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**Metals and O₂ in Initiation and Prevention of
Lipid Oxidation in Muscle Tissue**

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C. ABSTRACT: Metals and O_2 in Initiation and Prevention of Lipid Oxidation in Muscle Foods.

The research characterized and identified iron ions as the main initiator of muscle lipid peroxidation of turkey and fish. Levels of the transition metals, iron and copper, in various muscle fractions, were determined. "Free" iron ions seems to derive from ferritin, which releases iron ions during post-mortem storage. In order to release iron and activate it, ferric ions should be reduced to ferrous form. Methods to determine total reducing compounds in turkey and fish muscle tissues were developed. The amount of the reducing compounds in muscle tissues are in the range of 200-400 μM eq. to ascorbic acid. These reducing compounds accelerate lipid peroxidation dependent on iron ions, however inhibit those of H_2O_2 -activated myoglobin.

Results showed that the driving force of iron ions dependent of muscle lipid peroxidation are not only non-enzymatic but also enzymatic. Characterization of the major enzymic iron-reducing systems in fish and turkey muscle tissues were evaluated, namely the sarcoplasmic reticulum and mitochondria.

Iron ions activity towards lipid peroxidation is very much affected by the degree of iron solubility, redox potential and site-specific location (compartmentalization).

Our evidence suggests that complexes made by normal muscle metabolites with iron can have a great effect on the ability of the iron to catalyze lipid oxidation. The prooxidant and inhibitory effects of NaCl seem to derive from the ability of the salt to affect these factors.

It was possible to control lipid peroxidation by decreasing the activity of the catalytic chelatable iron. This effect was achieved by site-specific delocation or enzymic oxidation (ceruloplasmin) of iron ions to the ferric state. Results

show that lipid peroxidation takes place very rapidly during processing and antioxidants added were shown to reduce the degree of oxidation as measured both chemically and by sensory analysis.

Our preliminary study with turkey demonstrated that a decrease in nutritional iron, 3-7 weeks prior to slaughtering, could result in a significant reduction in lipid peroxidation in in-situ muscle tissue during storage.

This study indicates that by manipulating iron supplement in animal diets it will be possible to affect lipid peroxidation, and by this muscle food quality.

D. OBJECTIVES OF THE RESEARCH PROPOSAL

- a) To characterize iron compounds from muscle tissues which could initiate lipid peroxidation in in-situ muscle foods.
- b) To evaluate the generation of H_2O_2 and other active oxygen species in muscle tissues.
- c) To determine the relative roles of enzymic and non-enzymic lipid peroxidation in stored raw and cooked poultry and fish.
- d) To reveal the changes which occur post-mortem which accelerate lipid oxidation.
- e) To develop strategies for the utilization of enzymes and natural antioxidants for the control of lipid peroxidation of muscle foods in-situ under prolonged storage conditions.

E. INTRODUCTION

Lipid peroxidation is one of the primary mechanisms of quality deterioration in stored foods, especially in muscle tissues. The changes in quality can be manifested by deterioration in flavor (1), color (2), texture (3), nutritive value and the production of toxic compounds (4-6).

Stability of carcass fat and meat of broiler chicks and turkeys towards oxidation is markedly dependent on the type and level of dietary fat (7).

Off-flavor in cooked meats, as a result of lipid peroxidation, was first proposed by Tims and Watts (8). Wilson et. al (9) suggested that phospholipids play a major role in the development of off-flavor in turkey, chicken, beef and lamb, but that total lipid are the major contributor to off-flavors in pork. Igene and Pearson (10) verified that phospholipids were the major contributors to off-flavors. The lability of phospholipids to oxidation is at least partially due to their high content of PUFA, particularly arachidonic acid.

The reaction of ground state oxygen which is triplet is spin-forbidden with ground state molecules of singlet multiplicity such as PUFA. The initiation of lipid peroxidation could arise only if the PUFA molecules form a free radical, or triplet oxygen, is excited to singlet state. The most important question concerns the source of the first catalysts which lead to the formulation of the primary PUFA radicals.

