'Grady, M.N., Monahan, F.J., Mooney, M.T., Butler, F., Buckley, D.J., Kerry, J., Allen, P. and Keane, M.G. 1996. Inhibition of oxymyoglobin oxidation by vitamin E. In"meat for the consumer" published by MATFORSK, Norwegian Food Research Institute. p.100.

Olson, D.G. and Rust, R.E. 1973. Oxidative rancidity in dry-cured hams: effect of low pro-oxidant and antioxidant salt formulation. J. Food Sci. 38:251.

Osinchak, J.E., Hultin, H.O. Zajicek, O.T., Kelleher, S.D. and Huang, C.H. 1992. Effect of NaCl on catalysis of lipid oxidation by the soluble fraction of fish muscle. Free Radical Biol. Med. 12:35.

Puppo, A. and Halliwell, B. 1988. Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Is hemoglobin a biological Fenton reagent? Biochem. J. 249:185.

Rhee, K.S. 1988. Enzymic and nonenzymic catalysis of lipid oxidation in muscle foods. Food Technol. 42(6):127.

Rhee, K.S., Smith, G.C. and Terrell, R.N. 1983. Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork. J. Food Prot. 46:578.

Rhee, K.S. and Ziprin, Y.A. 1987. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. J. Food Sci. 52:1174.

Sadrzadeh, S.M.H., Graf, E., Panter, S.S., Hallaway, P.E. and Eaton, J.W. 1984. Hemoglobin: a biologic Fenton reagent. J. Biol. Chem. 259:14354.

Salih, A.M. 1986. Lipid stability in turkey meat as influenced by cooking, refrigerated and frozen storage, salt, metal cations and antioxidant. Ph.D. thesis, Michigan State Univ., East Lansing, MI.

Samokyszyn, V.M., Miller, D.M., Reif, D.W. and Aust, S.D. 1989. Inhibition of superoxide and ferritin-dependent lipid peroxidation by ceruloplasmin. J. Biol. Chem. 264:21.

Schricker, B.R., Miller, D.D. and Stouffer, J.R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47:740.

Shomer, I., Weinberg, Z.G. and Vasilever, R. 1987. Structural binding properties of silver carp muscle affected by NaCl and $CaCl_2$ treatments. Food microstruct. 6:199.

Smith, G.J. and Dunkley, W.L. 1962. Initiation of lipid peroxidation by a reduced metal ion. Arch. Biochem. Biophys. 98:46.

Tappel, A.L. 1952. Linoleate oxidation catalyzed by hog muscle and adipose tissue extract. Food Res. 17:550.

Torres, E., Pearson, A.M., Gray, J.I., Booren, A.M. and Shimokomaki, M. 1988. Effect of salt on oxidative changes in pre- and post-rigor ground beef. Meat Sci. 23:51.

Uri, N. 1961. In "Autoxidation and antioxidants" Lundberg, W.O. (ed), Wiley-Interscience, New York, p55.

Velinov, P.D., Zhikov, M.V. and Cassens, R.G. 1990. The effect of tumbling, sodium chloride and polyphosphates on the microstructure and appearance of whole-muscle processed meats. Food Mircrostruct. 9:91.

Wallace, W.J., Houtchens, R.A., Maxwell, J.C. and Caughey, W.S. 1982. Mechanism of autoxidation for hemoglobins and myoglobins. J. Biol. Chem. 257:4966.

Watts, B.M. and Peng, D. 1947. Lipoxidase activity of hog hemoglobin and muscle extract. J. Biol. Chem. 179:441.

CHAPTER I

A COMPARISON OF THREE ANALYTICAL PROCEDURES FOR DETERMINING NON-HEME IRON CONCENTRATION IN GROUND PORK

Abstract

Meat samples containing four concentrations of iron (0, 3, 6 and 9 ug Fe/g meat) were prepared by mixing ground pork with 10% of its weight of a solution containing 0, 33, 66 and 99 ug Fe/ml ferrous chloride and forming into patties. The modified Schricker, Igene, and the ferrozine procedures were used to quantitate the non-heme, heme and total iron concentrations. The modified Schricker method realized greater overall recoveries of added iron than the ferrozine and Igene methods in all pork samples. For similar pork samples, non-heme and heme iron distribution was different when evaluated by the different analytical methods. Greater non-heme iron (3.9 ug/g and 5.1 ug Fe/raw meat; 5.1 ug Fe/g cooked meat) and smaller heme iron concentrations (8.8 ug Fe/g raw meat; 7.9 ug Fe/g cooked meat) were found in the control patties (i.e., no addition of iron) using the modified Schricker method. On the other hand, the Igene method measured the smallest non-heme iron concentrations (1.1 ug Fe/g raw meat; 2.2 ug Fe/g cooked meat) and the highest heme iron concentrations (10.1 ug Fe/g raw meat; 10.4 ug Fe/g cooked meat). The data indicate that the procedures used for separating the non-heme and heme iron account for the differences in the iron concentration observed in the samples. The quantitation steps yield similar values.

Introduction

The accurate determination of heme and non-heme iron concentrations in foods is important not only for the assessment of iron bioavailability (Monsen, 1988; Davis et al., 1992) but also for the role of iron in the storage stability of foods. It was well established by Igene et al. (1979) that the major prooxidant in cooked meat was not myoglobin but free iron which was released from the heme pigment during heating. It had also been suggested that non-heme iron is essential for the prooxidant effect of NaCl in meat (Kanner et al., 1991b; Osinchak et al., 1992). To further study the role of iron forms in nutrition and lipid oxidation, an accurate non-heme iron measurement method is necessary.

The Schricker (Schricker et al., 1982), modified Schricker (Rhee and Ziprin, 1987), Igene et al. (1979), and ferrozine methods (Carter, 1979) are commonly used assays to measure the non-heme iron concentration in meat. In general, each method can be divided into sample preparation and iron quantitation steps. In the sample preparation step, non-heme iron is extracted from the sample by a metal chelator solution such as EDTA (Igene method) or phosphate/citrate (ferrozine method) or by acid solution (modified Schricker method). The extracted irons can be directly quantitated by atomic absorption spectroscopy or by forming a color complex with metal chelator (ferrozine or bathophenanthroline) followed by quantitation by spectrophotometery. Although these methods are widely used to measure non-heme iron in meat, they were originally used for quantitating total iron in water. The quantitation procedure (either atomic absorption or colorimetry) is the same for non-heme and total iron for each method. The only difference

in the methods is in the preparation of the samples for non-heme and total iron quantitation.

Non-heme iron analysis measurement has been evaluated by various researchers (Rhee and Ziprin,1987; Ahn et al., 1993; Carpenter and Clark, 1995) by adding iron to the meat system and several questions remain unanswered. Ahn et al. (1993) reported 3.6, 6.9 and 6.7 ug non-heme iron/g meat in raw turkey leg using the ferrozine, Schricker and modified Schricker assays, respectively. Rhee and Ziprin (1987) also reported that the modified Schricker method produced higher non-heme iron concentrations (3.27 ug Fe/g meat) than the Igene (Igene et al., 1979) method (1.16 ug Fe/g meat) in the beef semimembranosus muscle. It is not clear why different non-heme iron concentrations are reported for the same sample when different assays are used.

Sample preparation and quantitation procedures are the two variables that may contribute to the differences in non-heme iron concentrations attained by the various methods. Ahn et al. (1993) suggested that the modified Schricker method may overestimate non-heme iron concentrations because iron ions were released from the heme proteins during sample preparation. In contrast, Rhee and Ziprin (1987) suggested that the Igene method might underestimate the non-heme iron concentrations in meat due to its inability to extract all of the non-heme iron in meat. No evidence is available to verify that the difference in non-heme iron concentrations is due to different sample preparation procedures.

The Schricker assay generally detects greater non-heme iron concentrations than the ferrozine or Igene method. It was postulated by Rhee and Ziprin (1987) that the higher iron concentrations are due to the release of iron from heme pigments and

nonenzymatic browning when using the Schricker method. The nonenzymatic browning reaction produced a color complex which increased the apparent iron-chelator color complex absorption, and therefore, overestimated the non-heme iron concentration. They later modified the Schricker method by adding sodium nitrite to prevent iron release from heme pigments and applying a second blank to correct for the greater iron concentrations obtained because of nonenzymatic browning. Because the modified Schricker method still gives greater non-heme iron concentrations than the ferrozine and Igene methods, it is not clear whether other factors exist which will interfere with the iron quantitation step of the modified Schricker method.

The percent iron recovered when a known amount is added to meat is a common technique used to evaluate non-heme iron measurement methodology. However, iron recovery in the Igene method has not been examined and compared to the other methods. Whether the variability in non-heme iron concentrations for the same sample when analyzed by different methods is due to sample preparation or quantitation procedures has not been examined. The objective of this study was to evaluate the iron recovery of the Igene method and to determine whether higher non-heme iron concentrations obtained using the modified Schricker method are due to differences in sample preparation or differences in the quantitation procedure.

Materials and Methods

Materials

Three fresh boneless pork legs were obtained within 24 hr of slaughter from the Michigan State University Meat Laboratory. Ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl sulfonic acid)-1,2,4-triazine), bathophenanthroline disulfonic acid (sodium salt), and thioglycolic acid (96-99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid, ammonium acetate, nitric acid, ethylenediaminetetraacetic acid (EDTA, disodium salt) and perchloric acid were obtained from J.T. Baker Inc. (Phillipsburg, NJ). Sodium nitrite and hydrochloric acid were purchased from Mallinckrodt Inc. (Paris, KY). Neocuproine was obtained from Fisher Scientific Company (Fair Lawn, NJ). All chemicals were reagent grade. All glassware was immersed in 4 N hydrochloric acid solution overnight to remove any trace iron and then rinsed with distilled water to remove hydrochloric acid residues. Disposable plastic test tubes (Baxter Healthcare Corp., Romulus, MI) were used to prevent possible iron contamination during the analyses.

Sample preparation

The sample preparation procedures are illustrated in Figure 1-1. All visible fat was removed and the pork was cut into 1 cm x 1 cm cubes, overwrapped in a plastic bag and frozen at -26 °C overnight. The frozen pork cubes were placed in a cheesecloth and fractured using a hammer. The fractured pork was powdered by mixing with dry ice and homogenizing in a blender (Tekmar Company, Cincinnati, OH). Connective tissue was

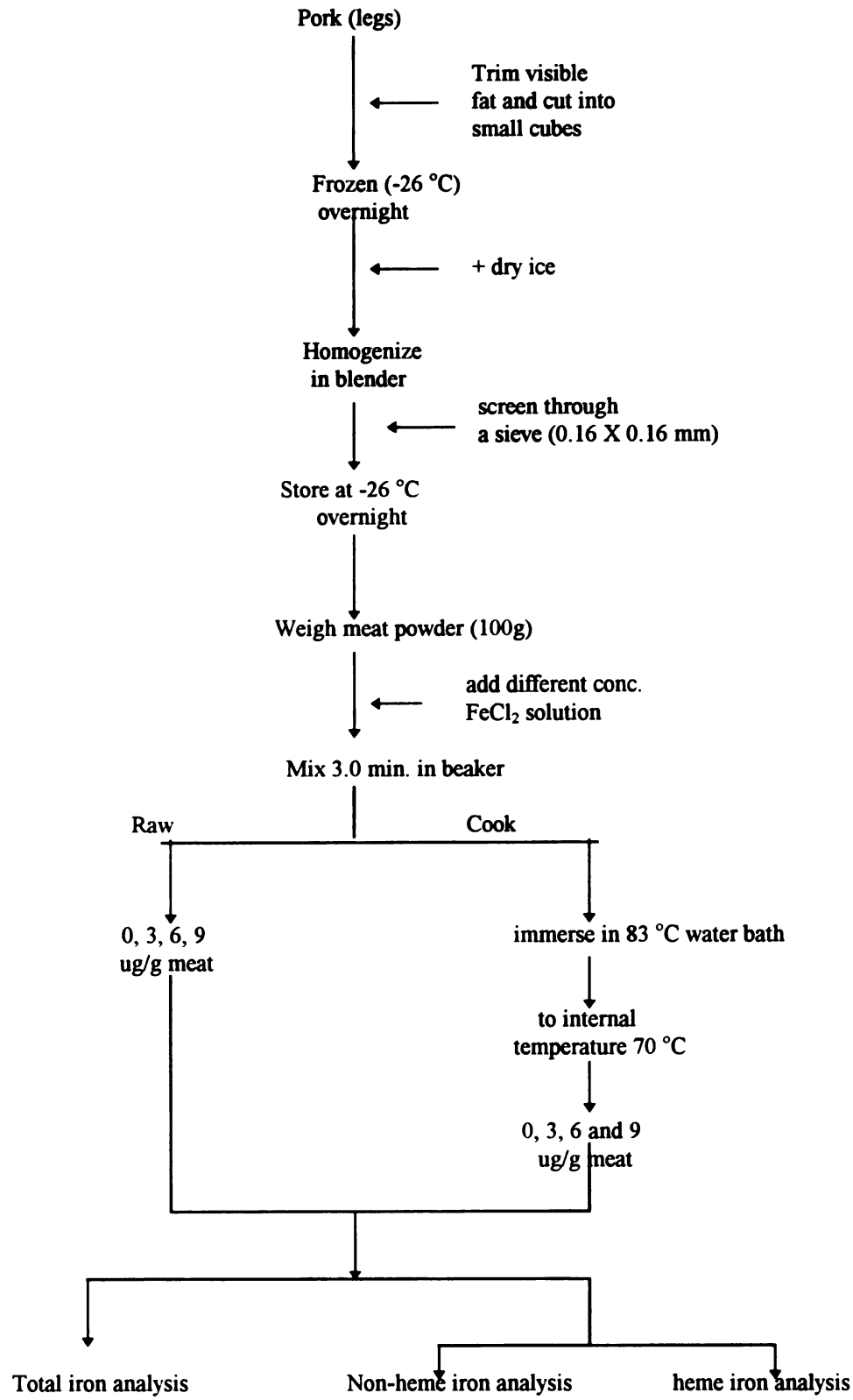

Figure 1-1 Sample preparation scheme for comparing modified Schricker, ferrozine and Igene methodology

