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ANTIOXIDANT PROPERTIES OF OLEORESIN
ROSEMARY IN RESTRUCTURED PORK STEAKS AND THE
RELATION BETWEEN SODIUM TRIPOLYPHOSPHATE AND OLEORESIN
ROSEMARY IN A MEAT FIBER MODEL SYSTEM

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Hsing-Feng Liu

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Alden Booren
Major professor

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BY

Hsing-Feng Liu

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ABSTRACT

ANTIOXIDANT PROPERTIES OF OLEORESIN ROSEMARY IN RESTRUCTURED PORK STEAKS AND THE RELATION BETWEEN SODIUM TRIPOLYPHOSPHATE AND OLEORESIN ROSEMARY IN A MEAT FIBER MODEL SYSTEM

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The antioxidant activity of the oleoresin rosemary (OR) with and without sodium tripolyphosphate (STPP) or citrate was studied when added to restructured pork steaks and in meat fiber model systems. Cooked restructured pork steaks were stored at 4°C for 8 days and lipid oxidation was followed using TBA analysis and taste panel scores. Raw restructured pork steaks were stored at - 30°C for 0, 2, 4, 8 months then cooked and lipid oxidation was evaluated. STPP provided significant protection against lipid oxidation during all storage treatments. Citrate demonstrated more antioxidant effect during frozen storage than refrigerated storage of restructured pork. No additional benefits in lipid stability were evident when OR and STPP were used in combination.

The synergism between STPP and OR was studied using meat fiber model systems from pork meat. Synergism was found in a model system using hemoglobin but not in the model system using ferric iron. Antioxidants in the model system demonstrated similar trends as were seen in restructured pork steaks.

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INTRODUCTION

Lipid oxidation is a major deteriorative reaction in foods. It often results in a significant quality loss. Lipid oxidation leads to the formation of short chain aldehydes, ketones, and fatty acids, as well as the development of some polymers, all of which are believed to contribute to oxidized flavors in meat, poultry, and fish. Warmed over flavor (WOF) was first studied by Timms and Watts (1958) who defined it as the rapid onset of rancidity in cooked meat during refrigerated storage. In recent years, the demand for restructured meat by the fast food and airline food catering industries has increased rapidly. Warmed over flavor is a major problem in these foods.

For many years the food industry has used synthetic phenolic antioxidants to improve the stability of lipid containing products. The most commonly used antioxidants are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). They are added to a wide variety of foods. However, they are quite volatile and easily decomposed at high temperatures. Consequently, they are not satisfactory for such common food products as French fries and potato chips. Recently consumers and processors have

been increasingly concerned about the safety of synthetic food additives, including the possible toxicity of the synthetic chemicals used as antioxidants. Consequently, the use of natural instead of synthetic antioxidants in processed food is popular to the consumer regardless of the safety of the natural antioxidant.

Scientists have proven that many herbs and spices possess antioxidant characteristics in fat and oil systems. Chipault et al. (1952) reported a study of 32 common spices used as antioxidants in lard, and showed that only rosemary and sage are effective as antioxidants. Chang et al. (1977) also reported that the antioxidant activity of the purified antioxidant prepared from sage is comparable to that of rosemary in lard. The use of rosemary extracts as an antioxidant in foods has already been reported by Rac and Ostric (1955), Berner and Jacobson (1973), Chang (1976), and Chang et al (1977). Wu et al. (1982) and Houlihan et al. (1984, 1985) have reported that naturally occurring compounds in rosemary extracts exhibit antioxidant properties greater than BHA and equal to BHT. Barbut et al. (1985) also reported that oleoresin rosemary (OR) was comparable to a commercial blend of BHA/BHT/citric acid in suppressing lipid autoxidation. However, the extracts of rosemary and sage usually have a strong odor and bitter taste. Therefore, particular interest has been focused on the significant antioxidant activity of low odor and flavor extracts from sage and rosemary.

Watts and Wong (1951) proposed the idea that ethylene-diaminetetraacetic acid (EDTA) or polyphosphates, both metal chelators, could act as antioxidants in synergism with ascorbic acid in aqueous fat systems. Chang and Watts (1950) also demonstrated that phosphates and ascorbic acid reacted synergistically to retard hemoglobin (Hb) catalysis of fats. It is well recognized that a synergistic effect exists when phenolic antioxidants are used together with certain organic acids such as citric acid or polyphosphates. This is attributed to the metal chelating function of these ingredients.