Under biological conditions, significant amounts ($0.1-1 \mu\text{M}$) of superoxide (O_2^-) can be generated (11). Superoxide cannot oxidize lipids directly but could indirectly inactivate catalase or peroxidase, and oxidize α -tocopherol and via this mechanism impair the protective effects of these compounds against lipid peroxidation.

The conjugated acid of O_2^- , i.e. HO_2 (pK_a 4.8) has been shown to initiate chain oxidation of linoleic and arachidonic acids (12).

Hydrogen peroxide is normally present as a metabolite at low concentration in aerobic cells. The amount of H_2O_2 is maintained at an approximate level of 10^{-11} to 10^{-7} M by catalase and glutathione peroxidase (13). We have demonstrated the production of H_2O_2 in turkey muscle tissues (14). H_2O_2 is not a strong oxidant, and has not been shown to react directly with PUFA. However, by

one-electron reduction, H_2O_2 is converted to a potent oxidizing compound, the $HO\cdot$ radical with an E_{PH7} of +2.18, capable of oxidizing lipids (15).

Biological ubiquitous transition metals, e.g. iron and copper, with their labile d-electron system, are well suited to catalyse redox reactions. Stable paramagnetic states, resulting from the presence of unpaired electrons, are common for transition metals and facilitate their reaction with radical substrates and oxygen (which is a bi-radical). Transition metals may initiate lipid peroxidation by several mechanism: (i) they may, via single electron transfer or hydrogen abstraction, generate a PUFA radical and thus remove the spin restriction between PUFA and oxygen, as exemplified by lipoxygenase; (ii) they may interact with oxygen to generate superoxide radical ($O_2\cdot$) and (H_2O_2); (iii) transition metals interact with oxygen (cytochrome P450), or with peroxides (peroxidase, catalase, myoglobin, hemoglobin, cyclooxygenase), and by transfer of an oxene from the oxygen or peroxide to the metal ion, raise the formal metal oxidation state from +3 to +4 or +5, which could oxidize PUFA molecules; (iv) transition metals facilitate the decomposition of hydrogen peroxide and lipid peroxides to hydroxyl radicals or alkoxyl radicals, which initiate and propagate lipid peroxidation.

The stable oxidation state of iron in aqueous systems are exclusively the ferric Fe^{+3} and ferrous Fe^{+2} forms. Free iron, especially ferric ions, are insoluble in water above pH 3 and, therefore, in order to participate in aqueous phase reactions they must be chelated in some form of a soluble ligand complex, such as ascorbate, citrate, ADP or EDTA. The redox chemistry of iron is itself directly affected by the coordinating ligands. The effects of these ligands on the electronic structure and reactivity are critical for the role iron plays in lipid peroxidation (16,17,18).

Enzymic microsomal lipid peroxidation. Lipid peroxidation in isolated muscle microsomes requires NADPH (NADH) cytochrome P450 reductase and molecular oxygen, and is markedly stimulated by $ADP-Fe^{+3}$ (19,20-22). Reduction of oxygen to produce active oxygen species during microsomal electron transfer reaction has been demonstrated by many investigators and was reviewed by us (16).

Lipoxygenase and cyclooxygenase. Animal lipoxygenase utilizes free arachidonic acid as substrate and generates a single stereospecific hydroperoxide as the product (for review see 16,23). Several lipoxygenases have been described as 5-,8-,9-15-Lipoxygenases. Gill and skin tissue from fish have been shown to contain a 12-lipoxygenase (24-26). More recently, a lipoxygenase was identified from chicken muscle (26). Cyclooxygenase generates prostanoids via the stereospecific introduction of oxygen into free arachidonic acid. As lipoxygenase, cyclooxygenase is also activated by a preformed lipid hydroperoxide. Cyclooxygenase is a dimeric heme protein which catalyzes both the conversion of arachidonic acid to prostaglandin G_2 (cyclooxygenase activity) and the subsequent reduction of this hydroperoxide to prostaglandin H_2 (peroxidase activity) (16).