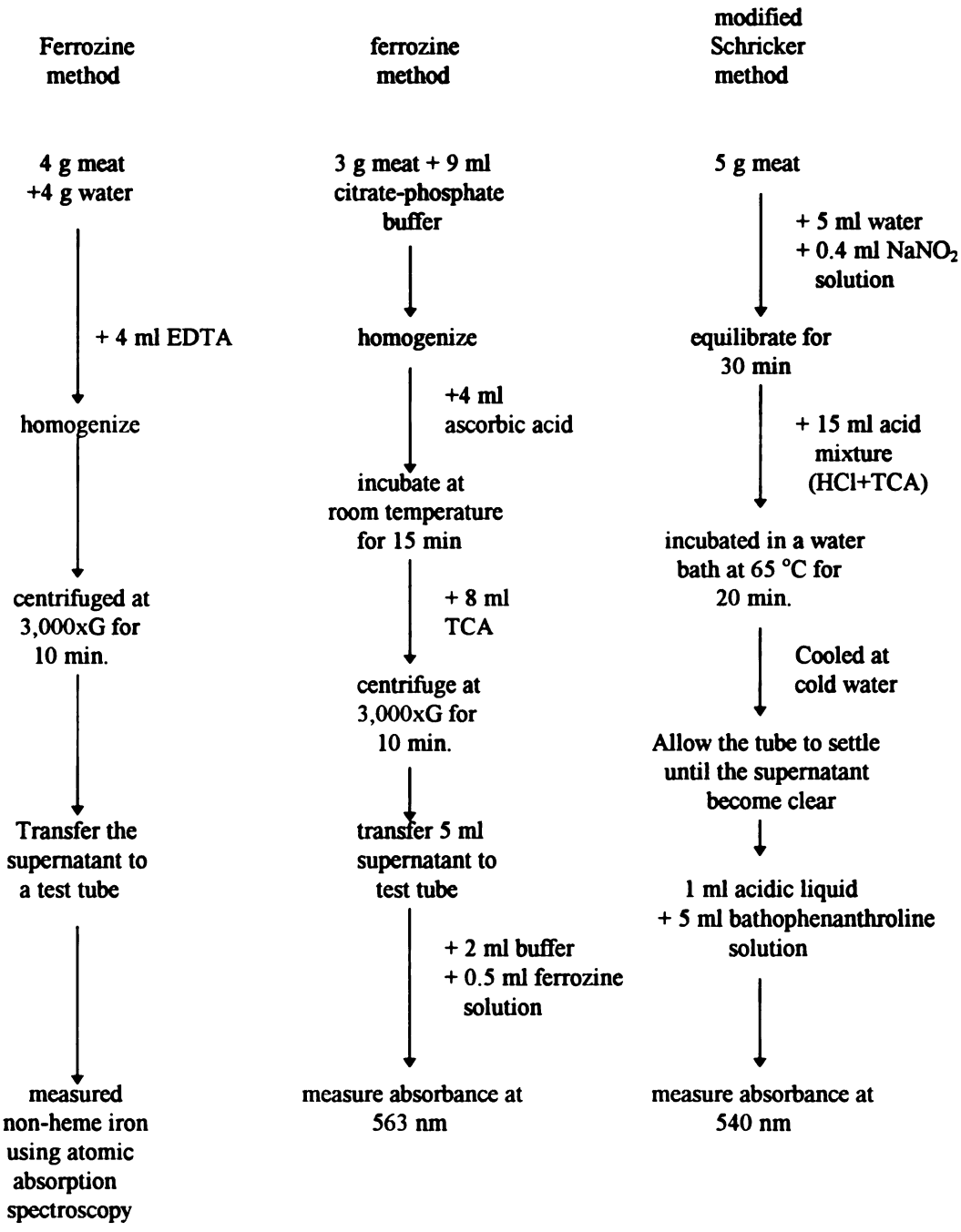

Figure 1-2 Flow diagram for different non-heme iron measurement procedures

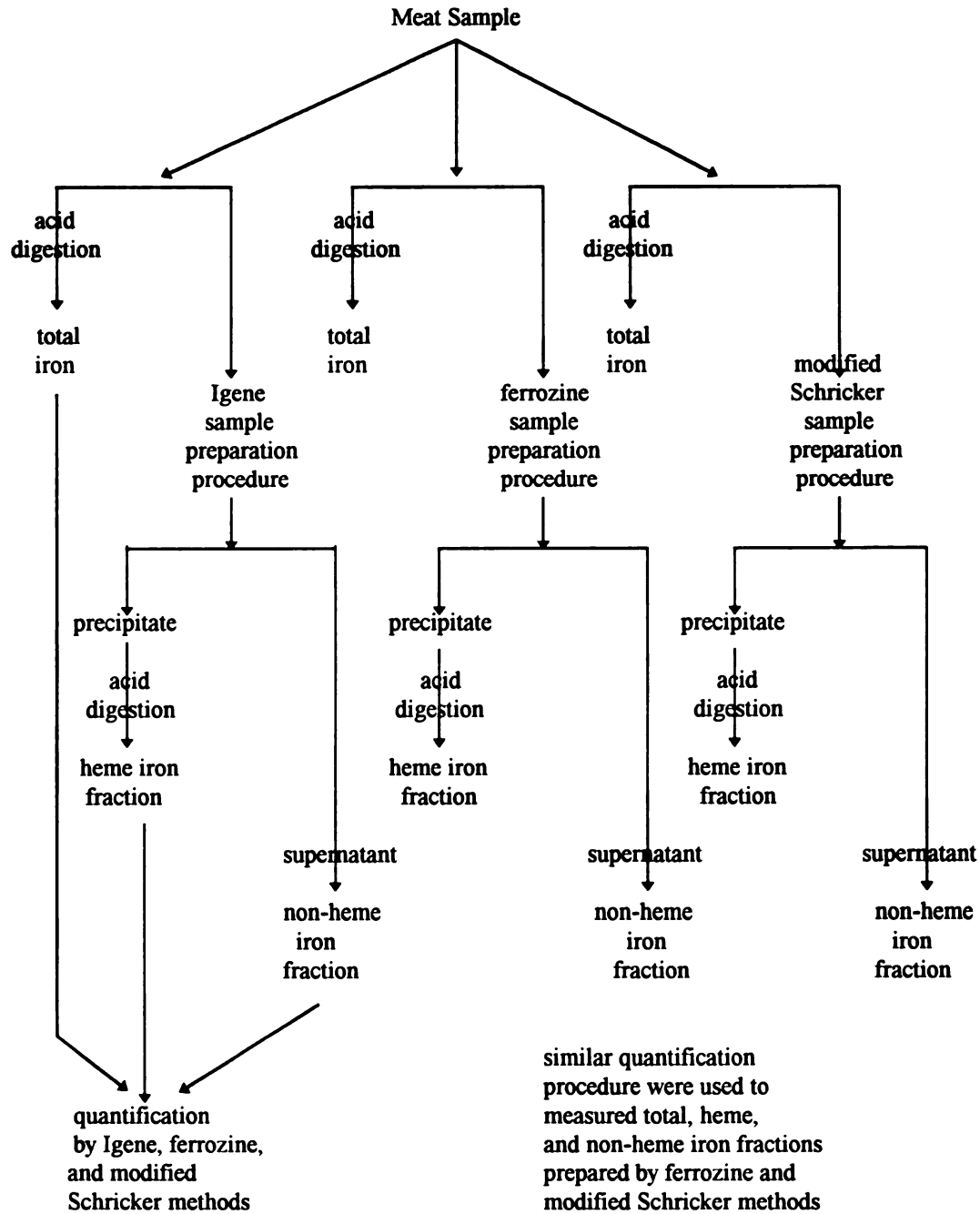

Figure 1-3 Flow chart for the evaluation of different iron quantitation procedures

removed by passing the powdered meat though a sieve (0.16 x 0.16 mm). The powdered tissues were stored overnight at -26 °C to permit evaporation of the remaining dry ice. Four treatments of iron (0, 3, 6 and 9 ug Fe/g meat) were added by hand-mixing 100g pork with 10 ml ferrous chloride solution (0, 33, 66 and 99 ug Fe/g meat) for 3 min with spatula in a 250 ml beaker. For the control samples (i.e., no added iron), 10 ml distilled water were added to the powdered meat and mixed in a similar fashion. Cooked samples were heated to an internal temperature of 70 °C in a water bath (temperature = 83 ± 2 °C).

For the quantitation of total iron, the samples were prepared using nitric acid and perchloric acid digestion (Igene et al., 1979). In the preparation step for non-heme iron fractions, samples were separated into supernatant (non-heme samples) according to Igene et al. (1979), ferrozine (Carter, 1971), and modified Schricker (Rhee and Ziprin, 1987) procedures and precipitate fractions (Figure 1-2). The recovery is calculated by [(measured non-heme iron in sample - non-heme iron in control) x 100%]/added non-heme iron. The precipitate was further digested by nitric acid and perchloric acid and identified as the heme iron fractions (Figure 1-3). Aliquots of the digests or extracts for total iron, heme iron and non-heme iron were quantitated by the Igene (atomic absorption), ferrozine and modified Schricker (colormetic) methods (Figure 1-3).

Data analysis

The experiments were designed as a four factor (quantitation method x preparation method x treatments x replication) randomized complete block design with balanced data (Gill, 1978). Three replicates were carried out in this study. Means, standard errors, sum of squares and mean square errors were calculated using the MSTAT-C microcomputer

statistical program (Michigan State University, 1989). The student t-test was used to make contrasts between treatments (Gill, 1978).

Results and Discussion

Recovery of added iron using three methods

Recovery data for added iron to raw and cooked samples using the three non-heme iron methods are presented in Figures 1-4 and 1-5, respectively. For the raw and cooked control samples, the modified Schricker realized greater ($p<0.05$) non-heme iron concentrations than the Igene method. This is consistent with iron data for beef that was reported by Rhee and Ziprin (1987). Greater non-heme iron concentrations were obtained by the modified Schricker method compared to the ferrozine method. These are consistent with turkey data reported by Ahn et al. (1993). These investigations demonstrated higher non-heme iron concentrations with the modified Schricker method than with the ferrozine method. A similar trend was found for the various iron treatments (Figures 1-4 and 1-5). The order of non-heme iron recovery for the methods was modified Schricker > ferrozine > Igene. These data suggest that a substantial portion of the added iron is not completely recovered by the Igene method. The recovery rate decreased significantly ($p<0.05$) when the levels of added iron was increased. It appears that the capacity for this method to extract non-heme iron may be exhausted as more iron is added. The lower recoveries may also be due to the added iron binding to protein macromolecules in meat systems (Kanner et al., 1991) and extraction procedures which are not strong enough to separate the iron-protein macromolecular interactions due to the low pH (pH = 5.6) in the extraction procedure. These results agree with Rhee and Ziprin's (1987) hypothesis which suggested that the Igene method underestimates the non-heme iron concentration of a meat sample.

The recovery of added iron using the ferrozine method is consistent with recoveries reported by Ahn et al. (1993) for cooked samples (100%). However, the recovery from raw samples is about 10% lower than the data of Ahn et al. (1993). The difference in iron recovery might be due to the differences in muscle species and sample preparation. To confirm this hypothesis, turkey was ground (instead of powdered) as described by Ahn et al. (1993) and the experiment with added iron was repeated. The percent recovery of added iron in the raw sample was similar to data (100%) previously reported by Ahn et al. (1993). Ahn's investigations suggested that because both the ferrozine and modified Schricker methods were able to completely recover the added iron from raw ground turkey, the differences in non-heme iron concentrations were not due to recovery. However, the data in the present study with indicates that the ferrozine method produced a different recovery (about 90%) when raw ground pork or different sample preparation procedures are used (Figures 1-4 and 1-5). The recovery of non-heme iron may be affected by species as well as physical sample preparation procedures.

Evaluation of three sample quantitation procedures

Both raw and cooked samples were separated into non-heme and heme fractions using methods described by Igene et al. (1979), Carter (1971) and Rhee and Ziprin (1987). The total, non-heme and heme iron fractions were quantitated using the Igene, ferrozine and modified Schricker methods (Tables 1-1 and 1-2). There were no significant differences ($p>0.05$) in total, non-heme and heme iron concentrations quantitated by the

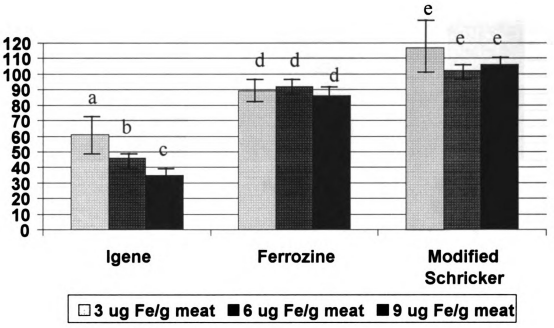

Figure 1-4. The recovery of added iron from raw ground pork using three procedures for determining non-heme iron (recovery with different letters within the same quantitation procedure are significant, $p<0.05$)

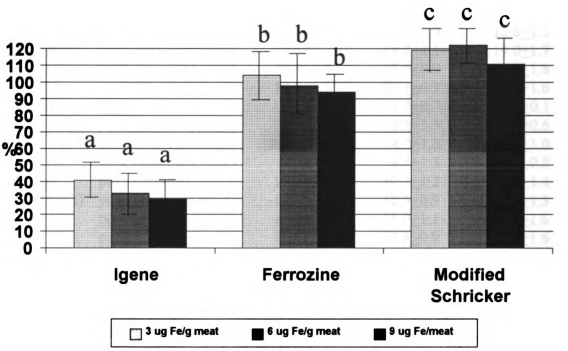

Figure 1-5. The recovery of added iron from cooked ground pork using three procedures for determining non-heme iron (recovery with different letters within the same quantitation procedure are significant, $p<0.05$)

Table 1-1. Comparison of iron quantitation procedures of raw pork extracted using the Igene method[1].

Fraction	Treatment	Igene[2,3]	ferrozine	modified Schricker
Total iron	pork (control)	10.2±0.4	11.0±0.9	10.8±1.3
(ug Fe/g meat)	pork (3ug Fe/g meat)	13.6±1.7	14.2±1.1	14.0±1.7
	pork (6ug Fe/g meat)	15.9±0.8	17.5±0.9	16.9±1.8
	pork (9ug Fe/g meat)	18.8±1.1	20.1±0.1	20.0±1.6
Non-heme iron	pork (control)	1.1±0.2	1.3±0.7	1.3±0.1
(ug Fe/g meat)	pork (3ug Fe/g meat)	2.9±0.5	3.2±1.3	3.3±0.6
	pork (6ug Fe/g meat)	3.8±0.3	4.0±1.5	3.9±1.0
	pork (9ug Fe/g meat)	4.2±0.3	4.4±1.3	4.7±0.5
Heme iron	pork (control)	10.1±0.7	10.2±1.2	9.8±1.4
(ug Fe/g meat)	pork (3ug Fe/g meat)	12.8±1.4	12.5±0.9	11.9±1.3
	pork (6ug Fe/g meat)	15.2±1.4	15.0±1.1	14.2±1.6
	pork (9ug Fe/g meat)	17.4±2.0	17.8±0.9	16.7±1.6

1. Sample separation into heme, non-heme and total iron using the Igene method and quantification of each fraction using the Igene, ferrozine, and modified Schricker methods
2. Mean ± standard deviation (n =3).
3. There is no significant difference ($p<0.05$) between quantitation procedures within each treatment in total, non-heme and heme iron.

Table 1-2. Comparison of iron quantitation procedures of cooked[1] pork extracted using the Igene method[2].

Fraction	Treatment	Igene[3,4]	ferrozine	modified Schricker
Total iron	pork (control)	11.4±2.5	12.4±1.8	14.7±2.8
(ug Fe/g meat)	pork (3ug Fe/g meat)	15.6±2.1	15.6±0.3	18.2±2.3
	pork (6ug Fe/g meat)	18.3±2.6	19.3±2.4	20.8±2.8
	pork (9ug Fe/g meat)	20.9±3.1	22.0±1.3	23.9±2.9
Non-heme iron	pork (control)	2.2±0.6	2.1±1.0	2.3±0.1
(ug Fe/g meat)	pork (3ug Fe/g meat)	3.4±0.9	3.4±0.9	3.2±0.1
	pork (6ug Fe/g meat)	4.2±1.3	3.9±1.1	3.6±0.1
	pork (9ug Fe/g meat)	4.8±1.6	4.3±1.3	4.2±0.3
Heme iron	pork (control)	10.4±2.0	9.4±1.8	10.4±1.2
(ug Fe/g meat)	pork (3ug Fe/g meat)	12.4±1.7	12.8±1.5	11.7±1.4
	pork (6ug Fe/g meat)	15.6±0.9	15.9±0.7	15.2±1.7
	pork (9ug Fe/g meat)	17.8±1.8	18.3±1.6	16.6±2.0

1. The cooked samples were heated to an internal temperature of 70°C in a 83°C water bath.
2. Sample separation into heme, non-heme and total iron using the Igene method and quantification of each fraction using the Igene, ferrozine, and modified Schricker methods.
3. Mean ± standard deviation (n =3).
4. There is no significant difference ($p>0.05$) between quantitation procedures within each treatment in total, non-heme and heme iron.

three methods for raw and cooked samples. These data agree with those of Carpenter and Clark (1995) who also reported there were no differences in iron concentrations obtained using ferrozine (colormetric) and atomic absorption spectroscopy quantitation methods.

Similar results were obtained for both raw and cooked samples prepared using the modified Schricker sample preparation procedures (Tables 1-5 and 1-6). However, non-heme iron concentrations of fractions prepared using the ferrozine procedure (Tables 1-3 and 1-4) were significantly ($p<0.05$) smaller when quantitated by the modified Schricker and Igene procedures than by the ferrozine method. This may be because a citrate-phosphate buffer used in the ferrozine procedure interferes with the modified Schricker method and because the ferrozine procedure does not use an acid digestion step. In the iron quantitation step a metal chelator (bathophenanthroline) was used to chelate metal and form a color complex in the modified Schricker method. Both citrate and phosphate are metal chelators which may compete with the bathophenanthroline for metal. Therefore, the sample preparation step may have competing chelating reactions which cause interference. Total and heme iron data indicate that the quantitation procedures are not responsible for the differences in non-heme iron concentrations reported for these different methods.

Evaluation of three sample preparation procedures

Data from samples, which were separated into heme and non-heme fractions and quantitated as described by Igene et al. (1979), Carter (1971) and Rhee and Ziprin (1987), are presented in Table 1-7. The non-heme iron concentrations in control

Table 1-3. Comparison of iron quantitation procedures of raw pork extracted using the ferrozine method[1].

Fraction	Treatment	Igene[2,3]	ferrozine	modified Schricker
Total iron	pork (control)	10.2±0.4	11.0±0.9	10.8±1.3
(ug Fe/g meat)	pork (3ug Fe/g meat)	13.6±1.7	14.2±1.1	14.0±1.7
	pork (6ug Fe/g meat)	15.9±0.8	17.5±0.9	16.9±1.8
	pork (9ug Fe/g meat)	18.8±1.1	20.1±0.1	20.0±1.6
Non-heme iron[4]	pork (control)	1.0±0.4^{A}	2.6±0.3^{B}	1.1±0.5^{A}
(ug Fe/g meat)	pork (3ug Fe/g meat)	2.3±0.3^{A}	5.3±0.2^{B}	2.5±0.5^{A}
	pork (6ug Fe/g meat)	3.3±0.4^{A}	8.2±0.3^{B}	3.8±0.4^{A}
	pork (9ug Fe/g meat)	4.5±0.5^{A}	10.4±0.4^{B}	5.0±0.4^{A}
Heme iron	pork (control)	8.3±1.1	10.3±0.7	11.5±2.3
(ug Fe/g meat)	pork (3ug Fe/g meat)	8.5±1.6	10.2±1.1	10.7±1.1
	pork (6ug Fe/g meat)	9.0±1.2	11.7±1.5	11.7±1.5
	pork (9ug Fe/g meat)	9.6±1.6	11.4±1.2	12.2±1.2

1. Sample separation into heme, non-heme and total iron using the ferrozine method and quantification of each fraction using the Igene, ferrozine, and modified Schricker methods
2. Mean ± standard deviation (n =3).
3. There are no significant differences ($p>0.05$) between quantitation procedures within each treatment in total and heme iron.
4. Non-heme iron concentrations in each row with the same capital letter superscript are not significantly different ($p<0.05$).

Table 1-4. Comparison of iron quantitation procedures of cooked[1] pork extracted using the ferrozine method[2].