The overall objectives of this study were to determine the antioxidant properties of oleoresin rosemary in restructured pork steaks and the possible synergism between sodium tripolyphosphate (STPP) and oleoresin rosemary in a meat model system. Specific objectives include: 1. To compare the antioxidative efficiency of OR in restructured pork steaks stored at $4^{o}C$ and - $30^{o}C$ with sage, citrate and STPP; 2. To examine two types (water soluble and water insoluble) of OR at two concentrations with or without STPP; 3. To compare tertiary butylhydroquinone (TBHQ)/STPP, when used as reference for antioxidative efficiency of OR; 4. To examine the possible synergistic relationship between OR and STPP by using a meat fiber model system; 5. To evaluate STPP, OR, and STPP/OR in a meat fiber model system with and without ferrous ion, or Hb (Love and Pearson, 1974) and without a metal catalyst.

REVIEW OF THE LITERATURE

Lipids in Muscle Structure

Lipids are found intermuscularly, intramuscularly, in adipose tissue, in neural tissue and in the blood. Triacylglycerides (the dominant species), phospholipids, sterols and sterol esters and other lipids in minute quantities are included.

Generally speaking, triacylglycerides serve as energy stores and phospholipids are the structural components of the cell membrane. Muscle triacylglycerides are composed of the glycerol esters of straight chain carboxylic acids having an even number of carbon atoms. Natural fats are composed principally of mixed triacylglycerides (i.e. the three fatty acids in the triacylglyceride are different).

Most fatty acids found in animal fats contain an even number of carbon atoms. Branched chain and odd numbered carbon fatty acids have been found at low levels in mutton and beef fats (Dugan, 1987). Saturated fatty acids and mono-unsaturated fatty acids are dominant in meat. The major unsaturated fatty acids are oleic, linoleic and linolenic acids (Pearson et al., 1977).

The major role of phospholipids in animal tissue is for structural and functional components of cells and membranes. Most of the phospholipids found in muscle are phosphoglycerides. These are diacyl esters of glycerol, which are esterified at one terminal carbon to phosphoric acid. The phosphoric acid is additionally esterified most frequently to choline, ethanolamine or serine. It appears that phospholipids may have important roles in flavor and in the keeping quality of meats as well as related processed foods. Younathan and Watts (1960) have suggested that they play a major role in accelerating flavor deterioration in cooked meats. The lability of the phospholipids is a result of their high unsaturated fatty acid content. Igene et al. (1980) in a meat fiber model system demonstrated that both triacylglycerides and phospholipids contribute to the development of rancidity. However, phospholipids make the greatest contribution in the early stage of lipid oxidation.

The Mechanism of Lipid Oxidation

A. Autoxidation

Bolland and Gee (1946) demonstrated the promotive effect of initiators and ultraviolet light as well as the inhibitory effect of radical scavengers in the lipid oxidation of olefins. Thus, they put beyond doubt that lipid oxidation is a free radical chain reaction.

In common with other radical chain reactions, lipid

oxidation can be divided into three separate processes: initiation, propagation and termination, as indicated below.

initiation: $X. + RH \text{ -----} > R. + XH$ .....(1)

propagation: $R. + O_2 \text{ -----} > RO_2.$ .....(2)

$RO_2. + RH \text{ ---} > ROOH + R.$ .....(3)

termination: $R'O_2. + R"O_2. \text{ --} > R_2CO + R_2CH_2OH + O_2$..(4)

$R'O_2. + R". \text{ -----} R'OOR"$stable products(5)

$R'. + R". \text{ -------} R'R"$.....(6)

X may be a transition metal ion, a radical generated by photolysis, high energy irradiation or a radical obtained by decomposition of a hydroperoxide.

(1) Initiation

There are many possible ways to proceed to the initiation reaction according to the thermodynamic theory. The initiation process can be divided into two types according to their substrate. Those which involve the substrate RH and those which involve the decomposition of the product ROOH.

(a) substrate RH

At the initiation reaction, when no metal was present, reaction (7) was considered as a possible initiation process (Bolland and Gee, 1946).

$RH + O_2 \text{ ---} > R. + HO_2.$ A= 35 Kcal....(7)

In 1949 Evans and Uri demonstrated that the reaction was endothermic and was therefore unlikely to contribute

significantly to the initiation process unless a catalyst was involved. On the other hand, metal catalyzed production of the radical R. from the substrate RH can take place by the process (8).

$$RH + M^{+3} \text{----->} R. + H^{+1} + M^{+2} \quad \text{.....(8)}$$

For methyl linoleate, the process (8) was estimated by Heaton and Uri (1961) to be exothermic and can therefore be an important initiation reaction.