Activated heme proteins. The ability of heme proteins (myoglobin, hemoglobin, cyt-c) to promote lipid peroxidation has been demonstrated by many researchers (27-29). Recently, it was discovered that the interaction of hydrogen peroxide with metmyoglobin and hemoglobin led to the generation of an active species - a ferryl compound which promotes membranal lipid peroxidation (30-31), and oxidation of other organic compounds of biological importance (32).

Initiation of lipid peroxidation in muscle models and tissues. Lipid peroxidation in muscle foods was attributed to heme catalysts (29,30). Lin and Watts (29) suggested, by using model systems of linoleate emulsions, that both heme and non-heme iron are important catalysts of lipid peroxidation in muscle

products. Sato and Hegarty (33) directed a study of muscle lipid peroxidation using water-washed muscle residue. They and others (33,34,35,36) proposed that non-heme iron plays a major role in the catalysis of muscle lipid peroxidation.

However, all these model systems suffer from many errors. Model systems using linoleate emulsion contain preformed hydroperoxides at different levels (37), and they could not simulate the process of initiation in muscle cells, which are mostly of membranous nature, containing unoxidized phospholipids. Model systems containing tissues better simulate the situation in muscle tissues. However, most of the researchers using this model system had omitted from it several important compounds which, during the exhaustive washing or dialysis of the muscle samples, were removed (33-36,38-39).

Processes which disrupt the membrane function expose it to oxygen, metal ions and enzymes, cause a rapid development of rancidity in fresh and cooked muscles (30-34,38-39).

Muscle lipid peroxidation in precooked products seems to only derive from an non-enzymic reaction dependent on "free" metal ions. Heme proteins seem to affect lipid peroxidation in muscle foods only after continuous heating, which decreases the amounts of the reducing compounds to a low level. At this stage, heme proteins especially affect the propagation process by breaking down hydroperoxides to free radicals (37).

Sources of iron for lipid peroxidation in biological systems. Biological oxidations are almost exclusively metal ion-promoted reactions and, in this respect, iron being the most abundant is commonly involved (16,19,40-46).

In mammalia, about two-thirds of iron is present in hemoglobin. A further 10% is found in myoglobin - a very small amount as prosthetic components in various iron-containing enzymes and in the transport plasma protein transferrin.

The remainder is present in intracellular storage proteins, ferritin and hemosiderin. A small pool of nonprotein-bound iron equilibrating between transferrin, cell cytoplasm, mitochondria and ferritin provide "free" iron at micromolar concentrations in tissues (47-49). More recently, it was found by us that the amount of "free" iron in turkey muscle is of 0.5-2.0 $\mu\text{g/g.f.wt}$ (49).

It seems that the low molecular weight "iron pool" or the "free" iron may, in fact, be the most important pools of redox active iron available to catalyze the destruction oxidation of biomolecules in biological systems (50).

The majority of intracellular iron is deposited in ferritin. Ferritin can contain up to 4,500 ferric ions per ferritin complex. Iron is mobilized from ferritin by reduction, but the physiological reductant remains unknown. Chelating agents are required, along with reductant, but again the physiological chelator is unknown. It is now hypothesized that iron release from ferritin by O_2 is the source of iron for the O_2^- and H_2O_2 -dependent (Haber-Weiss) reaction, which generate lipid peroxidation and other cytotoxic effects to biological systems (50,51).

Another major pool of iron in muscle cells is myoglobin. Gutteridge (52) and others (53-55) have shown that iron can be released from myoglobin or hemoglobin by H_2O_2 . Future research should be directed towards the low molecular weight forms of chelated iron, their properties, and the possibility that oxidative stress might occur by induction of excessive levels of this form of iron.

Control of lipid peroxidation. Available methods for controlling lipid peroxidation may be divided into two categories, i.e. those acting to control the level of oxygen and active oxygen species, and those acting to control activated catalysts and free radicals.