Fraction	Treatment	Igene [3,4]	ferrozine	modified Schricker
Total iron	pork (control)	11.4±2.5	12.4±1.8	14.7±2.8
(ug Fe/g meat)	pork (3ug Fe/g meat)	15.6±2.1	15.6±0.3	18.2±2.3
	pork (6ug Fe/g meat)	18.3±2.6	19.3±2.4	20.8±2.8
	pork (9ug Fe/g meat)	20.9±3.1	22.0±1.3	23.9±2.9
Non-heme iron[5]	pork (control)	2.3±0.9^{A}	4.0±0.3^{B}	3.1±1.7^{A}
(ug Fe/g meat)	pork (3ug Fe/g meat)	4.2±1.2^{A}	7.1±0.5^{B}	4.9±1.1^{A}
	pork (6ug Fe/g meat)	6.0±0.7^{A}	9.9±0.9^{B}	6.6±2.0^{A}
	pork (9ug Fe/g meat)	7.5±1.3^{A}	12.4±0.9^{B}	8.1±2.8^{A}
Heme iron	pork (control)	8.4±2.2	10.7±0.7	11.7±1.1
(ug Fe/g meat)	pork (3ug Fe/g meat)	9.0±2.8	10.6±0.7	12.0±1.1
	pork (6ug Fe/g meat)	9.7±1.9	11.9±0.3	13.1±0.4
	pork (9ug Fe/g meat)	9.2±2.4	11.9±0.3	13.1±1.1

1. The cooked samples were heated to an internal temperature of 70°C in a 83°C water bath.
2. Sample separation into heme, non-heme and total iron using the ferrozine method and quantification of each fraction using the Igene, ferrozine, and modified Schricker methods
3. Mean ± standard deviation (n =3).
4. There are no significant differences ($p>0.05$) between quantitation procedures within each treatment in total and heme iron.
5. Non-heme iron concentrations in each row with the same capital letter superscript are not significantly different ($p<0.05$).

Table 1-5.Comparison of iron quantitation procedures of raw pork extracted using the modified Schricker method[1].

Fraction	Treatment	Igene[2,3]	ferrozine	modified Schricker
Total iron	pork (control)	10.2±0.4	11.0±0.9	10.8±1.3
(ug Fe/g meat)	pork (3ug Fe/g meat)	13.6±1.7	14.2±1.1	14.0±1.7
	pork (6ug Fe/g meat)	15.9±0.8	17.5±0.9	16.9±1.8
	pork (9ug Fe/g meat)	18.8±1.1	20.1±0.1	20.0±1.6
Non-heme iron	pork (control)	4.2±0.3	4.8±0.5	3.9±0.3
(ug Fe/g meat)	pork (3ug Fe/g meat)	7.1±1.1	8.0±1.4	7.4±1.3
	pork (6ug Fe/g meat)	10.0±0.3	10.6±1.1	10.0±0.4
	pork (9ug Fe/g meat)	12.8±0.6	14.3±1.4	13.4±1.1
Heme iron	pork (control)	6.5±0.7	8.5±1.2	8.8±2.1
(ug Fe/g meat)	pork (3ug Fe/g meat)	6.7±2.6	8.6±1.5	8.7±1.8
	pork (6ug Fe/g meat)	7.3±2.1	8.7±1.3	8.9±1.6
	pork (9ug Fe/g meat)	7.8±1.3	9.2±1.3	9.8±1.0

1. Sample separation into heme, non-heme and total iron using the modified Schricker method and quantification of each fraction using the Igene, ferrozine, and modified Schricker methods
2. Mean ± standard deviation (n =3).
3. There are no significant differences ($p>0.05$) between quantitation procedures within each treatment in total, non-heme and heme iron.

Table 1-6.Comparison of iron quantitation procedures of cooked[1] pork extracted using the modified Schricker method[2].

Fraction	Treatment	Igene[3,4]	ferrozine	modified Schricker
Total iron	pork (control)	11.4±2.5	12.4±1.8	14.7±2.8
(ug Fe/g meat)	pork (3ug Fe/g meat)	15.6±2.1	15.6±0.3	18.2±2.3
	pork (6ug Fe/g meat)	18.3±2.6	19.3±2.4	20.8±2.8
	pork (9ug Fe/g meat)	20.9±3.1	22.0±1.3	23.9±2.9
Non-heme iron	pork (control)	5.3±0.9	6.4±1.4	5.1±0.4
(ug Fe/g meat)	pork (3ug Fe/g meat)	8.0±1.0	9.4±1.7	8.7±0.2
	pork (6ug Fe/g meat)	10.9±0.8	12.5±1.9	12.4±0.6
	pork (9ug Fe/g meat)	13.3±0.8	16.0±1.8	15.2±1.0
Heme iron	pork (control)	7.1±2.2	8.6±1.3	7.9±1.2
(ug Fe/g meat)	pork (3ug Fe/g meat)	8.1±1.5	9.1±1.6	8.4±1.2
	pork (6ug Fe/g meat)	7.6±2.1	9.3±1.9	8.9±2.0
	pork (9ug Fe/g meat)	8.8±1.8	10.2±1.6	9.5±1.5

1. The cooked samples were heated to an internal temperature of 70°C in a 83°C water bath.
2. Sample separation into heme, non-heme and total iron using the ferrozine method and quantification of each fraction using the Igene, ferrozine, and modified Schricker methods
3. Mean ± standard deviation (n =3).
4. There are no significant differences ($p>0.05$) between quantitation procedures within each treatment in total, non-heme and heme iron.

Table 1-7. Heme, non-heme and total iron concentrations in the raw pork prepared following by Igene, ferrozine and modified Schricker methods.

Fraction	Treatment	Igene[1]	ferrozine	modified Schricker
Total iron (ug Fe/g meat)	pork (control)	10.2±0.4	11.0±0.9	10.8±1.3
	pork (3ug Fe/g meat)	13.6±1.7	14.2±1.1	14.0±1.7
	pork (6ug Fe/g meat)	15.9±0.8	17.5±0.9	16.9±1.8
	pork (9ug Fe/g meat)	18.8±1.1	20.1±0.1	20.0±1.6
Non-heme iron (ug Fe/g meat)	pork (control)	$1.1\pm0.2^{a,A}$	$2.6\pm0.3^{h,B}$	$3.9\pm0.3^{m,C}$
	pork (3ug Fe/g meat)	$2.9\pm0.5^{b,A}$	$5.3\pm0.2^{i,B}$	$7.4\pm1.3^{n,C}$
	pork (6ug Fe/g meat)	$3.8\pm0.3^{b,c,A}$	$8.2\pm0.3^{j,B}$	$10.0\pm0.4^{o,C}$
	pork (9ug Fe/g meat)	$4.2\pm0.3^{c,A}$	$10.4\pm0.4^{k,B}$	$13.4\pm1.1^{p,C}$
Heme iron[2,3] (ug Fe/g meat)	pork (control)	$10.1\pm0.7^{d,A}$	$10.3\pm0.7^{l,A}$	$8.8\pm2.1^{q,B}$
	pork (3ug Fe/g meat)	$12.8\pm1.4^{e,A}$	$10.2\pm1.1^{l,A}$	$8.7\pm1.8^{q,B}$
	pork (6ug Fe/g meat)	$15.2\pm1.4^{f,A}$	$11.7\pm1.5^{l,B}$	$8.9\pm1.6^{q,C}$
	pork (9ug Fe/g meat)	$17.4\pm2.0^{g,A}$	$11.4\pm1.2^{l,B}$	$9.8\pm1.0^{q,C}$

1. Mean ± standard deviation (n =3).
2. Treatments with different superscript lower case letters within the same column and iron fraction are significantly different ($p<0.05$).
3. Treatments in each row with the same capital letter superscript are not significantly different ($p<0.05$).

samples prepared and quantitated by the Igene, ferrozine and modified Schricker method are 1.1, 2.6 and 3.9 ug Fe/g meat, respectively. Since there are no differences in the quantitation steps (colormetric and atomic absorption) of the three non-heme iron methods, the difference in the non-heme iron concentrations in the control samples are due to differences in the preparation of the samples. The modified Schricker procedure extracts more non-heme iron than does the ferrozine and the Igene methods. The same trend was also found for the samples containing the three levels of added iron. Data for the cooked pork samples, separated and quantitated as described above, are presented in Table 1-8. These data exhibited the same trend as the raw samples. The modified Schricker sample preparation method gave higher non-heme iron concentrations than the ferrozine and the Igene methods.

The heme iron concentrations have an inverse relationship when compared to non-heme iron (Tables 1-7 and 1-8). The trend for all heme samples is: Igene > ferrozine > modified Schricker methodology. The modified Schricker method, which gave the highest non-heme iron value, produced no significant differences ($p>0.05$) in heme iron concentration in all the iron addition treatments (Table 1-7). On the other hand, the Igene method realized the smallest non-heme iron recovery and significantly different ($p<0.05$) heme iron concentrations among the various treatments. The heme iron data (Table 17) indicates that the ferrozine and the Schricker methods extract more added iron. This statement can be made because no significant differences ($p>0.05$) were found in the heme iron fractions. The data for cooked pork, separated and quantitated as described above, are presented in Table 1-8. Similar trends as in raw samples

Table 1-8. Heme, non-heme and total iron concentrations in the cooked[1] pork prepared following by Igene, ferrozine and modified Schricker methods

Fraction	Treatment	Igene[2]	ferrozine	modified Schricker
Total iron (ug Fe/g meat)	pork (control)	11.4±2.5	12.4±1.8	14.7±2.8
	pork (3ug Fe/g meat)	15.6±2.1	15.6±0.3	18.2±2.3
	pork (6ug Fe/g meat)	18.3±2.6	19.3±2.4	20.8±2.8
	pork (9ug Fe/g meat)	20.9±3.1	22.0±1.3	23.9±2.9
Non-heme iron (ug Fe/g meat)	pork (control)	$2.2\pm0.6^{a,A}$	$4.0\pm0.3^{f,B}$	$5.1\pm0.4^{k,C}$
	pork (3ug Fe/g meat)	$3.4\pm0.9^{a,A}$	$7.1\pm0.5^{g,B}$	$8.7\pm0.2^{l,C}$
	pork (6ug Fe/g meat)	$4.2\pm1.3^{a,A}$	$9.9\pm0.9^{h,B}$	$12.4\pm0.6^{m,C}$
	pork (9ug Fe/g meat)	$4.8\pm1.6^{a,A}$	$12.4\pm0.9^{i,B}$	$15.2\pm1.0^{n,C}$
Heme iron[3,4] (ug Fe/g meat)	pork (control)	$10.4\pm2.0^{b,A}$	$10.7\pm0.7^{j,A}$	$7.9\pm1.2^{o,B}$
	pork (3ug Fe/g meat)	$12.4\pm1.7^{c,A}$	$10.6\pm0.7^{j,B}$	$8.4\pm1.2^{o,C}$
	pork (6ug Fe/g meat)	$15.6\pm0.9^{d,A}$	$11.9\pm0.3^{j,B}$	$8.9\pm2.0^{o,C}$
	pork (9ug Fe/g meat)	$17.8\pm1.8^{e,A}$	$11.9\pm0.3^{j,B}$	$9.5\pm1.5^{o,B}$

1. The cooked samples were heated to an internal temperature of 70°C in a 83°C water bath.
2. Mean ± standard deviation (n =3).
3. Treatments with different superscript letters within the same column and iron fractions are significant ($p<0.05$).
4. Treatments in each row with the same capital letter superscript are not significantly different ($p<0.05$).

were found. The Igene method gave the lowest non-heme iron concentrations and the highest heme iron concentrations among the non-heme iron measurement methods. The modified Schricker procedure produced the highest non-heme iron concentrations and the lowest heme iron concentrations compared to the other two non-heme iron measurement methods.

Another question which needs to be addressed is "which method will give non-heme iron values which are closest to the actual concentration in meat?". The Igene method should not be used because of its poor iron recovery. For the ferrozine method, recovery of the added iron ranged from about 90-100% depending on which specie or preparation step (grinding or powdering) was used. Ahn et al. (1993) reported that the recovery of non-heme iron from the iron-containing proteins such as ferritin is about 80-87% in ground turkey. Based on Ahn's data, the ferrozine method may underestimate the actual non-heme iron concentration in meat. For the modified Schricker method, Ahn et al. (1993) suggested that the modified Schricker method overestimates the actual non-heme iron present in the sample. This is most likely due to iron released from the heme pigments as a result of the strong acidic conditions and subsequent heating during extraction. However, there are no data to support the above hypothesis. On the other hand, Rhee and Ziprin (1987) presented data which indicate that sodium nitrite applied to the modified Schricker method can effectively prevent the release of the iron from heme proteins even under acidic and heating conditions. Small amounts of iron detected in the non-heme fraction were released from the heme fraction using this extraction procedure. Based on iron recovery and data reported by Rhee and Ziprin (1987), the modified Schricker method will minimally overestimate the actual non-heme iron in meat because

sodium nitrite can effectively prevent the release of iron from the heme proteins. The modified Schricker sample preparation method is the most effective way to completely extract non-heme iron from meat samples and was used in subsequent studies. Atomic absorption spectroscopy will be used for iron quantitation because it is rapid and it can handle many samples.

Summary and Conclusions

The sample preparation step, not the iron quantitation step, contributes to the differences in iron concentrations realized by the three methods under evaluation. The modified Schricker method produced higher values for non-heme iron because it extracted more iron from the sample than the ferrozine and Igene procedures. The Igene procedure was not an effective procedure to extract the non-heme iron. These data indicate that the ferrozine method may not completely recover added iron from either pork or turkey samples. The modified Schricker method is the most effective procedure to completely extract non-heme iron from meat.

References

Ahn, D.U., Wolfe, F.H. and Sim, J.S. 1993. Three methods for determining nonheme iron in turkey meat. J. Food Sci. 58:288.

Carpenter, C.E. and Clark, E. 1995. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. J. Agric. Food Chem. 43:1824.

Carter, P. 1971. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal. Biochem. 40:450.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H. and Ku, P.K. 1984. Some factors influencing the nonheme iron content of meat and its implications in oxidation. J. Food Sci. 49:581.

Davis, C.D., Malecki, E.A. and Greger, J.L. 1992. Interactions among dietary manganese, heme iron, and nonheme iron in women. Am. J. Clin. Nutr. 56:926.

Gill, J.L. 1978. "Design and Analysis of Experiments in the Animal and Medical Sciences." Iowa State Univ. Press, Ames, IA.

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27:838.

Kanner, J., Harel, S. and Jaffe. R. 1991. Lipid peroxidation of muscle food as affected by NaCl. J. Agic. Food Chem. 39:101.

Monsen, E.R. 1988. Iron nutrition and absorption:dietary factors which impact iron bioavailability. J. Am Diet. Asso. 88:786.

Osinchak, J.E., Hultin, H.O., Zajicek, O.T., Kelleher, S.D. and Huang, C.H. 1992. Effect of NaCl on catalysis of lipid oxidation by the soluble fraction of fish muscle. Free Radical Biol. Med. 12:35.

Rhee, K.S. and Ziprin, Y.A. 1987. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. J. Food Sci. 52:1174.

Schricker, B.R., Miller, D.D. and Stouffer, J.R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47:740.

CHAPTER II

THE EFFECT OF VARIOUS SALTS ON NON-HEME IRON CONCENTRATIONS AND LIPID OXIDATION IN GROUND PORK

Abstract

The effect of NaCl concentration (0, 8.8, 17.4 and 26.1 mg/g meat) and different salts (NaCl, KCl, NaBr and KBr) on non-heme iron concentration and lipid oxidation was studied in raw and cooked ground pork. As NaCl concentration increased from 0 to 26.1 mg/g meat , lipid oxidation increased ($p<0.05$) in both raw and cooked samples when monitored by thiobarbituric acid-reactive substances (TBARS) and peroxide values. Increasing NaCl concentration increased non-heme iron concentrations for raw samples at day 6 and for the cooked samples immediately after cooking (day 0). Treatments containing different salts had higher ($p<0.05$) TBARS and peroxide values than the control. Different salts (NaCl, NaBr and KBr) also significantly ($p<0.05$) increased non-heme iron concentrations in raw samples at day 6 of storage. At the same molarity, the prooxidant effect and non-heme iron concentrations between different salts were not significantly different.

Introduction

Sodium chloride has been established as a prooxidant in meat (King and Bosch, 1990; Arnold et al., 1991; Kanner et al., 1991; Osinchak et al., 1992; Ahn et al. 1993a,b;). However, its exact mechanism as a prooxidant is unclear. Recently, researchers have linked the prooxidant effect of NaCl to the release of iron ions in meat (Kanner et al., 1991; Osinchak et al., 1992). Kanner et al. (1991) proposed that NaCl increases the release of iron ions and makes them available for lipid oxidation; and they postulated that when free iron ions were added to ground turkey, a large portion of the added iron interacted with protein macromolecules. This interaction prevented iron ions from reacting with membranous lipids and acting as a catalyst of lipid oxidation. Sodium chloride interrupts the interaction between iron ions and protein macromolecules. Therefore, more free iron is available to interact with the lipid fraction and catalyze lipid oxidation. Osinchak et al. (1992) used a similar approach as Kanner et al. (1991) when studying the prooxidant effect of NaCl in a model system (phosphatidylcholine liposomes and mackerel press juice). They confirmed that the prooxidant effect of NaCl involved iron ions. In order to verify the hypothesis that the prooxidant effect of NaCl in meat involves iron, it is necessary to establish that NaCl does increase free iron in meat systems. There are few research data available relative to the effect of NaCl on the non-heme iron concentration in pork. One factor which adds to the difficulty of studying this question is the inherent difficulty with the non-heme iron measurement method itself as discussed in chapter I.