(b) substrate ROOH

In the case of initiation reactions involving ROOH only and without metal, there are two possible reactions, depending on whether one (process 9) or two (process 10) molecules of ROOH are involved. Currently, process 10 has been widely held to be an important initiation process (Bateman and Hughes, 1952; Walling and Heaton, 1965).

$$ROOH \text{----->} RO. + .OH \quad \text{.....(9)}$$

$$2ROOH \text{----->} RO. + H_2O + RO_2. \quad \text{.....(10)}$$

In the case of initiation reactions involving ROOH and metal ions, two radical producing reactions are possible. One (11) involves the metal in its lower oxidation state, and the other (12) involves the metal in its higher oxidation state (Hiatt et al., 1968a; Hiatt et al., 1968b):

$$ROOH + M^{+2} \text{----->} RO. + OH^{-1} + M^{+3} \text{.....(11)}$$

$$ROOH + M^{+3} \text{----->} ROO. + H^{+1} + M^{+2} \text{.....(12)}$$

From the thermodynamic and bond dissociation energy information, it can be deduced that the dominant initiation

processes in lipid oxidation of unsaturated lipids are the metal catalyzed reactions (8), (11) and (12). To substantiate the view that the metal catalyzed reaction is important in lipid oxidation, Uri (1956) indicated that even very pure unsaturated lipids, which oxidize slowly, contain trace amounts of metals.

Bolland (1949, 1950) investigated the activation energies of the propagation reaction of several unsaturated lipids. He found that the decrease in activation energy in going from a monoene to a 1,4-diene is particularly significant. We also know the bond dissociation energy for R= alkyl is some 20 kcal/mole higher than the corresponding alkenyl compounds. These facts may explain the observed (Gunstone and Hilditch, 1945) faster rates of oxidation of polyenoic fatty acids as compared to monenoic acids.

(2) Propagation

In the propagation step, reactions (2) and (3) form the basis of the chain reaction process. When the allyl radical (R.) was initiated by initiator, it reacts very quickly with O_2 with almost zero activation energy to form the peroxy radical (ROO.). Then the peroxy radical can abstract allylic hydrogen (H.) from another unsaturated fatty acid (RH) to form hydroperoxide (ROOH) and create another allyl radical (R.). Hydroperoxide (ROOH) can undergo further hydrolysis to RO., OH. or ROO. depending upon the environment.

Once peroxy radicals (ROO.) or free radicals (R.) have been formed, the rearrangement reaction will continue in the absence of significant chain termination because the reaction is essentially one of equilibration (Figure 1). Therefore, many different isomers of hydroperoxides will be formed when the reaction of each step has been completed.

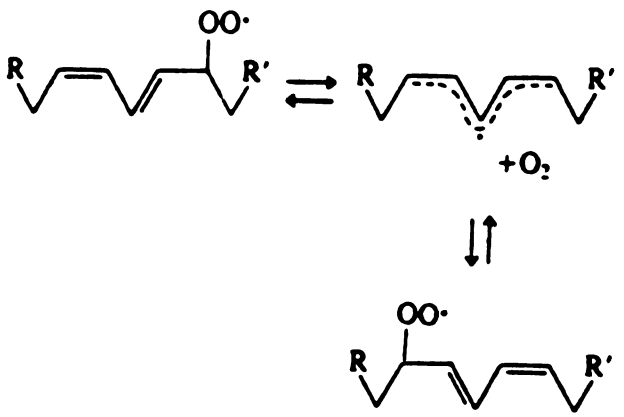

Figure 1. Isomerization of a peroxy radical

(3) Termination

During the termination step, free radicals are eliminated by metal ions or formation of nonradical products with other free radicals. Reviewing the termination processes involving R. or RO_2., the bimolecular reaction (13) is likely to be the most important termination reaction under the conditions without metal ions participating.

$$RO_2. + RO_2. \longrightarrow R_2CO + R_2CHOH + O_2 \quad \ldots\ldots\ldots\ldots (13)$$

Russell (1956, 1957) used the isotope methodology to study the termination of secondary peroxy radicals and

demonstrated the intermediacy of a tetroxide in the reaction which gives rise to a molecule of a ketone, a secondary alcohol and oxygen confirming reaction (13). The other important chain termination pathway is reaction (14).

$$ROO. + M^2 \text{ ---> } ROO\text{-}M^3 \quad \ldots\ldots (14)$$

A transition metal in its lower oxidation state combines with a peroxy radical to form stable products (Ingold, 1963; Kamiya et al., 1963; Kamiya and Ingold, 1964). Reaction (14) may serve to explain the antioxidative effect of high concentrations of metal ions in the lipid oxidation of unsaturated lipids.