Control of oxygen and oxygen species. The level of available oxygen may be controlled in foods by specific processing, such as deaeration or vacuum-packaging. It may be also controlled by oxygen scavengers, such as glucose oxidase/catalase system which reduces oxygen to H_2O .

The main protective mechanisms used by cells in vivo against the toxic effects of oxygen are superoxide dismutase (SOD), catalase, and glutathione peroxidase enzymes (11,56,57,58).

Control of active catalysts and free radicals. Iron is mostly kept tightly bound to functional proteins, however a small pool is in transit between storage proteins (ferritin), transport proteins (transferrin), and functional proteins (59). Chelating agents can significantly affect the kinetics of lipid peroxidation induced by metals (60). Numerous studies with EDTA have demonstrated the complexity which it imparts upon the reactivity of iron. This was summarized by us (16,31). Polyphosphates are well-known chelators of iron, which could control lipid peroxidation in minced, cooked meat (60).

For convenience, antioxidants are divided into two classes. Preventative, or primary antioxidants, reduce the rate of initiation of free radical chains, usually by converting the free radical producing hydroperoxidic products, $ROOH$, to innocuous products, such as alcohols (16).

Chain-breaking or secondary antioxidants trap the chain-propagation radicals. The majority of chain-breaking antioxidants are phenols. The major synthetic antioxidants in common foods used are BHA (butylhydroxyanisole), BHT (butylhydroxytoluene), TBHQ (t-butylhydroxyquinone), propyl gallate, and ascorbyl palmitate. Vitamin E (α -tocopherol) is a natural fat-soluble molecule that concentrates in the hydrophobic interior of biological membranes (61). Tocopherols are effective antioxidants in many foods; they have been tested and found effective in bacon, baked goods, butterfat, lard, and numerous other foods

(62). Stability of carcass fat and meat of broiler chicks and turkeys towards oxidation is markedly dependent on the amount of dietary vitamin E and the type of synthetic antioxidant used. Increasing the amount of dietary vitamin E and using the synthetic antioxidant, ethoxyquin, but not BHT and Endox[®], improves meat and fat stability (63,64). Synthetic oxidants, such as BHA, BHT and TBHQ, and natural-occurring substances may be used successfully to prevent lipid peroxidation in muscle tissue, but only in minced products (9,79). Lipid peroxidation could be inhibited very effectively in cured meat by nitrite (60), which generates NO[•]. Nitric oxide was found by us and others to inactivate iron catalysts as promoters of lipid peroxidation (65).

3. Results

Lipid peroxidation in model system of microsomes extracted from muscle tissues (sarcosomes). Our studies were concentrated at the beginning of the research on understanding better the potential activities of several prooxidants which might affect the catalysis of lipid peroxidation in muscle tissues. We chose to use microsomes as a substrate for lipid peroxidation because phospholipids are the major part of the lipids oxidized in-situ muscle foods.

It was found earlier by us that H₂O₂ is generated in muscle tissues and this compound could activate myoglobin to an active compound which could initiate membranial lipid peroxidation (Harel & Kanner 1986). The condition by which activated metmyoglobin could initiate membranial lipid peroxidation was described in our papers (#1).

As muscle tissues contain non-heme iron and reducing compounds, we evaluated the potential activities of an "iron-redox cycle" catalyst to initiate membranial lipid peroxidation and the oxidation of KTBA (2 keto-4(mercaptomethyl) butyric acid in free solution. The oxidation of KTBA to ethylene was enhanced by

iron-ascorbate, especially in the presence of EDTA. As expected for reactions involving hydroxyl radicals ($\text{HO}\cdot$), ethylene production was inhibited by several $\text{HO}\cdot$ scavengers. The generation of ethylene from KTBA was also inhibited by superoxide dismutase (SOD), and catalase. These results demonstrated that the oxidation of KTBA in the presence of iron-ascorbate and EDTA is dependent in part by superoxide, H_2O_2 and $\text{HO}\cdot$ radicals, (#2).