Salts other than NaCl, such as KCl and $MgCl_2$, have also been shown to promote lipid oxidation in meat systems (Rhee et al., 1983; Ahn et al., 1993a; King and Bosch,

1990). Because of the similarity in the chemical composition of these alkali and alkali-earth halides (LiCl, NaCl, NaF, KCl, NaBr and NaI), researchers have attempted to investigate their prooxidant mechanisms by examining the effects of the salts and the effects of their anions and cations on lipid oxidation in meat systems (Ellis et al., 1968; Osinchak et al., 1992; Wettasinghe and Shahidi, 1996). Different salts have different prooxidant effect in lipid oxidation in meat (Rhee et al., 1983; King and Bosch, 1990). However, the consensus in the literature regarding their relative prooxidant effect in lipid oxidation indicates that extensive differences exist. For instance, KCl had no effect on rancidity development in raw ground pork during frozen storage (Chang and Watts, 1949, 1950; Watts and Peng, 1947). However, Rhee et al. (1983) reported KCl promoted lipid oxidation in raw ground pork but had no prooxidant effect in cooked ground pork. King and Bosch (1990) reported that KCl promoted lipid oxidation in ground turkey. Under specific storage conditions the prooxidant effect of different salts is also not consistent. For example, the prooxidant effect of $MgCl_2$ is higher than NaCl in cooked ground pork during refrigerated storage. However, the prooxidant effect of $MgCl_2$ is lower than NaCl during frozen storage (Rhee et al., 1983). It is not understood why these differences occur as the prooxidant effect of various salts is studied. It is also not clear whether these salts share the same prooxidant mechanism as NaCl, or if their prooxidant effects on lipid oxidation are due to their varying ability to release iron ions.

Since the prooxidant effect of NaCl is related to concentration, treatments with higher NaCl concentrations had greater lipid oxidation (King and Bosch, 1990; Torres et al., 1988). The first objective of this study is to examine the effect of NaCl concentration on lipid oxidation and non-heme iron release in ground pork. The second objective of this

research is to study the effect of different salts on non-heme iron concentrations and lipid oxidation.

Materials and Methods

Pork (legs) from market hogs were obtained from a local meat company within 48 hr of slaughter. The fresh legs were sealed in plastic bags and covered with ice during transportation. Bathophenanthroline disulfonic acid (sodium salt), thioglycolic acid (96-99%), NaCl, KCl NaBr and KBr were purchased from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid, ammonium acetate, nitric acid and perchloric acid were obtained from J.T. Baker Inc. (Phillipsburg, NJ). Sodium nitrite and hydrochloric acid were purchased from Mallinckrodt Inc.(Paris, KY). All chemicals were reagent grade. Possible contamination of glassware with trace quantities of iron was eliminated by immersing in 4 N hydrochloric acid solution overnight and rinsing with distilled water the following day. Disposable plastic test tubes (Baxter Diagnostics Inc., McGraw Park, IL) were used to prevent possible iron contamination.

Sample preparation

Bone and skin were removed from the legs immediately after arriving at Michigan State University. All visible fat was removed and the pork was ground (Hobart, Troy, OH) twice (through a 9 mm and a 3 mm plate). For the NaCl concentration study, 150g ground pork and 15 ml of NaCl solutions of varying concentrations (1.65, 3.27 or 4.91 M) were hand mixed using a spoon in a 250 ml beaker for 1.5 min at 4 °C to give target NaCl concentrations of 8.8, 17.4 and 26.1 mg/g meat (equal to about 0.15, 0.30 and 0.45 M NaCl, respectively). For the control (no NaCl), 15 ml of distilled water were added to the pork and mixed in a similar manner. For the second study, 150 g ground pork were mixed with 15 ml of 1.65 M salt solutions (NaCl, KCl, NaBr and KBr) to reach a target of

8.8 mg salt/g meat (equal to a 0.15 M salt concentration). For the control, 15 ml distilled water were added to the ground pork and similarly mixed. In the cooked study, 165 g samples were left in the 250 ml beaker after salt incorporation, covered with aluminum foil and a thermometer was inserted to the center of the meat to monitor the internal temperature during cooking. The samples were immersed in a 83 ± 2 °C water bath and cooked to an internal temperature of 70 °C (Igene et al.1979; Apte and Morrissey, 1987). Samples were stored in a refrigerator (4 °C) during the study.

Lipid oxidation was monitored by measuring the development of TBARS (thiobarbituric acid-reactive substances) and peroxide values. The TBARS measurement was based on the method of Tarladgis et al. (1960) as modified by Crackel et al. (1988). Propyl gallate and EDTA were added to the sample in the modified method (Crackel et al., 1988) to prevent any further lipid oxidation during sample preparation and TBARS were expressed as mg malonaldehyde/Kg sample. Peroxide values were measured according to Shantha and Decker (1994). The method is based on the principle of the rapid peroxide-mediated oxidation of ferrous to ferric iron. The latter, in the presence of cyanide ion, forms a ferric-cyanide color complex which can be measured using a spectrophotometer. Total iron fractions were prepared as described by Igene et al. (1979). This method utilizes a nitric acid and perchloric acid digestion followed by quantitation utilizing atomic absorption spectroscopy. Non-heme iron was determined according to the modified Schricker method (Rhee and Ziprin, 1987) and quantitated using atomic absorption spectroscopy as discussed in chapter I. Non-heme iron ions were first extracted from the samples using a HCl/TCA acid solution in 65 °C water bath for 20 hr (Figure 1-2). The non-heme iron concentration of the acid extract was directly

measured using atomic absorption spectroscopy. Lipid oxidation and non-heme iron were monitored on days 0, 3 and 6 for raw samples and days 0, 1 and 2 for cooked samples. Storage time designated as day 0 represents analyses carried out immediately after the salt or distilled water was mixed with the meat.

Data analysis

The experiment utilized a split-plot design with repeated measurements (Gill, 1978). Means, standard errors, sum of squares, mean square errors and the least significant difference (LSD) test were calculated using the MSTAT-C microcomputer statistical program (Michigan State University, 1989). Three replicates were carried out in each study.

Results and Discussion

Effect of NaCl concentrations on TBARS and Peroxide values

The effects of NaCl concentrations on lipid oxidation in the raw and cooked pork systems were monitored by measuring TBARS and peroxide values (Tables 2-1 and 2-2). For the raw pork study, the addition of NaCl significantly ($p<0.05$) increased TBARS and peroxide values during the 6 day refrigerated storage (4 °C) period. On the other hand, the treatment without NaCl (control) produced almost constant values during the storage period (Table 2-1). At day 0 there were no significant differences ($p<0.05$) in the extent of lipid oxidation between pork sample treatments monitored by both TBARS and peroxide values. After 3 days of refrigerated storage, the addition of NaCl (8.8, 17.4 and 26.1 mg/g meat) to the pork resulted in higher TBARS values than the control. Increasing NaCl concentrations produced greater ($p<0.05$) TBARS (26.1 mg NaCl/g meat > 17.4 mg NaCl/g meat > 8.8 mg NaCl/g meat > control). These data are consistent with those reported by Rhee et al. (1983) and Akamittath et al. (1990) indicating that NaCl is a prooxidant. Increasing NaCl concentration increased lipid oxidation (King and Bosch, 1990; Kuo and Ockerman, 1985; Torres et al., 1988). At day 6, the TBARS values for the 26.1 mg NaCl/g meat treatments were not statistically different from those for the 17.4 mg NaCl/g meat treatments. However, all values were significantly ($p<0.05$) greater than the control. Trends established by peroxide value measurements were similar to those obtained by measuring TBARS (Table 2-1). Statistical signficance was not the same for TBARS and peroxide value methods.

Table 2-1. The effect of NaCl concentrations on TBARS and peroxide values in raw ground pork during refrigerated storage (4 °C)

	TBARS[1] (mg malonaldehyde/kg meat)		
Treatment	Day 0[2,3]	Day 3	Day 6
1. pork (control)	0.3^{aA}	0.3^{aA}	0.3^{aA}
2. pork (8.8 mg NaCl/g meat)	0.3^{aA}	2.7^{bB}	6.0^{bC}
3. pork (17.4 mg NaCl/g meat)	0.5^{aA}	4.2^{cB}	8.7^{cC}
4. pork (26.1 mg NaCl/g meat)	0.7^{aA}	5.6^{dB}	9.1^{cC}
	Peroxide values[1] (milliequivalents of peroxide values/kg sample)		
	Day 0	Day 3	Day 6
1. pork (control)	1.6^{aA}	2.3^{aA}	2.4^{aA}
2. pork (8.8 mg NaCl/g meat)	2.4^{aA}	3.2^{aA}	9.5^{bB}
3. pork (17.4 mg NaCl/g meat)	3.5^{aA}	4.6^{abA}	14.5^{cB}
4. pork (26.1 mg NaCl/g meat)	3.1^{aA}	7.2^{bA}	18.8^{cB}

1. TBARS mean square error = 0.72, peroxide values mean square error = 2.12.
2. Means with different superscript letters within the same column are significantly different ($p<0.05$).
3. TBARS or peroxide values in each row with the same capital letter superscript are not significantly different ($p<0.05$).

For cooked samples (Table 2-2), similar trends in lipid oxidation between treatments were observed as was for the raw samples. Increasing NaCl concentrations significantly ($p<0.05$) increased lipid oxidation as measured by both TBARS and peroxide values during two days of refrigerated storage. However, the most significant differences in the lipid oxidation between treatments occurred at the early stage of storage (day 0) rather than at the later stages of storage. For example, at day 0, NaCl treatments (8.8, 17.4 and 26.1 mg NaCl/g meat) had significantly higher TBARS and peroxide values than the control. After 1 day of refrigerated storage, there was no significant difference in lipid oxidation between 8.8 mg NaCl/g meat treatment and the control monitored by TBARS and peroxide values. The NaCl concentration effect in TBARS and peroxide values are either less apparent or not significant between cooked treatments when compared to the raw treatments (Tables 2-1 and 2-2). This phenomenon is consistent with reports by other researchers that NaCl or other salts promote lipid oxidation in raw samples but may or may not in cooked samples. For example, Torres et al. (1988) reported no differences in lipid oxidation in cooked post-rigor beef treated with different NaCl concentrations (0 - 4 %) but documented differences in the raw samples. Ahn et al. (1993b) reported there were no differences in lipid oxidation in cooked turkey containing 0 and 2 % NaCl. Several explanations for the differences in conclusions by the investigators regarding the effect of NaCl on lipid oxidation in cooked meat are possible. First, lipid oxidation is a free radical reaction and the initiation reaction is the rate-determining step. Once the reaction is initiated, the importance of the presence of the prooxidant may be reduced. Cooking can provide the energy to initiate lipid oxidation in meat. Although NaCl has a prooxidant effect by itself, the NaCl effect is confounded with cooking effects and the

Table 2-2. The effect of NaCl concentrations TBARS and peroxide values in cooked[1] ground pork during refrigerated storage (4 °C)

Treatment	TBARS[2] (mg malonaldehyde/kg meat) Day 0[3,4]	Day 1	Day 2
1. pork (control)	1.7^{aA}	3.8^{aB}	5.2^{aC}
2. pork (8.8 mg NaCl/g meat)	2.3^{bA}	4.4^{abB}	6.0^{aC}
3. pork (17.4 mg NaCl/g meat)	3.2^{cA}	5.6^{bcB}	7.6^{bC}
4. pork (26.1 mg NaCl/g meat)	3.3^{cA}	6.3^{cB}	7.9^{bC}
	Peroxide values[2] (milliequivalents of peroxide values/kg sample)		
	Day 0	Day 1	Day 2
1. pork (control)	2.8^{aA}	6.3^{aB}	9.8^{aC}
2. pork (8.8 mg NaCl/g meat)	3.8^{bA}	6.9^{abB}	9.8^{aC}
3. pork (17.4 mg NaCl/g meat)	4.6^{bcA}	7.7^{bcB}	9.9^{aC}
4. pork (26.1 mg NaCl/g meat)	5.1^{cA}	8.1^{cB}	11.1^{aC}

1. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
2. TBARS mean square error = 0.49, peroxide values mean square error = 1.68.
3. Means with different superscript letters within the same column are significantly different ($p<0.05$).
4. TBARS or peroxide values in each row with the same capital letter superscript are not significantly different ($p<0.05$).

NaCl effect on lipid oxidation is less dramatic for cooked samples (Tables 2-1 and 2-2). Second, it is possible that heating and NaCl may have similar mechanisms for promoting lipid oxidation. For example, both NaCl and heating break down the meat microstructure (Ofstad et al., 1995; Velinov et al., 1990) and release iron ions (Kanner et al., 1991; Osinchak et al., 1992; Love and Pearson, 1974). Therefore, the prooxidant effect of NaCl will be less significant after heating because the microstructure has already been broken down with or without NaCl addition. The exception may be when a high concentration of NaCl is used. A third possibility is attributed to the experimental error due to differences in the meat systems or species examined, complexity of the model system, and concentrations of salt used (Srinivasan and Xiong, 1996).

For example, Wettasinghe and Shahidi (1996) reported that lean pork treated with NaCl at a concentration of 100 meq/kg sample exhibited a prooxidative effect after day 3 of refrigerated storage. However, lean pork treated with NaCl at day 1 had significantly ($p<0.05$) lower lipid oxidation than the control (antioxidant effect). This observation is in direct conflict with the hypothesis that NaCl is a prooxidant. This observation may be due to experimental error.

Effect of NaCl on non-heme iron concentration

There were no differences in total iron concentrations within raw or cooked samples (Tables 2-3 and 2-4). This indicates that salt or other materials used in this study do not contribute to the total iron concentrations in the meat systems. In raw, control samples (no salt) , non-heme iron concentration did not increase during 6 days of refrigerated storage (Table 2-3). In raw samples containing salt, non-heme iron

concentrations significantly increased during the refrigerated storage period from day 3 to 6. Decker and Hultin (1990) reported that low-molecular weight fraction (<10 kilodalton) iron increased significantly in mackerel muscle during 7 days storage at 0 °C. This increase may be attributed to the presence of ascorbate and the superoxide radicals which were reported to release iron from ferritin (Biemond et al., 1986; Boyer and McCleary, 1987) and hydrogen peroxide which may cause release of heme iron (Rhee et al., 1987) during storage. Sodium chloride seems to have accelerated this process. Therefore, non-heme iron concentrations in the NaCl-added treatments increase significantly during storage (Table 2-3). There were no differences in the non-heme iron concentrations between treatments with or without NaCl on days 0 and 3. This is consistent with data reported by Ahn et al (1993c) which indicated that NaCl does not affect the non-heme iron concentrations in raw turkey meat. On day 6, the NaCl treatments had higher non-heme iron concentrations than the control. However, only treatments with 17.4 and 26.1 mg NaCl/g meat had non-heme iron concentrations that were significantly ($p<0.05$) greater than the control.

In the cooked samples (Table 2-4), 17.4 and 26.1 mg NaCl/g meat treatments significantly ($p < 0.05$) increased non-heme iron concentrations in ground pork at day 0. This observation is also in agreement with Ahn et al. (1993c) who found that NaCl increased the amounts of non-heme iron in cooked turkey leg meat and mechanically deboned turkey meat. However, because the total iron concentration in the cooked samples also increased, part of the iron increase in cooked samples may be a concentration effect due to the water or lipid loss during cooking.

Table 2-3. Total and non-heme iron concentration of raw ground pork during refrigerated storage (4 °C)

Treatment	Non-heme iron[1] (ug Fe/g meat)			Total iron (ug Fe/g meat)
	Day 0[2,3]	Day 3	Day 6	
1. pork (control)	5.0^{aA}	5.3^{aA}	5.7^{aA}	11.2^{a}
2. pork (8.8 mg NaCl/g meat)	4.8^{aA}	5.3^{aA}	7.2^{abB}	10.9^{a}
3. pork (17.4 mg NaCl/g meat)	5.2^{aA}	5.8^{aA}	7.6^{bB}	11.9^{a}
4. pork (26.1 mg NaCl/g meat)	5.9^{aA}	5.9^{aA}	8.3^{cB}	11.4^{a}

1. Non-heme iron mean square error = 0.65.
2. Means with different superscript letters within the same column are significantly different ($p<0.05$).
3. Non-heme iron concentration in each row with the same capital letter superscript are not significantly different ($p<0.05$).

Table 2-4. Total iron and non-heme iron concentration of cooked[1] ground pork during refrigerated storage (4 °C)

Treatment	Non-heme iron (ug Fe/g meat)[2]			Total iron (ug Fe/g meat)
	Day 0[3,4]	Day 1	Day 2	
1. pork (control)	5.3^{aA}	6.6^{aA}	5.6^{aA}	13.6^{a}
2. pork (8.8 mg NaCl/g meat)	5.9^{abA}	7.1^{aA}	6.4^{abA}	14.7^{a}
3. pork (17.4 mg NaCl/g meat)	7.7^{bA}	6.7^{aA}	7.8^{bA}	13.6^{a}
4. pork (26.1 mg NaCl/g meat)	8.1^{bA}	7.1^{aA}	7.1^{abA}	14.0^{a}

1. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
2. Non-heme iron mean square error = 1.90.
3. Means with different superscript letters within the same column are significantly different ($p<0.05$).
4. Non-heme iron concentration in each row with the same capital letter superscript are not significantly different ($p<0.05$).

The relationship between lipid oxidation, non-heme iron concentration and NaCl concentration

The data in Tables 2-1 and 2-2 indicate that increasing NaCl concentration will increase TBARS and peroxide values in raw and cooked samples. As NaCl concentrations increased in the cooked samples at day 0, the non-heme iron concentration increased and measures of lipid oxidation increased proportionately. Greater NaCl concentrations resulted in greater lipid oxidation and non-heme iron concentrations at day 0. In the raw pork study, increasing NaCl concentrations produced significantly higher TBARS and peroxide values than the control after 3 days of refrigerated storage. NaCl affected non-heme iron concentrations in samples stored for 6 days at 4 °C. The response of non-heme iron release to increases in NaCl concentrations in raw pork samples was slower when compared to the response of lipid oxidation to the NaCl concentration effect. This may be because most of the loosely bound non-heme irons were removed by NaCl at day 0. It took longer time for NaCl to release the tightly bound non-heme iron from the meat. Therefore, the effect of NaCl on non-heme iron release was only apparent at day 6. Since there was good agreement between lipid oxidation and non-heme iron concentrations in cooked samples at day 0 and in raw samples at day 6 as discussed above, the hypothesis that the prooxidant mechanism of NaCl is to increase the availability of iron to catalyze lipid oxidation in meat is valid.