B. Photooxidation

In addition to autoxidation (thermal induced lipid oxidation), another pathway of lipid oxidation is photooxidation. The function of light in photooxidation is to increase the initiation rate by formation of radicals resulting from light absorption by species other than lipids. There are two types of reactions. In the first type, direct photooxidation, the overall effect is the generation of a free radical R. (allyl radical) from the lipid molecule. The products are identical to those of lipid oxidation (Chan, 1977). In the second type of reaction, sensitized photooxidation, singlet state (excited state) oxygen is generated as a result of energy transfer between the triplet state (ground state) oxygen and the excited species generated after light absorption. Singlet

oxygen reacts with unsaturated lipids by the "ene" reaction (Figure 2) to form hydroperoxides (Neff and Frankel, 1980).

C. Hydroperoxides

In 1954 Bateman provided experimental evidence to confirm the "hydroperoxide hypothesis". That is, the product of the reaction between an unsaturated lipid and oxygen is a hydroperoxide. This discovery was a landmark in the study of the lipid oxidation of unsaturated lipids. Once the theory was established, the manifestation of lipid oxidation could be understood in terms of a primary reaction between oxygen and the unsaturated lipid to form a hydroperoxide, which then undergoes further reactions with or without the participation of other compounds.

Hydroperoxides are the primary products of lipid oxidation. In enzymatic oxidation one dominant hydroperoxide was produced. Nonenzymic oxidation of unsaturated lipids yield a mixture of hydroperoxides that differ in the position of the OOH group and the geometrical isomerism of the double bonds. These hydroperoxides can be decomposed by many routes leading to the formation of volatile and nonvolatile products (Figure 3). The first step involves a homolytic cleavage of the O-O bond of the hydroperoxide to yield the alkoxy and hydroxy free radical. The alkoxy radical can go further heterolytic processes and form volatile compounds such as alcohols, ketones and hydrocarbons (Figure 3) or they can polymerize and form macromolecular compounds.

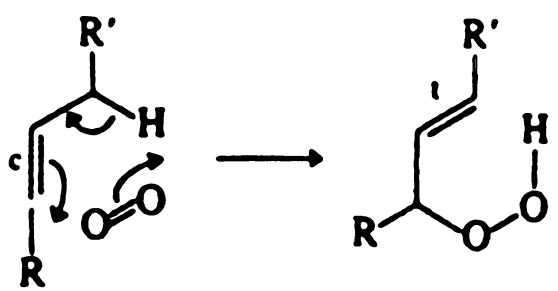

Figure. 2. Sensitized photooxidation

The singlet oxygen molecule is joined onto one end of a double bond simultaneously as it abstracts an allylic proton. A new double bond is formed between the allylic position and the other end of what was the C=C bond. In the photooxidation reaction, the double bonds behave as isolated C=C units rather than the 1,4-diene system. Oxygenation occurs at both ends of all the double bonds.

Badings (1959), Frankel et al. (1961) and Swoboda and Lea (1965) explained the formation of volatile compounds with the decomposition of the monohydroperoxides by homolytic reaction (Figure 3). Polyunsaturated fatty acids form hydroperoxy epidioxides and undergo fragmentation to yield volatile compounds (Figure 4). Frankel et al (1982) compared the volatile products from decomposition of monohydroperoxides with those from hydroperoxides and showed only small differences in kinds of compounds.

The range of volatile compounds that are formed during the lipid oxidation is dependent upon: 1. The composition of the monohydroperoxides and the hydroperoxy epidioxides; 2. The extent and nature of the rearrangement of the hydroperoxy group in the monohydroperoxides; and 3. The extent and nature of oxidative cleavage of double bonds.

Lipid Oxidation in Food

Lipid oxidation is very complex in meat. Some meat components or additives such as amino acids and enzymes act as antioxidants , while others such as salt and heme iron act as prooxidants. During processing, some antioxidants are produced by Maillard reactions, however, heat treatment can also activate non enzymatic lipid oxidation catalysts such as hemoproteins. Therefore, lipid oxidation in meat is due to a combination of prooxidant and antioxidant factors.