Muscle membrane lipid peroxidation was stimulated by iron-ascorbate. Lipid peroxidation was inhibited only slightly by $\text{HO}\cdot$ scavengers, and SOD or catalase failed to inhibit the reaction. EDTA which enhanced the generation of the hydroxyl radicals, inhibited almost totally the stimulation of membranal lipid peroxidation. Lipid peroxidation was also inhibited by antioxidants such as ascorbyl palmitate or BHT, (#2).

Microsomal enzymic lipid peroxidation dependent on NADPH or NADH and iron ions was also evaluated in our studies and summarized (Hazan 1986; Hazan and Kanner 1986), see abstract enclosed, (#3).

Membrane lipid peroxidation initiated by three pathways was inhibited by several compounds in order to find inhibitors which could differentiate between the prooxidants which catalyze lipid peroxidation in in-situ muscle tissues.

Desferrioxamine (DFO) is an excellent chelating agent for ferric ions, Fe^{+3} , with a stability constant of 10^{31} . In clinical medicine, DFO was proved to be of great value especially in diseases such as beta-thalassaemia. Desferrioxamine was found to inactivate iron, eliminating the "iron redox cycle" and enzymic microsomal lipid peroxidation. These results indicated that DFO could be a good candidate for differentiation between the free iron ions and heme protein catalysts in in-situ muscle tissues, (#4).

It was a great surprise for us when we found that DFO inhibited also very efficient initiation of membranal lipid peroxidation by H_2O_2 -activated

hemoproteins and peroxidase compound I. Kinetic studies using the spectra changes in the Soret region of metmyoglobin suggest a mechanism by which H_2O_2 reacts with the iron-heme to form an intermediate of oxy-ferryl myoglobin that subsequently reacts with DFO to return the activated compound to the resting state. These activities of DFO resemble the reaction of other electron donors.

From all the compounds tests by us to identify prooxidants in in-situ muscle tissues, it was found that EDTA and ceruloplasmin could be used for evaluating the role of free iron or heme proteins in these reactions. In membrane model systems and in minced turkey muscle before or after heating, lipid peroxidation was inhibited by relatively low concentration of EDTA. This low concentration of EDTA did not inhibit lipid peroxidation by H_2O_2 -activated myoglobin. In addition, it was found that ceruloplasmin, which affects membranal lipid peroxidation dependent on iron ions, significantly inhibits lipid peroxidation in turkey minced muscle tissues.

Both results demonstrated that the main catalyst in muscle tissues seems to be "free" iron ions and not hemoproteins (see papers #5,6,7).

In order to evaluate the amount of catalytic "free" iron ions in muscle tissues, we had adopted the "bleomycin method" to identify and determine these active ions. The results demonstrated that turkey and chicken muscle contain significant amounts of free catalytic iron. The amounts of free iron in turkey and chicken dark muscle were found to be 2.5 and 0.5 $\mu\text{g/g}$, and those of light muscles 0.9 and 0.2 $\mu\text{g/g}$, respectively. Turkey and chicken dark and white muscle stored at 4°C for 1 week release free iron ions, the concentration of which increases by 2-3-fold over fresh samples (see paper #6). It was assumed by us that, in part, the source of free iron ions in muscle cells is ferritin and hemoproteins.

We had estimated the amount of ferritin in dark and light turkey muscle tissues. Ferritin was isolated by heating, centrifugation, precipitation by $(\text{NH}_4)_2\text{SO}_4$ and separated by column chromatography and gel-electrophoresis.