The effect of various salts on lipid oxidation and non-heme iron concentration in pork

In the raw pork study, there were no differences in lipid oxidation and non-heme iron concentrations between various salts treatments at day 0 (Tables 2-5, 2-6). After day 3, lipid oxidation in treatments with the various salts (NaCl, KCl, NaBr and KBr) was significantly ($p<0.05$) higher than the control when monitored by TBARS. However, only NaCl and NaBr treatments were significantly ($p<0.05$) higher than the control when monitored by the peroxide values. The deviation between TBARS and peroxide values may be because the variation for peroxide values methodology was greater than TBARS methodology in this study (TBARS mean square error = 0.865 and peroxide values = 2.682). For the corresponding non-heme iron concentrations at day 3, only the 8.8 mg NaCl/g meat treatment was significantly different from the control (Table 2-6). The other three salt treatments had higher, but not significant, non-heme iron concentrations than the control. This may be due to the sensitivity of the non-heme iron measurement method as discussed above. There were no significant differences in TBARS and non-heme iron concentrations between various salt treatments. At day 6, the various salt treatments had higher TBARS and peroxide values than the control except the KBr treatment when monitored by peroxide values (Table 2-5). The non-heme iron concentrations also reflect a similar trend as lipid oxidation except for 8.8 mg KCl/g meat (Table 2-6). There were no significant differences in non-heme iron concentration and lipid oxidation (TBARS and peroxide values) among various salt treatments. In general, these data demonstrated that salts (KCl, NaBr and KBr) other than the NaCl can also promote lipid

Table 2-5. The effect of different salts on TBARS and peroxide values in raw ground pork during refrigerated storage (4 °C)

Treatment	TBARS[1] (mg malonaldehyde/kg meat)		
	Day 0[2,3]	Day 3	Day 6
1. control	0.3^{aA}	0.3^{aA}	0.3^{aA}
2. control (8.8 mg NaCl/g meat)	0.4^{aA}	1.9^{bB}	3.5^{bC}
3. control (11.2 mg KCl/g meat)	0.4^{aA}	1.8^{bB}	3.4^{bC}
4. control (15.5 mg NaBr/g meat)	0.5^{aA}	1.3^{bA}	3.1^{bB}
5. control (17.9 mg KBr/g meat)	0.9^{aA}	1.2^{bA}	2.4^{bB}
	Peroxide values[1] (milliequivalents of peroxide values/kg sample)		
	Day 0	Day 3	Day 6
1. control	2.0^{aA}	2.3^{aA}	3.2^{aA}
2. control (8.8 mg NaCl/g meat)	2.8^{aA}	7.3^{bB}	8.9^{bB}
3. control (11.2 mg KCl/g meat)	2.2^{aA}	5.9^{abcB}	8.8^{bC}
4. control (15.5 mg KCl/g meat)	1.8^{aA}	6.4^{bcB}	7.2^{bB}
5. control (17.9 mg KCl/g meat)	1.8^{aA}	3.2^{acA}	5.7^{abB}

1. TBARS mean square error = 0.87, peroxide values mean square = 2.68.
2. Means with different superscript letters within the same column are significantly different ($p<0.05$).
3. TBARS or peroxide values in each row with the same capital letter superscript are not significantly different ($p<0.05$).

Table 2-6. Total and non-heme iron concentration in raw ground pork during refrigerated storage (4 °C)

Treatment	Non-heme iron[1] (ug Fe/g meat)			Total iron (ug Fe/g meat)
	Day 0[2,3]	Day 3	Day 6	
1. control	3.6^{aA}	4.1^{aA}	3.9^{aA}	11.8^{a}
2. control (8.8 mg NaCl/g meat)	3.9^{aA}	5.4^{bB}	6.2^{bB}	11.6^{a}
3. control (11.2 mg KCl/g meat)	4.7^{aA}	4.9^{abA}	5.2^{ab}	11.3^{a}
4. control (15.5 mg KCl/g meat)	4.0^{aA}	5.2^{abB}	6.6^{bC}	11.2^{a}
5. control (17.9 mg KCl/g meat)	4.3^{aA}	5.2^{abAB}	6.0^{bB}	11.9^{a}

1. Non-heme iron mean square error = 1.07.
2. Means with different superscript letters within the same column are significantly different ($p<0.05$).
3. Non-heme iron concentration in each row with the same capital letter superscript are not significantly different ($p<0.05$).

oxidation and increase the non-heme iron concentrations in the meat systems. However, the differences in prooxidant activity and non-heme iron concentration were not significant at the same molarity between these various salts. These data are in agreement with Srinivasan and Xiong (1996) who reported that no significant differences in lipid oxidation ($p > 0.05$) were observed between various salts (NaCl, NaBr, Na_2SO_4, KCl and LiCl) in salted beef heart surimi during refrigerated storage at low ionic strength (0.1 M). However, the results are different from Rhee et al. (1983) who reported that NaCl promoted more extensive lipid oxidation than KCl in meat at the same molarity. Wettasinghe and Shahidi (1996) also indicated that lean pork treated with NaCl, NaBr, KCl and KBr at concentrations of 100 and 200 meq/kg sample had different degrees of lipid oxidation during refrigerated storage. The prooxidant activity order between these salts (NaCl, NaBr, KCl and KBr) are not always constant during the storage. The conflicting observations on the role of various salts in lipid oxidation may be due to differences in the meat systems or species examined, complexity of the model system, and concentrations of salt used (Srinivasan and Xiong, 1996). For example, Srinivasan and Xiong (1996) reported when various salts were added at a low concentration (0.1 M) to buffer-washed surimi, they exhibited only a minimal effect on lipid oxidation. When added at a much higher concentration (0.6 M), these salts stimulated lipid oxidation to various extents. When adding salt on a weight percentage basis rather than on a molar basis, King and Bosch (1990) reported that 2 % NaCl in cooked ground turkey was more oxidized than turkey containing 2 % KCl. These differences may be attributed to the fact that the same percentage weight of the various salts will result in different molarities (NaCl > KCl > NaBr > KBr) because each salt has a different molecular weight.

For cooked meat samples, various salt treatments had higher TBARS and peroxide values than the control at day 0 (Table 2-7). After 1 day of refrigerated storage, the differences in lipid oxidation between control and various salt treatments were not significant. However, all TBARS values were above 3.5 and all peroxide values were above 8.0. The salt effect was less significant in the cooked samples compared to the raw samples as was discussed previously. For the corresponding non-heme iron data at day 0, treatments with various salts had a trend of higher non-heme iron concentrations than the control (Table 2-8). However, only the KBr treatment was significant ($p<0.05$). After 2 days of refrigerated storage, NaCl, KCl and KBr treatments had significantly higher non-heme iron concentrations than control.

The data demonstrating the effect of various salts on lipid oxidation and non-heme iron concentration in pork are similar to the NaCl data presented above (Tables 2-1, 2-2, 2-3 and 2-4). Various salts will increase lipid oxidation in both raw and cooked samples when monitored by TBARS and peroxide values. Various salts also significantly ($p<0.05$) increased the non-heme iron concentrations in raw pork at 6 days of storage but only immediately after addition in the cooked samples. Because of the similar response of these various salts (NaCl, KCl, NaBr and KBr) on lipid oxidation and non-heme iron concentration in the meat systems, it is possible that these salts share the same prooxidant mechanism as NaCl. The mechanism proposed is that the prooxidant effect of salt is related to non-heme iron release.

Table 2-7. The effect of different salts on TBARS and peroxide values in cooked[1] ground pork during refrigerated storage (4 °C)

Treatment	TBARS (mg malonaldehyde/kg meat)[2]		
	Day 0[3,4]	Day 1	Day 2
1. control	1.9^{bA}	3.6^{aB}	4.6^{aC}
2. control (8.8 mg NaCl/g meat)	2.8^{aA}	4.7^{aB}	5.3^{aB}
3. control (11.2 mg KCl/g meat)	2.7^{aA}	4.3^{aB}	4.5^{aB}
4. control (15.5 mg KCl/g meat)	3.6^{aA}	5.1^{aB}	5.0^{aB}
5. control (17.9 mg KCl/g meat)	3.4^{aA}	5.1^{aB}	5.3^{aB}
	Peroxide values[2] (milliequivalents of peroxide values/kg sample)		
	Day 0	Day 1	Day 2
1. control	4.1^{aA}	8.2^{aB}	10.2^{aC}
2. control (8.8 mg NaCl/g meat)	5.6^{bA}	9.6^{aB}	10.5^{aB}
3. control (11.2 mg KCl/g meat)	5.3^{bA}	9.0^{aB}	10.7^{aC}
4. control (15.5 mg KCl/g meat)	6.1^{bA}	8.9^{aB}	10.8^{aC}
5. control (17.9 mg KCl/g meat)	5.3^{bA}	9.6^{aB}	10.3^{aB}

1. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
2. TBARS mean square error = 0.82, peroxide values mean square error = 0.95.
3. Means with different superscript letters within the same column are significantly different ($p<0.05$).
4. TBARS or peroxide values in each row with the same capital letter superscript are not significantly different ($p<0.05$).

Table 2-8. Total iron and Non-heme iron concentration in cooked[1] ground pork during refrigerated storage (4 °C)

Treatment	Non-heme iron[2] (ug Fe/g meat)			Total iron (ug Fe/g meat)
	Day 0[3,4]	Day 1	Day 2	
1. control	5.0^{aA}	6.4^{aA}	5.6^{aA}	12.6^{a}
2. control (8.8 mg NaCl/g meat)	5.6^{abA}	6.7^{aA}	6.9^{bA}	12.6^{a}
3. control (11.2 mg KCl/g meat)	6.4^{abA}	6.0^{aA}	6.6^{bA}	13.3^{a}
4. control (15.5 mg KCl/g meat)	6.1^{abA}	6.7^{aA}	6.4^{abA}	12.6^{a}
5. control (17.9 mg KCl/g meat)	7.1^{bA}	6.5^{aA}	6.7^{bA}	12.1^{a}

1. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
2. Non-heme iron mean square error = 1.89.
3. Means with different superscript letters within the same column are significantly different ($p<0.05$).
4. Non-heme iron concentration in each row with the same capital letter superscript are not significantly different ($p<0.05$).

Summary and Conclusions

The addition of NaCl increased lipid oxidation in both raw and cooked samples as well as non-heme iron concentrations. The corresponding non-heme iron analyses indicated that non-heme iron concentration increased significantly immediately for cooked samples and after 6 days of storage for raw samples. Salts (KCl, NaBr and KBr), other than NaCl, also had similar effects as NaCl with respect to lipid oxidation and non-heme iron concentration. Based upon data presented, it is possible that the prooxidant effect of salt is to make more iron available to catalyze lipid oxidation.

References

Ahn, D.U., Wolfe, F.H. and Sim, J.S. 1993a. Prevention of lipid oxidation in pre-cooked turkey meat patties with hot packaging and antioxidant combinations. J. Food Sci. 58:283.

Ahn, D.U., Ajuyah, A., Wolfe, F.H. and Sim, J.S. 1993b. Oxygen availability affects prooxidant catalyzed lipid oxidation of cooked turkey patties. J. Food Sci. 58:278.

Ahn, D.U., Wolfe, F.H. and Sim, J.S. 1993c. Three methods for determining nonheme iron in turkey meat. J. Food Sci. 58:288.

Akamittath, J.G., Brekke, C.J. and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513.

Apte, S. and Morrissey, P.A. 1987. Effect of haemoglobin and ferritin on lipid oxidation in raw and cooked muscle systems. Food Chem. 25:127.

Arnold, A.R., Bascetta, E. and Gunstone, F.D. 1991. Effect of sodium chloride on prooxidant activity of copper (II) in peroxidation of phospholipid liposomes. J. Food Sci. 56:571.

Biemond, P., Swaak, J.G., Beudorff, C.M. and Koster, J.F. 1986. Superoxide-dependent and -independent mechanisms of iron mobilization from ferritin by xanthine oxidase. Biochem. J. 239:169.

Boyer, R.F. and McCleary, C.J. 1987. Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. Free Radical Biology & Medicine. 3:389.

Chang, I. and Watts, B.M. 1949. Antioxidants in the hemoglobin catalyzed oxidation of unsaturated fats. Food Technol. 3:332.

Chang, I. and Watts, B.M. 1950. Some effects of salt and moisture on rancidity of fat. Food Res. 15:313.

Crackel, R.L., Gray, J.I., Pearson, A.M., Booren, A.M. and Buckley, D.J. 1988. Some further observations on the TBARS test as an index of lipid oxidation in meats. Food Chem. 28:138.

Decker, E.A. and Hultin, H.O. 1990. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. J. Food Sci. 55:947.

Ellis, R., Currie, G.T., Thornton, F.E., Bollinger, N.C. and Gaddis, A.M. 1968. Carbonyls in oxidizing fat. II. The effect of the pro-oxidant activity of sodium chloride on pork tissue. J. Food Sci. 33:555.

Gill, J.L. 1978. "Design and Analysis of Experiments in the Animal and Medical Sciences." Iowa State Univ. Press, Ames, IA.

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. 1979. Influence of Heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27:838.

Kanner, J., Harel, S. and Jaffe. R. 1991. Lipid peroxidation of Muscle food as affected by NaCl. J. Agic. Food Chem. 39:101.

King, A.J. and Bosch, N. 1990. Effect of NaCl and KCl on rancidity of dark turkey meat heated by microwave. J. Food Sci. 55:1549.

Kuo, J.C. and Ockerman, H.W. 1985. Effects of rigor state, salt level and storage time on chemical and sensory traits of frozen and freeze-dried ground beef. J. Food Prot. 48:142.

Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and non-heme iron as prooxidants in cooked meat. J. Agric. Food Chem. 22:1032.

Ofstad, R., Kidman, S., Myklebust, R., Olsen, R.L. and Hermansson, A.M. 1995. Liquid-holding capacity and structural changes in comminuted salmon (Salmo Salar) muscle as influenced by pH, salt and temperature. Lebens. Wissen. Technol. 28:329.

Osinchak, J.E., Hultin, H.O., Zajicek, O.T., Kelleher, S.D. and Huang, C.H. 1992. Effect of NaCl on catalysis of lipid oxidation by the soluble fraction of fish muscle. Free Radical Biol. Med. 12:35.

Rhee , K.S., Smith, G.C. and Terrell, R.N. 1983. Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork. J. Food Prot. 46:578.

Rhee, K.S. and Ziprin, Y.A. 1987. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. J. Food Sci. 52:1174.

Rhee, K.S., Zibru, Y.A. and Ordonez, G. 1987. Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin-hydrogen peroxide, nonheme iron and enzyme systems. J. Agric. Food Chem. 35:1013.

Shantha, N.C. and Decker, E.A. 1994. Rapid, sensitive, iron-base spectrophotometric methods for determination of peroxide values of food lipids. J. AOAC Int. 77:421.

Srinivasan, S. and Xiong, Y.L. 1996. Sodium chloride-mediated lipid oxidation in beef heart surimi-like material. J. Agric. Food Chem. 44:1697.

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

Torres, E., Pearson, A.M., Gray, J.I., Booren, A.M. and Shimokomaki, M. 1988. Effect of salt on oxidative changes in pre- and post-rigor ground beef. Meat Sci. 23:151.

Velinov. P.D., Zhikov, M.V. and Cassens, R.G. 1990. The effect of tumbling, sodium chloride and polyphosphates on the microstructure and appearance of whole-muscle processed meats. Food Structure. 9:91.

Watts, B.M. and Peng, D. 1947. Lipoxidase activity of hog hemoglobin and muscle extract. J. Biol. Chem. 179:441.

Wettasinghe, M. and Shahidi, F. 1996. Oxidative stability of cooked comminuted lean pork as affected by alkali and alkali-earth halides. J. Food Sci. 61:1160.

CHAPTER III

THE EFFECTS OF CHLORIDE AND BROMIDE SALTS ON NON-HEME IRON CONCENTRATION AND LIPID OXIDATION IN A MODEL SYSTEM CONTAINING VARIOUS FORMS OF IRON

Abstract

The effects of NaCl, KCl, NaBr and KBr salts on non-heme iron release from hemoglobin and myoglobin and on recovery of added iron were studied in raw and cooked water-washed muscle fiber model systems. Lipid oxidation was monitored by the thiobarbituric acid-reactive substances (TBARS) method, and iron was determined using atomic absorption spectroscopy. The addition of various chloride and bromide salts to the model system containing heme-containing proteins increased TBARS values (1.58 for myoglobin alone, 4.02 for myoglobin + NaCl after 6 days). However, the addition of these salts had no effect on the release of non-heme iron from hemoglobin and myoglobin in either the raw or cooked samples (7.0 ug Fe/g meat for myoglobin alone, 7.2 ug Fe/g meat for myoglobin + NaCl after 2 days). The addition of various salts to the cooked model system containing 3 parts per million added iron produced greater TBARS values compared to the model systems containing added iron alone. They also did not significantly increase the non-heme iron concentration. Because sodium chloride promoted lipid oxidation without increasing the non-heme iron fraction in the model system. These data suggest that salt does not increase lipid oxidation by releasing iron from heme proteins.