The amount of ferritin in dark and light muscle was found to be before chromatography elution 250 and 128 ug protein/g muscle tissue. Muscle ferritin in dark and light tissues contain 1.6 and 0.35 ug/g iron. Compared with liver ferritin, muscle ferritin contains only about 1-5% of iron/mol (liver ferritin contains 2000-2400 iron atoms/mol). Iron-ferritin decreases in dark and light muscle tissue during storage at 4°C for 10 days, from 1.6 and 0.5 ug/g to 0.6 and 0.1 ug/g muscle, respectively. These results demonstrated that a large part of the "free" iron ions in muscle tissues release from ferritin molecules (Kanner and Dole 1987, unpublished data).

We have also determined the potential release of iron from hemeproteins by H_2O_2 . The reaction of H_2O_2 with resting metmyoglobin (MetMb), methaemoglobin (MetHb) and cytochrome-c (Cyt-c) was studied in the Soret and visible regions. The differences between the original and the final peak heights of the native haemproteins at 408 nm was found to be directly proportional to the loss of iron from the molecule. The release of iron from haemproteins was studied in a system generating H_2O_2 continuously at a low rate by an enzymic system, or by addition of large amounts of H_2O_2 , Cytochrome-c, methaemoglobin and metmyoglobin during interaction with H_2O_2 at a concentration of 200 μM release 40%, 20% and 3%, respectively, of molecular iron after 10 min. The inhibition of haem degradation and iron release by enzymatically-generated H_2O_2 was determined using several hydroxyl radical scavengers, reducing agents and antioxidant enzymes, such as superoxide dismutase, catalase and caeruloplasmin (see paper #8).

The generation of ferryl or hydroxyl radicals during interaction of haemproteins with H_2O_2 was also studied (see paper #9).

The prooxidative effect of NaCl on minced turkey muscle lipid peroxidation was demonstrated using two methods, TBA RC and conjugated dienes. The prooxidative effect of NaCl was inhibited by EDTA and ceruloplasmin. Both inhibitors indicated that the effect of NaCl was inhibited by EDTA and ceruloplasmin. Both inhibitors indicated that the effect of NaCl is to enhance the activity of iron ions toward lipid peroxidation. In model systems of washed muscle residue in the presence of ascorbic acid and iron, NaCl decreased lipid peroxidation. However, if muscle cytosol was added to the model system, NaCl enhanced lipid peroxidation. The effects of NaCl on both systems and in minced turkey muscles seems to derive from its capability to work as an ion-exchanger (see paper #10).

Our studies on the control of lipid peroxidation of minced turkey muscle tissue showed that the reaction could be decreased or prevented by the addition of antioxidants, such as BHA, BHT, ascorbyl palmitate, chelators such as sodium citrate, phytate and desferrioxamine, or enzymes such as SOD with catalase and ceruloplasmin (see abstract paper #11).

The effect of cytosol was also studies by us. Researchers utilized water-extracted muscle residue as a model system to study the mechanism by which lipid peroxidation occurs in muscle foods. These model systems were used especially to understand the catalysis of lipid peroxidation by heme or non-heme iron compounds.

It is apparent that aqueous extraction of muscle tissues might also have extracted several essential compounds such as enzymes, H_2O_2 , reducing compounds or chelating agents which may affect the overall catalysis of muscle lipid peroxidation in-situ.

We have developed a method to determine total reducing agents in muscle tissues. The results showed that turkey muscle tissue contains a high

concentration of reducing agents (200-400 nmole/g tissue, eq. to ascorbic acid). The amount of the reducing compounds reduced drastically during heating or storage of muscle food at 4°C (see paper #12).

The effect of the cytosolic fraction was determined in three model systems, and contained washed muscle residue and prooxidative systems such as 1) iron-ascorbic acid (iron-redox cycle); 2) enzymic, NADPH, iron-ADP; 3) H_2O_2 -activated myoglobin.

The cytosol was found to inhibit only partially the first two systems, however it inhibited totally lipid peroxidation by H_2O_2 -activated myoglobin. The cytosolic extract was separated into a low and high molecular weight fraction and both fractions exhibited inhibitory effects toward H_2O_2 -activated myoglobin. Membrane lipid peroxidation initiated by the iron-redox cycle was inhibited partially by the high molecular weight fraction but accelerated by the low molecular weight fraction (see paper #13).