Introduction

Sodium chloride has been reported to promote lipid oxidation in different species of meat (King and Bosch, 1990; Arnold et al., 1991; Kanner et al., 1991; Ahn et al. 1993a, b). For example, lipid oxidation in raw pork patties during frozen storage was rapidly accelerated by 1% salt concentration (Andersen and Skibsted, 1991). Raw turkey meat patties with 2% NaCl had greater TBARS values than turkey meat patties without NaCl (Ahn et al., 1993b). Recently, it has been suggested that the prooxidant mechanism of NaCl is to increase the free iron concentration by either breaking iron-protein macromolecular interactions (Kanner et al.,1991) or by releasing iron from high molecular weight (>10 kilodalton)proteins (Osinchak et al., 1992; Ahn et al., 1993c).

Kanner et al. (1991) suggested that when free iron ions were added to minced turkey dark muscle, a large part of the added iron interacted with protein macromolecules. This interaction prevented iron ions from contacting with membranous lipids and acting as a catalyst of lipid oxidation. Addition of NaCl interrupted the interaction between the iron and macromolecules. Therefore, more free iron ions were available to interact with the lipid fraction, thereby enhancing lipid oxidation. Osinchak et al. (1992) used a different model system (phosphatidylcholine liposomes) but a similar approach to study the prooxidant effect of NaCl. They confirmed that iron ions were involved in NaCl-mediated lipid oxidation. They also observed that using NaCl and a high molecular weight (>10 kilodalton) fraction of mackerel press juice as a prooxidant in the model system increased the amount of lipid oxidation 7-8 times compared to using the high molecular weight (>10 kilodalton) fraction of mackerel press juice alone. They hypothesized that iron was released from iron-containing protein in the high molecular weight fraction (>10

kilodalton) of mackerel muscle press juice when NaCl was present. The free iron subsequently catalyzed lipid oxidation in this model system.

Iron in the high molecular weight iron-containing proteins such as hemoglobin, myoglobin and ferritin account for 51 to 74% of the total iron in muscle depending on the type of muscle (Hazell, 1982). It has been reported that these proteins will release iron during cooking (Love and Pearson, 1974; Oellingrath, 1988; Buchowski et al., 1988) or during storage (Decker and Hultin, 1990). Cooking denatures the heme proteins, degrades the heme, and subsequently releases iron (Oellingrath, 1988). Salt may have a similar effect as heat in denaturing the heme-containing proteins (Kijowski and Mast, 1988; Ahn and Maurer, 1989). Salt can alter the stability of meat proteins, causing a reduction in the denaturation temperature (Quinn et al. 1980; Kijowski and Mast, 1988). Ahn and Maurer (1989) reported that NaCl will decrease the heat stability of hemoglobin and myoglobin.

Although Kanner et al. (1991) and Osinchak et al.(1992) suggested that the prooxidant effect of NaCl is to increase the availability of iron ions by either breaking the iron-protein macromolecule or releasing iron from the high molecular iron-containing proteins, there are no data available which verifies the source of free iron in a meat system when NaCl is present. The objective of this study is to verify the free iron source by examining the effects of various salts on iron release from hemoglobin and myoglobin as well as to study the recovery of added iron in a meat system. Specific objectives are: 1. To determine the effect of chloride and bromide salts on iron release from heme proteins in a water-washed muscle fiber model system. 2. To determine the effect of these salts on iron recovery in a water-washed muscle fiber model system. 3. To compare the effect of

chloride and bromide salts on non-heme iron release from heme proteins as well as the recovery of the added iron with lipid oxidation.

Materials and Methods

Pork legs were obtained from a local packer within 48 hr after slaughter. Bone and skin were removed from the legs the following day after arriving at Michigan State University. The boneless pork was stored at -10 °C before use. Bathophenanthroline disulfonic acid (sodium salt), hemoglobin, myoglobin, thioglycolic acid (96-99%), NaCl, KCl, NaBr and KBr were purchased from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid, ammonium acetate, nitric acid and perchloric acid were obtained from J.T. Baker Inc. (Phillipsburg, NJ). Sodium nitrite and hydrochloric acid were from purchased Mallinckrodt Inc. (Paris, KY). All chemicals were reagent grade. All the glassware used in this study was immersed overnight in a 4 N hydrochloric acid solution to remove potential trace iron and rinsed with distilled water to remove hydrochloric acid residues. Disposable plastic test tubes (Baxter Diagnostics Inc., McGraw Park, IL) were used instead of glass test tubes to prevent possible iron contamination.

Sample preparation

Experiment I

Water-washed muscle fibers (WF) were prepared according to the method of Hazell (1982)(Figure 3-1). One hundred twenty g WF, 12 ml 1.65M salt solution (NaCl, KCl, NaBr or KBr) and/or heme proteins (5 mg hemoglobin or myoglobin/g wet fiber) were mixed in a 250 ml beaker for 1.5 min then held refrigerated for 30 min. In order to more easily study the effect of NaCl on non-heme iron release from heme proteins, higher heme proteins than naturally exists in pork were used in this study. The target iron concentration in treatments containing heme proteins was 16.5 ug Fe/g meat (Hazell,

1982). The target salt concentration in each treatment that contained salt was 8.8 mg NaCl/g meat, 11.2 mg KCl/g meat, 15.5 mg NaBr/g meat and 17.9 mg KBr/g meat (equal to 0.15 M salt in meat). For the control, distilled water instead of a salt solution was added to the WF and handled in a similar fashion. Iron release and lipid oxidation were studied in both raw and cooked systems. The samples were cooked by placing the samples in a 250 ml beaker, covering with aluminum foil and heating to an internal temperature of 70 °C in a 83 °C water bath. Cooking time was approximately 50 min. All samples were stored at 4°C during the storage study.

Lipid oxidation was monitored by the 2-thiobarbituric acid procedure (Tarladgis et al., 1960) as modified by Crackel et al. (1988). Total iron in each sample was determined using the extraction method of Igene et al. (1979), which utilizes nitric acid and perchloric acid digestion followed by quantitation by atomic absorption spectroscopy. The non-heme iron fraction of each sample was prepared using the modified Schricker method (Rhee and Ziprin,1987) and quantitated using atomic absorption spectroscopy.

Experiment II

Hemoglobin or myoglobin solutions were prepared to target 16.5 ug Fe/g meat (approximately 5 mg hemoglobin or myoglobin per ml water). Different salts (NaCl, KCl, NaBr and KBr) were added to the heme protein solutions to reach a 17.4 mg salt/g meat. Samples (15 ml) were cooked in a 88-90 °C water bath to an internal temperature of 70 °C. For the hemoglobin stability test, 15 ml of the hemoglobin solution were cooked in a 88-90 °C water bath to internal temperatures of 75, 85 and 87.5 °C (Chen et al., 1984). The non-heme iron concentrations in the cooked heme proteins solution were quantitated by the method of Igene et al. (1979).

Experiment III

Three ug Fe/g meat was incorporated into the WF by mixing 120 g of WF with a 12 ml 33 ug Fe/g meat ferrous sulfate solution in a 250 ml beaker for 1.5 min. The samples were refrigerated for 30 min and cooked as described previously. Lipid oxidation was monitored by the TBARS method (Tarladgis et al., 1960) as modified by Crackel et al. (1988). Non-heme iron was extracted according to Igene et al. (1979) and quantitated using atomic absorption spectroscopy.

Data Analysis

The experiment was performed using a split-plot design with repeated measures (Gill, 1978). Means, standard errors, sum of squares and mean square errors were calculated using the MSTAT-C microcomputer statistical program (Michigan State University, 1989). The Bonferroni t test was used to compare selected contrasts.

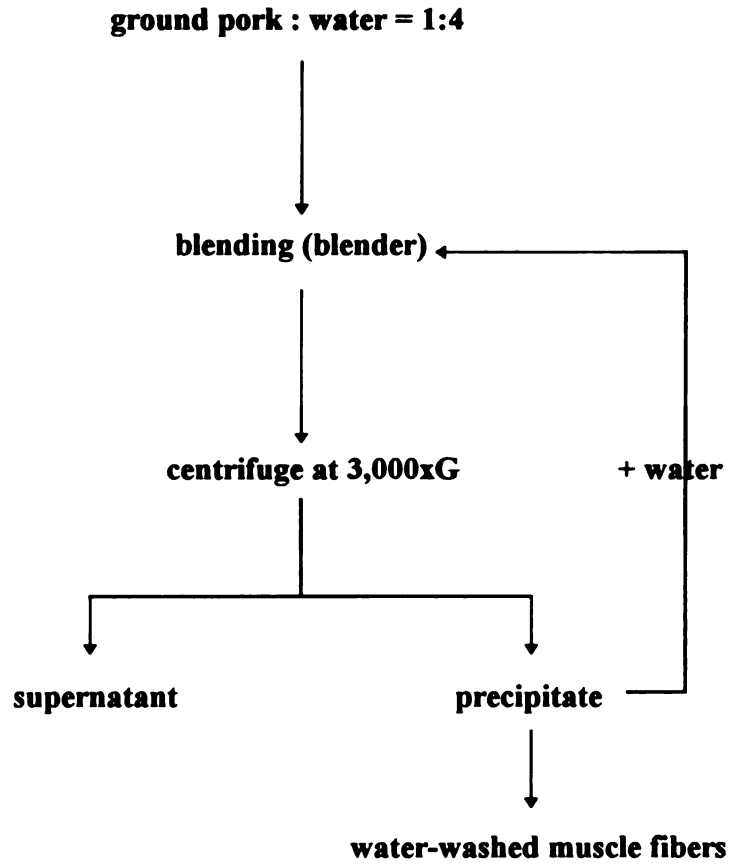

Figure 3-1 Water-washed muscle fibers preparation procedure

Results and Discussion

The effect of salt on non-heme iron concentrations in the WF system containing heme proteins

Water-washed muscle fibers systems were used to study the effect of salt on non-heme iron release from heme protein. This model system most closest resembles the meat system when comparing with other model systems such as liposome systems, emulsions of methyl linoleate and linoleic acid systems. Total and non-heme iron concentrations for the raw and cooked WF model systems in the presence of various salts and heme proteins are presented in Tables 3-1 and 2-2, respectively. Hemoglobin and myoglobin treatments had an average of 16.2 ug Fe/g meat more total iron than the WF (control). This is close to the 16.5 ug Fe/g meat which was targeted. Addition of hemoglobin and myoglobin increased ($p<0.05$) non-heme iron in raw samples at day 0 (Table 3-1). This observation is in agreement with Ahn et al. (1993c) who reported that the addition of hemoglobin to turkey breast meat increased the free iron in that system. They attributed the iron increase to the damaged heme proteins and which may

have released iron during the sample preparation procedure (Chen et al., 1984; Ahn et al., 1993c). There were no differences ($p<0.05$) in non-heme iron concentrations present in either the myoglobin or hemoglobin treatments in this study (Table 3-1). Neither salt addition nor different salts had any effect on the non-heme iron concentration in treatments containing either heme protein at day 0. This is consistent with data reported by Ahn et al (1993c) which indicated that NaCl does not affect the non-heme iron

Table3-1. Total and non-heme iron in a raw water-washed muscle fiber (WF) model system in the presence of selected heme proteins[1] or salts[2]

Treatment	Non-heme iron (ug Fe/g meat)			Total iron
	Day 0[3,4,5]	Day 3	Day 6	
1. WF (control)	4.5[a]	4.9[a]	4.0[a]	5.9[a]
2. WF + Mb (myoglobin)	6.5[b]	7.2[b]	5.1[b]	21.4[b]
3. WF + Mb + NaCl	6.2[b]	7.7[b]	6.7[b]	21.0[b]
4. WF + Mb + KCl	7.1[b]	7.7[b]	6.7[b]	20.9[b]
5. WF + Mb + NaBr	5.9[b]	6.9[b]	5.9[b]	20.7[b]
6. WF + Mb + KBr	6.4[b]	7.6[b]	6.4[b]	21.5[b]
7. WF + Hb (hemoglobin)	6.4[b]	7.9[b]	5.7[b]	22.4[b]
8. WF + Hb + NaCl	6.4[b]	7.3[b]	6.6[b]	22.5[b]
9. WF + Hb + KCl	7.0[b]	7.9[b]	6.1[b]	21.4[b]
10. WF + Hb + NaBr	5.9[b]	7.8[b]	6.5[b]	22.5[b]
11. WF + Hb + KBr	6.6[b]	7.2[b]	5.8[b]	21.9[b]

1. Hemoglobin and myoglobin solutions were prepared to target 16.5 ug Fe/g meat or 5 mg myoglobin or hemoglobin/g meat.
2. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg salt/g meat.
3. Samples were held at 4 °C.
4. Mean square error = 0.98.
5. Mean values within the same column with different superscripts are different ($p < 0.05$).

concentrations in raw turkey meat. After 6 days of refrigerated storage at 4 °C, salt did heme iron release from hemoglobin treatments was greater ($p<0.05$) than from myoglobin treatments in the cooked samples. This may be because hemoglobin is more heat sensitive than myoglobin (Lewis, 1926; Oellingrath, 1988). Oellingrath (1988) reported that hemoglobin-heme was more heat sensitive than myoglobin-heme between 78 °C and 100 °C. Therefore, more non-heme iron was released from hemoglobin than myoglobin in cooking. Neither salt addition nor different salts significantly increased the non-heme iron concentrations in the cooked treatments containing either hemoglobin or myoglobin during 2 days of refrigerated storage at 4 °C (Table 3-2). Ahn et al. (1993c) reported that with 2% NaCl, the amounts of non-heme iron in cooked turkey leg meat and mechanically deboned turkey meat were 3.5-4.0 μg/g greater than those without NaCl. They attributed the increase in non-heme iron to NaCl addition which destabilized the heme pigment and therefore released iron. The differences between the current study and the one performed by Ahn et al. (1993c) may be because a different meat system or cooking temperature were used. In the Ahn et al. (1993c) study, the experiment was conducted with turkey meat and samples cooked in a 350 °C oven to an internal temperature of 80 °C. In this study, WF from pork was used and samples were cooked in a 83 °C water bath to an internal temperature of only 70 °C. It is also possible that the increase in non-heme iron in Ahn's study came from sources other than the heme proteins such as the water-insoluble fraction of meat. In the Ahn et al. (1993c) study, the experiment was conducted in a meat system. No specific evidence was presented indicating that the non-heme iron increase was from heme pigments when NaCl was present. On the other hand, Decker et al. (1993) demonstrated that non-heme iron in the water-insoluble fraction of beef diaphragm

Table 3-2. Total and non-heme iron in a cooked[1] water-washed muscle fiber (WF) model system in the presence of selected heme proteins[2] or salts[3]

Treatment	Non-heme iron (ug Fe/g meat)			Total iron
	Day 0[4,5,6]	Day 1	Day 2	
1. WF (control)	5.0[a]	5.4[a]	6.1[a]	7.0[a]
2. WF + Mb (myoglobin)	6.7[b]	6.7[b]	7.0[b]	23.8[b]
3. WF + Mb + NaCl	6.4[b]	7.5[b]	7.2[b]	22.7[b]
4. WF + Mb + KCl	6.2[b]	7.6[b]	7.3[b]	22.7[b]
5. WF + Mb + NaBr	5.9[b]	7.3[b]	7.4[b]	21.7[b]
6. WF + Mb + KBr	6.7[b]	7.3[b]	7.6[b]	22.8[b]
7. WF + Hb (hemoglobin)	7.0[c]	8.2[c]	7.8[c]	23.0[b]
8. WF + Hb + NaCl	7.3[c]	8.3[c]	7.7[c]	23.4[b]
9. WF + Hb + KCl	7.1[c]	7.7[c]	8.5[c]	23.4[b]
10. WF + Hb + NaBr	7.7[c]	7.7[c]	8.4[c]	23.7[b]
11. WF + Hb + KBr	7.1[c]	8.1[c]	8.5[c]	22.7[b]

1. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
2. Hemoglobin and myoglobin solutions were prepared to target 16.5 ug Fe/g meat or approximately 5 mg myoglobin or hemoglobin/g meat.
3. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg salt/g meat .
4. Samples were held at 4 °C.
5. Mean square error = 0.86.
6. Mean values within the same column with different superscripts are different ($p < 0.05$).

muscle can be released and will catalyze lipid oxidation. Hazell (1982) indicated that water-insoluble iron accounted for 58% of the total iron in chicken. Carpenter et al. (1995) reported that about 57 % (7.4 ug Fe/g meat iron) of iron in turkey is non-heme iron. However, Kanner et al. (1988) reported that only a small portion of the non-heme iron in dark turkey meat is water-soluble (2.5 ug Fe/g meat iron). This study indicated that the majority of the non-heme iron in turkey is in the water-insoluble fraction and could be the source of the non-heme iron.

To confirm that NaCl did not accelerate the release of iron from heme-containing proteins in the WF model system, hemoglobin and myoglobin solutions (targeting 16.5 ug Fe/g water) were cooked with greater salt concentrations (17.4 mg salt/g meat) or temperatures (75, 85 and 87.5 °C). In previous studies ground pork significant differences were always found at this salt concentration. Both hemoglobin and myoglobin were stable in 17.4 mg NaCl/g meat during heating (Table 3-3). Salt and heating to 70 °C did not increase ($p<0.05$) non-heme iron release from these proteins (Table 3-3). When hemoglobin solutions targeting the same iron concentration were cooked to internal temperatures of 75, 85 and 87.5 °C in a 88-90 °C water bath, the corresponding non-heme iron concentrations were 0.52, 0.52 and 0.54 ug Fe/g meat, respectively. Non-heme iron analysis indicated that cooking at these temperatures and NaCl concentrations did not significantly increase non-heme iron concentrations. This study demonstrated that heme-containing proteins were stable at cooking temperatures and NaCl concentrations consistent with Oellingrath (1988). Oellingrath (1988) reported that the heme degradation in metmyoglobin was less than 25% during 2 hr of heating at 78 °C in a test tube. When heated to 100 °C for 2 hr, the reduction of heme in metmyoglobin was similar to the

Table 3-3. Effect of cooking[1] and different salts[2] on the iron release from heme[3] proteins in a model system stored at 4 °C for 2 days

	Non-heme iron (ug Fe/g meat)[4]	
Treatment	Raw[5]	Cooked
1. Mb (myoglobin)	0.3[a]	0.3[a]
2. Mb + NaCl	0.3[a]	0.3[a]
3. Mb + KCl	0.3[a]	0.4[a]
4. Mb + NaBr	0.3[a]	0.2[a]
5. Mb + KBr	0.3[a]	0.3[a]
6. Hb (hemoglobin)	1.2[b]	1.2[b]
7. Hb + NaCl	1.2[b]	1.2[b]
8. Hb + KCl	1.2[b]	1.4[b]
9. Hb + NaBr	1.1[b]	1.4[b]
10. Hb + KBr	1.2[b]	1.2[b]

1. The samples were placed into test tubes and cooked in a 88 °C water bath to an internal temperature of 70 °C.
2. NaCl, KCl, NaBr and KBr concentrations were 0.3mg salt/g meat .
3. Hemoglobin and myoglobin solutions were prepared to target 16.5 ug Fe/g meat or approximately 5 ml myoglobin or hemoglobin/ml water.
4. Mean square error = 0.02.
5. Mean values within the same column with different superscripts are different ($p < 0.05$).

reduction found at 78 °C, while methemoglobin solutions decreased about 35% after 2 hr of cooking at 100 °C. Janky and Froning (1973) reported that only 13.7 percent of the metmyoglobin in solution was denatured at 70 °C. This study indicated that the majority of heme-containing proteins were stable even when held at 100 °C for 2 hr.

One factor which may have an important impact on non-heme iron concentration in meat during cooking is the cooking rate (Chen et al., 1984; Buchowski et al., 1988). Chen et al. (1984) demonstrated that slow heating increased the amount of non-heme iron more than rapid heating in muscle pigment extracts. They suspected that heating rate may have changed coagulation of the myoglobin molecule in such a way that the heme iron could not be cleaved from the globin moiety. In this study, when the temperature in the hemoglobin solutions were raised to internal temperatures of 75, 85 and 87.5 °C in a 88-90 °C water bath in approximately 50 sec., there were no significant differences in the non-heme iron concentrations. These data were consistent with Chen et al (1984) who reported that rapid heating did not increase the concentration of non-heme iron. However, recently published research concerning the effect of the rate of cooking on the non-heme iron concentration has produced opposite results. Conforti and Giuffrida (1995) reported that the rate of cooking (slow vs fast cooking) did not have an effect ($p<0.05$) on the non-heme iron concentration of drumsticks or breasts.

Based on the cooking time, temperature and heating rate in our study, it is possible that heme-containing proteins were stable at these temperatures and cooking rate. However, these studies were conducted in a model system. There is always a limitation whether the data gathered from a simple model system can be representative of what really happens in the complex meat system. Many components in a meat system are not

present in the model system. For example, Schricker et al. (1982) reported that heating 3 g of beef for 20 min in a boiling water bath increased the non-heme iron concentration of the fresh beef muscle from 9.9 to 20.9 μg/g and that of aged beef muscle from 11.5 to 16.0 μg/g. The cooking conditions (100 °C for 20 min) in Schricker's study was less severe than the cooking conditions (100 °C for 2 hr) in Oellingrath's study. However, the non-heme iron concentration increase in a meat system (Schricker et al., 1982) is far greater than the increase in a model system (Oellingrath, 1988). Oellingrath (1988) reported that the denaturation temperatures for myoglobin in meat systems (Slinde, 1987) were lower than those determined in the highly diluted myoglobin solution model systems. He attributed the difference in the myoglobin denaturation temperature to the presence of other less thermally stable proteins in meat which may destabilize the hemeproteins due to interaction between denatured protein and undenatured myoglobin (Ledward, 1978). Han et al. (1995) reported that ascorbic acid, which was not present in the heme protein model system, caused iron release from hemoglobin. They reported that after 5 hr incubation at 37 °C, about 4.7% of total heme iron had been liberated from 3.2 mM hemoglobin by 5.0 mM ascorbic acid.

The non-heme iron concentrations in cooked samples were greater ($p<0.05$) than raw samples (Tables 3-1 vs. 3-2). It has been suggested by other researchers that cooking will denature the heme proteins, degrade the heme and subsequently release iron (Igene et al., 1979; Chen et al., 1984; Oellingrath, 1988). Buchowski et al. (1988) studied the effect of heating (60, 77 and 97 °C) on the iron distribution between meat and broth. They reported that total, heme and non-heme iron concentrations in cooked beef samples all increase after cooking. For the beef samples cooked to 97 °C, heme iron concentrations

were greater than raw samples. Since heme structure will be degraded and subsequently release free iron during the cooking process, it is not likely that heme iron concentration in the cooked sample will be higher than the raw sample unless food components were lost. The amount of total iron in meat is fixed and the total iron concentration is unlikely to increase during cooking unless some components are lost during cooking. Therefore, the increase in total, heme and non-heme iron in cooked samples in the Buchowski et al. (1988) study probably was a concentration effect due to water and/or lipid losses during the cooking process. Because cooking losses are a problem in muscle samples, it is hard to determine which portion of the non-heme iron increase is due to heme degradation and which is attributed to a concentration effect. In this study because of the relative stability of heme iron in hemoglobin and myoglobin during cooking and because water loss occurred during the cooking process, the effect of cooking on the heme degradation and non-heme iron release is small.

The effect of salt on lipid oxidation in the presence of heme iron

The effect of chloride and bromide salts on lipid oxidation in raw and cooked treatments containing heme proteins is presented in Tables 3-4 and 3-5. For raw samples, the WF (control) had similar TBARS values during the 6 day refrigerated storage period. There were no differences between heme protein and salt treatments on day 0. After 3 days storage, all treatments containing myoglobin and hemoglobin had greater ($p<0.05$) TBARS values than the control. These data are consistent with Johns et al. (1989) who reported that heme proteins are prooxidants in meat. Treatments containing myoglobin and various salts had greater ($p<0.05$) TBARS values than treatments containing

myoglobin alone (Table 3-4). However, there were no significant differences in non-heme iron concentration between myoglobin treatments and treatments containing myoglobin and various salts (Table 3-1). This indicates that NaCl did not promote lipid oxidation in the model system by releasing free iron from heme pigments. Other mechanisms are involved.

For the cooked samples (Table 3-5), there were no differences between these treatments at day 0. At day 2 of refrigerated storage, hemoglobin/salt or myoglobin/salt treatments had greater ($p<0.05$) TBARS values than hemoglobin or myoglobin treatments. This is consistent with Ahn et al. (1993b) who reported that treatments containing hemoglobin and NaCl had greater TBARS values than the hemoglobin only treatments. As was consistent with raw samples, the cooked samples had no significant differences in the non-heme iron concentrations between treatments containing heme proteins and treatments containing heme proteins and various salts (Table 3-2).

Effect of salt on non-heme iron recovery and lipid oxidation in a model system

Total iron in the raw samples containing added iron was approximately 3.5 ug Fe/g meat greater than the control (Table 3-6). It was close to the target added iron level of 3 ug Fe/g meat. At day 0, the non-heme iron recovery was 2.6 ug Fe/g meat (74%). The addition of NaCl or other salts did not significantly ($P < 0.05$) affect the recovery of added iron in the model system. This observation is consistent with Ahn et al.(1993b) who reported that added NaCl had no effect on the non-heme iron concentrations in treatments with different levels of added iron. A similar non-significant recovery was found after 6 days of refrigerated storage would be expected. The effect of chloride and bromide salts

Table 3-4. The effect of heme proteins[1] and different salts[2] on TBARS values in a raw water-washed muscle fiber (WF) model system

	TBARS (mg malonaldehyde/Kg meat)		
Treatment	Day 0[3,4,5]	Day 3	Day 6
1. WF (control)	0.69[a]	0.78[a]	0.67[a]
2. WF + Mb (myoglobin)	0.84[a]	1.59[b]	1.58[b]
3. WF + Mb + NaCl	0.87[a]	2.89[c]	4.02[c]
4. WF + Mb + KCl	0.93[a]	3.10[c]	3.43[c]
5. WF + Mb + NaBr	0.85[a]	2.57[c]	2.19[c]
6. WF + Mb + KBr	0.86[a]	2.22[c]	2.44[c]
7. WF + Hb (hemoglobin)	0.80[a]	1.00[d]	1.03[d]
8. WF + Hb + NaCl	0.79[a]	1.06[d]	1.23[d]
9. WF + Hb + KCl	0.78[a]	0.94[d]	1.11[d]
10. WF + Hb + NaBr	0.78[a]	0.94[d]	0.90[d]
11. WF + Hb + KBr	0.83[a]	1.09[d]	0.90[d]

1. Hemoglobin and myoglobin solutions were prepared to target 16.5 ug Fe/g meat or approximately 5 ml myoglobin or hemoglobin/ml water.
2. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg salt/g meat .
3. Samples were held at 4 °C.
4. Mean square error = 0.37.
5. Mean values within the same column with different superscripts are different ($p < 0.05$).

Table 3-5. The effect of heme proteins[1] and different salts[2] on TBARS values in a cooked[3] water-washed muscle fiber (WF) model system

	TBARS (mg malonaldehyde/Kg meat)		
Treatment	Day 0[4,5,6]	Day 1	Day 2
1. WF (control)	0.46[a]	0.55[a]	0.66[a]
2. WF + Mb (myoglobin)	0.38[a]	0.59[a]	0.81[b]
3. WF + Mb + NaCl	0.33[a]	0.79[b]	1.59[c]
4. WF + Mb + KCl	0.44[a]	0.82[b]	1.49[c]
5. WF + Mb + NaBr	0.42[a]	0.90[b]	1.68[c]
6. WF + Mb + KBr	0.43[a]	0.90[b]	1.69[c]
7. WF + Hb (hemoglobin)	0.35[a]	0.62[a]	0.80[b]
8. WF + Hb + NaCl	0.36[a]	0.80[b]	1.30[c]
9. WF + Hb + KCl	0.31[a]	0.72[b]	1.13[c]
10. WF + Hb + NaBr	0.35[a]	0.89[b]	1.41[c]
11. WF + Hb + KBr	0.33[a]	0.95[b]	1.59[c]

1. Hemoglobin and myoglobin solution were prepared to target 16.5 ug Fe/g meat or approximately 5 ml myoglobin or hemoglobin /ml water.
2. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg salt/g meat .
3. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
4. Samples were held at 4 °C.
5. Mean square error = 0.24.
6. Mean values within the same column with different superscripts are different ($p < 0.05$).

Table 3-6. The effect of chloride and bromide salts[1] on the recovery of added iron[2] in a raw water-washed muscle fiber (WF) model system

	Non-heme iron(ug Fe/g meat)			Total iron
Treatment	Day 0[3,4,5]	Day 3	Day 6	
1. WF	2.2[a]	2.0[a]	2.1[a]	5.3[a]
2. WF + Fe	4.8[b]	4.0[b]	5.1[b]	9.1[b]
3. WF + Fe + NaCl	4.2[b]	4.1[b]	4.5[b]	8.7[b]
4. WF + Fe + KCl	4.4[b]	3.9[b]	4.4[b]	8.8[b]
5. WF + Fe + NaBr	4.1[b]	3.6[b]	4.8[b]	8.8[b]
6. WF + Fe + KBr	3.6[b]	4.0[b]	4.2[b]	8.5[b]

1. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg/g meat .
2. $FeSO_4$ was added to target 3ug Fe/g meat.
3. Samples were held at 4 °C.
4. Mean square error = 0.13.
5. Mean values within the same column with different superscripts are different ($p < 0.05$).

on the recovery of the added iron in cooked samples (Table 3-7) was similar to that for raw samples (Table 3-6). Kanner et al. (1991) suspected that when free iron ions are added to the WF, the iron may be either bound to protein macromolecules or may be somehow trapped in the WF system. They proposed that the prooxidant effect of NaCl is to release free ionic iron from iron-binding macromolecules. Because the addition of NaCl or other salts do not increase the recoveries of added iron in the model system, the ability of NaCl to release free iron from iron-protein macromolecular interaction was not demonstrated in this model system. These data provide indirect evidence which indicates that the Kanner et al. (1991) theory is not likely. This study was conducted using a model system which is limited to predict what really happens in a meat system as discussed previously. On the other hand, all evidence which supports Kanner's hypothesis was indirect. Additional direct evidence is needed to prove Kanner's hypothesis. Kanner et al. (1991) reported that in preparation of the WF, the amount of chelatable iron in the eluent extracted from muscle by 0.3 mg NaCl/g meat was double that extracted by distilled water. They suggested that because 0.3 M NaCl solution washed out more chelatable iron than distilled water, it was possible that NaCl can more efficiently extract the added iron from minced muscles. However, they did not verify whether the greater amounts of chelatable iron in the eluent was because NaCl broke the iron-protein macromolecular interaction in the meat system first and subsequently released iron into the eluent or because the iron-protein macromolecular complexes were washed out by NaCl first and the iron in the complexes was released by the chelating agent later.

There were no significant differences in TBARS values for raw samples (Table 3-8) at day 0. After 3 days of refrigerated storage treatments containing added iron had

Table 3-7. The effect of chloride and bromide salts[1] on the recovery of added iron[2] in a cooked[3] water-washed muscle fiber (WF) model system

Treatment	Non-heme iron (ug Fe/g meat) Day 0[4,5,6]	Day 1	Day 2	Total iron
1. WF	2.1[a]	2.1[a]	2.2[a]	6.9[a]
2. WF + Fe	4.6[b]	5.3[b]	5.9[b]	11.0[b]
3. WF + Fe + NaCl	4.8[b]	5.4[b]	5.0[b]	11.4[b]
4. WF + Fe + KCl	4.1[b]	4.1[b]	4.5[b]	11.2[b]
5. WF + Fe + NaBr	5.0[b]	4.8[b]	5.3[b]	12.6[b]
6. WF + Fe + KBr	4.2[b]	4.0[b]	3.8[b]	10.9[b]

1. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg/g meat .
2. FeSO4 was added to target 3ug Fe/g meat in samples.
3. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
4. Samples were held at 4 °C.
5. Mean square error = 0.11.
6. Mean values within the same column with different superscripts are different ($p < 0.05$).

greater ($p<0.05$) TBARS values than the control. This is consistent with Sato and Hegarty (1971) who suggested that non-heme iron promotes lipid oxidation. After 6 days storage, iron/salt treatments were similar to iron-only treatments. In the cooked model system (Table 3-9), similar trends were found except that iron treatments with salt had significantly greater ($p<0.05$) TBARS values than the treatment with only added iron after 2 days of refrigerated storage. However, the non-heme iron concentrations in the iron/salt treatments were not significantly greater than the iron only treatments (Tables 3-7). These results again confirm that NaCl promotes lipid oxidation by mechanisms other than by increasing the non-heme iron concentration in meat.

Kanner et al. (1991) reported that increasing the concentration of NaCl enhanced lipid oxidation in raw minced muscle, especially after a freeze-thaw cycle. They suggested that NaCl and freeze-thaw cycles may cause fusion of the intracellular compounds and the destruction of the cell structure which further enhances lipid oxidation (Shomer et al., 1987). However, the major focus of their paper is that the prooxidant effect of NaCl is to increase the availability of iron ions during lipid oxidation. The important impact of NaCl on structure integrity related lipid oxidation had not been emphasized. It is generally believed that lipid oxidation in muscle foods is initiated in the highly unsaturated phospholipid fraction in subcellular membranes (Gray and Pearson, 1987). Any disruption of muscle membrane integrity by mechanical deboning, mincing, restructuring, or cooking alters cellular compartmentalization. These processes will increase the chance of interaction between prooxidant and unsaturated fatty acids and cause the increase of lipid oxidation. Chang and Watts (1950) suggested that salt may affect the physical state of meat in such a way that hemoglobin would be brought into closer contact with the fat and

Table 3-8. The effect of iron[1] and chloride and bromide salts[2] on TBARS values in a raw water-washed muscle fiber (WF) model system

Treatment	TBARS (mg malonaldehyde/Kg meat)		
	Day 0[3,4,5]	Day 3	Day 6
1. WF	0.60[a]	0.75[a]	0.78[a]
2. WF + Fe	0.93[a]	1.61[b]	1.67[b]
3. WF + Fe + NaCl	0.86[a]	1.61[b]	1.94[b]
4. WF + Fe + KCl	0.88[a]	1.63[b]	1.85[b]
5. WF + Fe + NaBr	0.86[a]	1.53[b]	1.72[b]
6. WF + Fe + KBr	0.86[a]	1.48[b]	1.62[b]

1. FeSO4 was added to target 3ug Fe/g meat in samples.
2. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg/g meat .
3. Samples were held at 4 °C.
4. Mean square error = 0.03.
5. Mean values within the same column with different superscripts are different ($p < 0.05$).