These results demonstrated the high potential of the reducing compounds in muscle tissue to prevent the activation of muscle lipid peroxidation by activated hemeproteins. Furthermore, it was found by us that myoglobin and hemoglobin could prevent the initiation of hydroxyl radicals in a model system contained H_2O_2 iron-redox cycle and by this to prevent lipid peroxidation (see paper #14).

As "free" iron ions were found to be the main catalysts of muscle lipid peroxidation, we had conducted a preliminary study to evaluate whether the stability of turkey muscle to the process of lipid peroxidation could be improved when the iron supplement is omitted from the diet fed during the last growth period.

The results demonstrate that a decrease in nutritional Fe 3-7 weeks prior to slaughtering could result in a reduction of more than 50% in lipid

peroxidation in in-situ turkey dark muscle. Turkey light muscle, which is by its nature more stable to lipid peroxidation, was affected during the first experiment but not during the second.

Removal of the Fe supplement from the diet fed during 3-7 weeks prior to slaughtering did not affect body weight or blood hematocrit, although the latter was slightly decreased, (#15).

A review on: "Initiation of lipid peroxidation in biological systems" is attached, (#16).

Summary

The objectives of our proposal "Metals and O_2 in Initiation and Prevention of Lipid Oxidation in Muscle" was to identify the nature of the iron compounds in muscle tissue and factors which activate or inhibit them. The overall goal was to develop strategies for the utilization of natural antioxidants for the control of lipid peroxidation of muscle foods in-situ under prolonged storage conditions.

Our studies on model systems gave us the possibility to understand better the involvement of prooxidants and antioxidants in the process of membranal lipid peroxidation. These studies help us also to chose compounds which could characterize the nature of the prooxidants in in-situ muscle tissue.

It was found that the main catalyzer of lipid peroxidation in turkey muscle tissue is "free" iron ion. The source of these iron ions seems to derive from ferritin.

In order to activate iron, ferric ion should be reduced to ferrous form. Redox compounds are the ultimate driving force in obtaining high concentration of ferrous ions, which are essential for the generation of oxy radicals, initiation of lipid peroxidation and accelerating the propagation step.

We developed a method to determine total reducing compounds in muscle tissue, which is dependent on iron ions. The amount of reducing compounds in turkey muscle tissues are in the range of 200-400 μM , eq. to ascorbic acid. These reducing compounds accelerate lipid peroxidation dependent on iron ions, however, inhibit those of H_2O_2 -activated myoglobin.

Reducing compounds which turn ferric to ferrous ions could derive also from the activation of a membranal enzyme (cytochrome NADPH reductase), a reaction which initiates lipid peroxidation.

Iron ions activity toward lipid peroxidation is affected very much by the degree of iron solubility, redox potential and site-specific location.

The pro- and antioxidative activities of NaCl seems to derive from the ability of the salt to affect these factors.

It was found by us that it is possible to control lipid peroxidation in muscle tissue by decreasing the activity of the catalytic free iron. EDTA, sodium citrate and phytate seem to be affected especially by iron delocation from membranous phospholipids to the cytosol. In the cytosol, enzymes such as catalase, glutathione peroxidase and myoglobin, in the presence of reducing compounds such as ascorbic acid and glutathione, control oxidative reactions.

Ceruloplasmin deactivates iron ion catalysis by its ability to chelate iron and its ferroxidase activity, which turns ferrous to ferric form.

Antioxidants, such as BHT, TBHQ, are well-known additives which could control in part lipid peroxidation in minced muscle tissues. We found that ascorbyl palmitate work as a very efficient antioxidant in minced muscle tissues. As ascorbyl palmitate breaks down to natural compound in the digestive system, it is possible to use it in unlimited concentration.

Finally, our preliminary study demonstrated that a decrease in nutritional iron 3-7 weeks prior to slaughtering could result in a significant reduction in lipid peroxidation in in-situ turkey muscle tissue during storage.