Table 3-9. The effect of iron[a] and chloride and bromide salts[b] on TBARS values in cooked[c] water-washed muscle fiber (WF) model system

	TBARS (mg malonaldehyde/Kg meat)		
Treatment	Day 0[4,5]	Day 1	Day 2
1. WF	1.19[a]	1.57[a]	1.73[a]
2. WF + Fe	1.89[b]	2.79[b]	3.07[b]
3. WF + Fe + NaCl	2.17[b]	3.24[b]	3.64[c]
4. WF + Fe + KCl	2.09[b]	3.21[b]	3.53[c]
5. WF + Fe + NaBr	2.17[b]	3.41[b]	3.37[c]
6. WF + Fe + KBr	2.07[b]	3.23[b]	3.68[c]

1. FeSO4 was added to target 3ug Fe/g meat in samples.
2. NaCl, KCl, NaBr and KBr concentrations were 8.8 mg/g meat .
3. The cooked samples were heated to an internal temperature of 70 °C in a 83 °C water bath.
4. Samples were held at 4 °C.
5. Mean square error = 0.03.

subsequently promote lipid oxidation. This study demonstrated that salt can promote lipid oxidation in the model system containing added iron (Tables 3-9) or heme proteins (Tables 3-4 and 3-5) without increasing the non-heme iron concentrations in the system. However, salt alone, without another prooxidant such as iron or heme proteins, will not promote lipid oxidation in the water-washed model system (Kanner et al., 1991). This demonstrates the importance of a catalyst such as free iron or heme proteins for the prooxidant effect of NaCl to occur. Since NaCl can promote lipid oxidation without increasing the non-heme iron concentration in the system, we believe the major function of NaCl in lipid oxidation is to reduce the integrity of muscle tissue instead of making more iron available to catalyze lipid oxidation (Kanner et al., 1991). Therefore, lipids are more accessible for the attack of the prooxidants (free iron). However, more research is needed to verify this theory.

Summary and Conclusions

The effects of chloride and bromide salts on non-heme iron release from hemoglobin and myoglobin and on the recovery of the added iron were studied in a raw and cooked WF model system. The addition of selected chloride and bromide salts did not increase the release of the non-heme iron from either hemoglobin or myoglobin in this model. Various salts also did not increase the recovery of the added iron. However, the addition of the same salts to the treatments with heme proteins or added iron significantly increased lipid oxidation. This indicates that NaCl does not promote the lipid oxidation by increasing the non-heme iron concentration in meat. Other mechanisms must be proposed to explain why NaCl is a prooxidant in this model system.

References

Ahn, D.U. and Maurer, A.J. 1989. Effects of sodium chloride, phosphate, and dextrose on the heat stability of purified myoglobin, hemoglobin, and cytochrome c. Poultry Sci. 68:1218.

Ahn, D.U, Wolfe, F.H. and Sim, J.S. 1993a. Prevention of lipid oxidation in pre-cooked turkey meat patties with hot packaging and antioxidant combinations. J. Food Sci. 58:283.

Ahn, D.U., Ajuyah, A., Wolfe, F.H. and Sim, J.S. 1993b. Oxygen availability affects prooxidant catalyzed lipid oxidation of cooked turkey patties. J. Food Sci. 58:278.

Ahn, D.U., Wolfe, F.H. and Sim, J.S. 1993c. Three methods for determining nonheme iron in turkey meat. J. Food Sci. 58:288.

Andersen, H.J. and Skibsted, L.H. 1991. Oxidative stability of frozen pork patties. Effect of light and added salt. J. Food Sci. 56:1182.

Arnold, A.R., Bascetta, E. and Gunstone, F.D. 1991. Effect of sodium chloride on prooxidant activity of copper (II) in peroxidation of phospholipid liposomes. J. Food Sci. 56:571.

Buchowski, M.S., Mahoney, A.W., Carpenter, C.E. and Cornforth, D.P. 1988. Heating and the distribution of total and heme iron between meat and broth. J. Food Sci. 53:43.

Carpenter, C.E. and Clark, E. 1995. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. J. Agric. Food Chem. 43:1824.

Chang, I. and Watts, B.M. 1950. Some effects of salt and moisture on rancidity of fat. Food Res. 15:313.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H. and Ku, P.K. 1984. Some factors influencing the nonheme iron content of meat and its implications in oxidation. J. Food Sci. 49:581.

Conforti, F.D. and Giuffrida, M.L. 1995. Determination of oxidative changes in precooked chicken parts by nonheme iron content and thiobarbituric acid value. Poultry Sci. 74:1224.

Crackel, R.L., Gray, J.I., Pearson, A.M., Booren, A.M. and Buckley, D.J. 1988. Some further observations on the TBARS test as an index of lipid oxidation in meats. Food Chem. 28:138.

Decker, E.A., Crum, A.D., Shantha, N.C. and Morrissey, P.A. 1993. Catalysis of lipid oxidation by iron from an insoluble fraction of beef diaphragm muscle. J. Food Sci. 58:233.

Decker, E.A. and Hultin, H.O. 1990. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. J. Food Sci. 55:947.

Gill, J.L. 1978. "Design and Analysis of Experiments in the Animal and Medical Sciences." Iowa State Univ. Press, Ames, IA.

Gray, J.I. and Pearson, A.M. 1987. Rancidity and warmed-over flavor. Adv. Meat Res. 3:221.

Han, D., McMillin, K.W., Godber, J.S., Bidner, T.D., Younathan, M.T. and Hart, L.T. 1995. Lipid stability of beef model systems with heating and iron fractions. J. Food Sci. 60:599.

Hazell, T. 1982. Iron and zinc compounds in the muscle of meats of beef, lamb, pork and chicken. J. Sci. Food Agric. 33:1049.

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. 1979. Influence of Heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27:838.

Janky, D.M. and Froning, G.W. 1973. The effect of pH and certain additives on heat denaturation of turkey meat myoglobin. Poultry Sci. 52:152.

Johns, A.M., Birkinshaw, L.H. and Ledward, D.A. 1989. Catalysts of lipid oxidation in meat products. Meat Sci. 25:209.

Kanner, J., Hazan, B. and Doll, L. 1988. Catalytic "free" iron ions in muscle foods. J. Agric. Food Chem. 36:412.

Kanner, J., Harel, S. and Jaffe. R. 1991. Lipid peroxidation of Muscle food as affected by NaCl. J. Agric. Food Chem. 39:101.

Kijowski, J.M. and Mast, M.G. 1988. Effect of sodium chloride and phosphates on the thermal properties of chicken meat proteins. J. Food Sci. 53:367.

King, A.J. and Bosch, N. 1990. Effect of NaCl and KCl on rancidity of dark turkey meat heated by microwave. J. Food Sci. 55:1549.

Ledward, D.A. 1978. Scanning calorimetric studies of some protein-protein interactions involving myoglobin. Meat Sci. 2:241.

Lewis, P.S. 1926. The kinetics of protein denaturation. Part 1. The effect of variation in the hydrogen ion concentration on the velocity of the heat denaturation of oxyhaemoglobin. Biochem. J. 20:965.

Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and non-heme iron as prooxidants in cooked meat. J. Agric. Food Chem. 22:1032.

Oellingrath, I.M. 1988. Heat degradation of heme in methemoglobin and metmyoglobin model systems measured by reversed-phase ion-pair high performance liquid chromatography. J. Food Sci. 53:40.

Osinchak, J.E., Hultin, H.O., Zajicek, O.T., Kelleher, S.D. and Huang, C.H. 1992. Effect of NaCl on catalysis of lipid oxidation by the soluble fraction of fish muscle. Free Radical Biology & Medicine. 12:35.

Quinn, J.R., Raymond, D.P. and Harwalkar, V.R. 1980. Differential scanning calorimetry of meat proteins as affected by processing treatment. J. Food Sci. 45:1145.

Rhee, K.S. and Ziprin, Y.A. 1987. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. J. Food Sci. 52:1174

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

Schricker, B.R., Miller, D.D. and Stouffer, J.R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47:740.

Slinde, E. 1987. Color of blank salami sausage. Dissociation of heme from myoglobin and hemoglobin. J. Food Sci. 52:1152.

Shomer, I., Weinberg, Z.G. and Vasilever, R. 1987. Structural binding properties of silver carp muscle affected by NaCl and CaCl2 treatments. Food Microstruct. 6:199.

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

SUMMARY AND CONCLUSIONS

A series of studies was conducted to investigate the prooxidant mechanism of NaCl, with focus on the effect of NaCl on non-heme iron concentrations in ground pork and in a WF model system as well as its relationship to lipid oxidation. Our hypothesis was that the prooxidant mechanism of NaCl in meat is primarily to make more iron ions available to promote lipid oxidation. This is done by: 1. Facilitating the release of iron ions from iron-containing proteins (hemoglobin and myoglobin); and/or 2. Breaking protein-iron interactions in a meat system.

Three methods for quantitating non-heme iron were evaluated to better understand why these methods of analysis produce different values for non-heme iron when analyzing the same sample. The extraction procedures for separating non-heme and heme iron were responsible for the differences in non-heme iron values. The quantitation procedures for these methods produced non-heme iron values which were not different from each other. The data presented in this study indicated that the ferrozine method may not completely recover added iron from ground pork. It was also recommended that the Igene method should not be used because of its poor iron recovery. The modified Schricker's method

was found to be the most effective procedure to completely extract non-heme iron from meat.

The effects of NaCl on the non-heme iron concentrations and lipid oxidation were studied in ground pork to verify that the prooxidant effect of NaCl in meat is to make more iron ions available to catalyze lipid oxidation. Increasing NaCl concentrations increased lipid oxidation and non-heme iron concentrations in both raw and cooked samples. Non-heme iron analyses indicated significant ($p<0.05$) increases in non-heme iron content at day 6 for raw treatments and day 0 for the cooked treatments. NaCl, KCl, NaBr and KBr addition resulted in similar effects with respect to lipid oxidation and non-heme iron release. However, the exact cause for the non-heme iron increase was not determined. It is possible that the increase in non-heme iron is caused either by a concentration effect during cooking or by the addition of salt.

If NaCl causes non-heme iron increases in the meat, the next step is to examine other possible iron sources such as ferritin and water-insoluble iron. A new non-heme iron measurement method must be developed to resolve this question. The ferrozine and modified Schricker methods are not capable of identifying whether non-heme iron release is due to the effect of salt because most of the iron in the ferritin will be extracted because of the extraction procedure used in the methods studied in our first study.

The effects of NaCl on the non-heme iron released from the possible non-heme iron protein sources (hemoglobin and myoglobin) and on the recovery of the added iron in a WF model system were studied. The addition of chloride and bromide salts had no effect on the release of non-heme iron from hemoglobin and myoglobin in either the raw and cooked samples in this model system. These salts had no effect on the recovery of the

added iron. However, the addition of the salts to the treatments with heme proteins or added iron significantly increased lipid oxidation compared to treatments with heme proteins or added iron alone. This indicates that NaCl can promote the lipid oxidation without increasing the non-heme iron concentration in meat.

It was our hypothesis that the prooxidant effect of NaCl in meat is primarily to make more iron ions available to catalyze lipid oxidation. In this study, the addition of NaCl in ground pork caused small increases in the non-heme iron concentration in ground pork. However, in an uncooked system this increase in the non-heme iron concentration was only significant after 6 days of refrigeration. The exact cause for the non-heme iron increases in ground pork (both cooked and uncooked studies) is still not determined. It is possible that the non-heme iron increase is caused by the addition of salt. However, it is also possible that it is the concentration effect during cooking which causes the non-heme iron increase. Data from the WF model system indicated that various salts had no effect on the non-heme iron release from heme proteins. Salt promotes lipid oxidation without increasing non-heme iron concentrations in the WF model system. Therefore, increases in non-heme iron availability in the meat system may not be a major pathway and can not be solely responsible for the dramatic increase in lipid oxidation when NaCl was present. Our data indicate it is likely that another prooxidant mechanism for NaCl exists. However, this study only provides these observations in a model system and does not provide similar observations in meat. It is unlikely that the effect of salt in meat will produce different results. However, further research is needed to confirm this.

Based on this study, we suggest that the major function of NaCl in lipid oxidation is to make muscle tissue more vulnerable for the attack of the prooxidants (free iron or

heme proteins). It is likely that free iron ion, heme proteins or other prooxidants already exist in the meat system. When NaCl is added to the meat, the system is more susceptible lipid oxidation due to microstructure changes. Therefore, even a small amount of free iron will catalyze lipid oxidation because the lipids are more susceptible to oxidative change. This salt-mediated lipid oxidation is the result of synergism between meat microstructure damage and the presence of catalysts such as iron.

FUTURE RESEARCH

1. To further study the effect of NaCl on the non-heme iron concentration in the meat system.
    - To determine whether the non-heme iron concentration increase in ground pork is caused by concentration effects due to moisture loss during cooking or by the addition of salt.
    - To develop a new procedure for measuring non-heme iron which detects the release of iron from all iron sources including ferritin and water-insoluble fractions of meat.
    - To determine the effect of NaCl on the release of non-heme iron from ferritin or the water-insolube fraction of meat.
2. To verify the hypothesis that the prooxidant effect of NaCl is due to muscle microstructure damage and the presence of catalysts such as free iron.

3. To re-evaluate the mechanism of cooking on the lipid oxidation.
    - To emphasize non-heme iron release in the meat system when cooking losses are constant.
    - To determine the effect of cooking on the release of free iron from the ferritin and water-insoluble fractions of meat.
    - To study the effect of heat-activated heme protein as a catalyst for lipid oxidation in both model systems and meat systems.
    - To study the effect of loss of membrane integrity losses due to heating on lipid oxidation.

APPENDICES

APPENDIX A

Modified Schricker non-heme iron procedure (Rhee and Ziprin, 1987)

1. Five grams of very fine ground meat (in triplicate) were weighed into test tubes with screw caps.

2. The meat was mixed thoroughly in each tube with 0.4 ml $NaNO_2$ reagent (156 ug $NaNO_2$/g meat based on the meat weight) and 5 ml distilled water.

3. Allow to equilibrate for 30 min.

4. Fifteen ml of the acid mixture [HCL (6N):trichloroacetic acid (40%) = 1:1] were added to each tube and the tube was tightly stoppered.

5. The tubes were incubated in a water bath-shaker at 65 °C for 20 hr and then cooled to room temperature in cool water.

6. Allow the tubes to settle until the supernatant become clear.

7. One ml of the acidic liquid supernatant was transferred to a small test tube and 5 ml bathophenanthroline disulfonate reagent solution added.

8. The absorbance of the supernatant was read at 540 nm against the reagent blank (1 ml acid mixture + 5 ml color reagent).

APPENDIX B

Ferrozine non-heme iron procedure (Carter, 1971)

1. Three grams of ground meat (in duplicate) and 9 ml 0.1 M citrate-phosphate buffer were put into 50 ml centrifuge tube.
2. Homogenize using a polytron for 2 seconds at top speed.
3. Added 4 ml 2% ascorbic acid solution.
4. Incubate for 15 min. at room temperature.
5. Add 8 ml trichloroacetic acid solution and mix thoroughly.
6. Centrifuge at 3000 G for 10 min.
7. Transfer 5 ml supernatant into test tube.
8. Add 2 ml buffer, 0.5 ml ferrozine reagent solution (0.3% ferrozine + 0.3% neocuproine) and mix thoroughly.
9. Allow to stand for 5 min.
10. The absorbance of the supernatant was read at 563 nm.

APPENDIX C

Igene non-heme iron procedure (Igene et al., 1979)

1. Eight grams of ground meat and distilled water (in duplicate) were weighed into test tubes.
2. Add 4 ml 2% EDTA solution.
3. Homogenize using a polytron for 2 seconds at top speed.
4. Added 4 ml 12.5% trichloroacetic acid.
5. Centrifuged at 3000 G for 10 min.
6. Transfer the supernatant to another test tube.
7. Measure free iron using atomic absorption spectroscopy

APPENDIX D

Total iron analysis procedure

1. 3 gram of ground pork were placed in a flask.

2. 3 ml perchloric acid and 15 ml nitric acid were added to the flask.

3. The sample was digested on a heat plate.

4. Dilute the digested solution to an approximate 1 to 10 ug Fe/g solution range.

5. Measure the iron concentration in atomic absorption spectroscopy.

APPENDIX E

The recovery of added iron from raw ground pork using three procedures for determining non-heme iron.

	Non-heme iron (ug Fe/g meat)[a]			Recovery (%)[b]		
	Igene	Ferrozine	modified Schricker	Igene	Ferrozine	modified Schricker
pork (control)	1.1±0.2	2.6±0.3	3.9±0.3			
pork (3 ug Fe/g meat)	2.9±0.5	5.3±0.2	7.4±1.3	61±16	89±8	117±35
pork (6 ug Fe/g meat)	3.8±0.3	8.2±0.3	10.0±0.4	46± 3	92±5	102±2
pork (9 ug Fe/g meat)	4.2±0.3	10.4±0.4	13.4±1.1	35± 2	86±3	106±9

a.Mean ± standard deviation (n =3)

b.Recovery (%) = (measured non-heme iron in treatment - control) x 100/added non-heme iron

APPENDIX F

The recovery of added iron from cooked[a] ground pork using three procedures for determining non-heme iron

	Non-heme iron (ug Fe/g meat)[b]			Recovery (%)[c]		
	Igene	Ferrozine	modified Schricker	Igene	Ferrozine	modified Schricker
pork (control)	2.2±0.6	4.0±0.3	5.1±0.4			
pork (3 ug Fe/g meat)	3.4±0.9	7.1±0.5	8.7±0.2	41±11	104±15	119±16
pork (6 ug Fe/g meat)	4.2±1.3	9.9±0.9	12.4±0.6	33±13	98±18	122±13
pork (9 ug Fe/g meat)	4.8±1.6	12.4±0.9	15.2±1.0	29±12	94±10	111±15

a. The cooked samples were heated to internal temperature to 70C in a water bath (temperature = 83 C).

b. Mean ± standard deviation (n =3)

c. Recovery (%) = (measured non-heme iron in treatment - control) x 100/added non-heme iron