This study leaves us to believe that it will be possible in the future, by manipulating iron and other compounds in the animal diets, to increase significantly the stability and quality of muscle foods.

F. DESCRIPTION OF THE COOPERATION

The current BARD study achieved much of its purpose by the cooperation of the investigators. Both investigators exchanged visits. Several important observations from the results achieved in one lab. were transmitted to the other. This information stimulated the research and brought it to a final success.

The possible effect of histidine, NaCl methods developed to isolate muscle mitochondria or determine reducing compounds are only a part of the important exchange of knowledge between the two investigators.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS

The research characterized and identified free iron ions as the main initiator of muscle lipid peroxidation of turkey and fish. Methods were developed to differentiate between catalysts such as activated hemeproteins and "free" iron ions. Results showed that the driving force of iron ions dependent on muscle lipid peroxidation are enzymatic and non-enzymatic reactions. In heated muscle products, the non-enzymatic iron-redox cycle seems to be the most important systems which is responsible for the acceleration of lipid peroxidation.

The research evaluated the generation of H_2O_2 and other oxy-radicals such as $HO\cdot$ or oxene ferryl ion and the activity of those radicals toward the oxidation of bio-molecules.

Some processing factors which influence the activity of free iron ions, such as temperature, pH, NaCl, chelating agents and O_2 , were evaluated.

Our evidence suggests that complexes made by normal muscle metabolites with iron can have a great influence on the ability of iron ions to catalyze lipid peroxidation. The prooxidant and antioxidant effects of NaCl seems to derive from the ability of the salt to affect these factors.

Knowledge gained both in the laboratories of the American group led by Prof. Hultin and the Israeli one, have been applied to muscle system, i.e. minced turkey or production of washed, minced fish muscle (surimi) from a fatty-type fish. Results show that lipid peroxidation takes place very rapidly during processing, and antioxidants added specifically to counteract some of the changes observed in a muscle system in vitro were shown to reduce the degree of oxidation as measured both chemically and by sensory analysis.

Our studies assess the application of naturally-occurring antioxidants in controlling the process of lipid peroxidation in minced muscle tissues, several of these antioxidants were: superoxide dismutase, catalase, ceruloplasmin, sodium citrate, sodium phytate (ascorbyl-palmitate) and an extract from sage.

Sodium citrate, phytate, ascorbyl-palmitate and sage extract all seem to be of great important and should be introduced in the future as natural (or like-natural) antioxidants in muscle foods.

A study of the Israeli group showed that a decrease in nutritional iron supplementation prior to slaughtering results in a reduction of lipid peroxidation in in-situ muscle tissue.

Our study indicated that by manipulation of iron supplement in the diet and maybe other nutrients which affect the prooxidative and antioxidative tone of muscle tissues, it will be possible to improve in the future the quality of muscle foods.

Although the general importance of lipid peroxidation in muscle tissue deterioration is well recognized and is often used as a measure of predicting

shelf-life and quality control, the role of various forms of iron compounds in the initiation process of lipid peroxidation was not understood. Our previous BARD-supported research elucidated the mechanism of enzymic and non-enzymic lipid peroxidation of membrane lipids and the control of those systems *in vitro*. Our goal was to understand the involvement of iron (metal) compounds *in situ*, and the control of inhibition of lipid peroxidation in muscle foods by adding enzymes and natural antioxidants in situ.

Poultry and fish represent important commodities in the U.S. and Israel. The use of these materials in the manufacture of pre-cooked and restructured food products can be anticipated to increase in both the domestic and foreign markets of the two countries. With these types of products as well as pre-cooked institutional foods, the problem of lipid peroxidation will be a major determinant of shelf life.

This research led to new techniques to inhibit and prevent this lipid peroxidation. The results have opened avenues that hold significant promise for allowing control of this major deteriorative reaction in muscle foods.

H. RELEVANT BIBLIOGRAPHY ON RESEARCH AREA

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