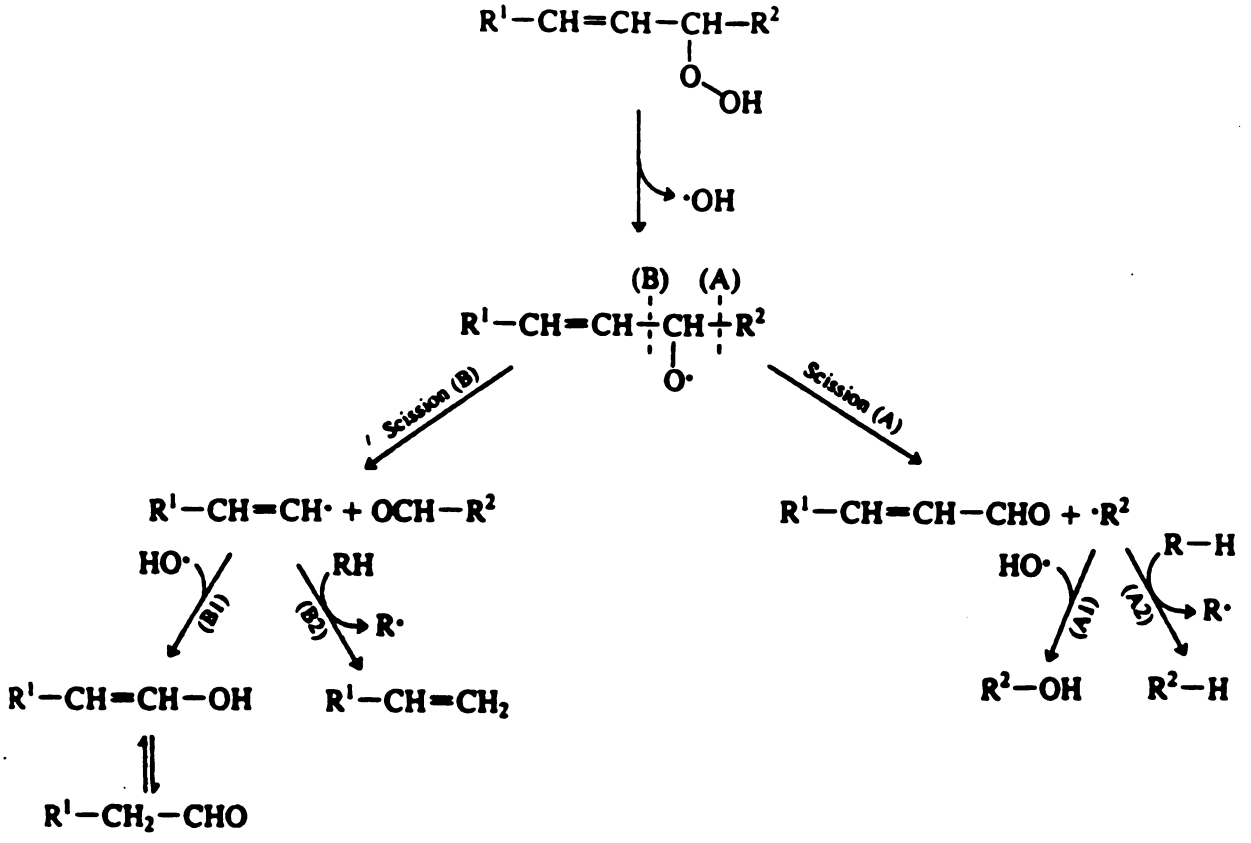

Figure 3. Reaction scheme proposed for the homolytic cleavage of monohydroperoxides

A. Lipid Oxidation in Raw Materials

In raw meat, heme iron, non heme iron and enzymes are the three major catalytic systems involved in lipid oxidation. The role of heme iron and non heme iron as a lipid oxidation catalyst is still disputed and will be discussed later. The major role of muscle microsomes in lipid oxidation is to maintain iron in its reduced form which is more prooxidative through the enzymatic dehydrogenation of NADPH, which is reduced in another enzymatic cycle (Hultin, 1980). In plants, lipoxygenase is a major cause of off flavor. Lipoxygenase can introduce oxygen into free fatty acids to primarily produce hydroperoxides.

Some food components such as milk proteins and soy proteins also act as antioxidants (Pratt, 1972). Amino acids such as histidine and alanine (Marcuse, 1960) also have an antioxidant effect. This effect depends upon a number of factors such as the presence and concentration of trace metals, pH, temperature and the concentration of the amino acid itself. Plant pigments like flavonoids and many spices or herbs have been found to have antioxidative properties. The food enzymes glucose oxidase, catalase (Sarett, 1960) and superoxide dismutase (Hicks et al., 1977) also play an antioxidant role.

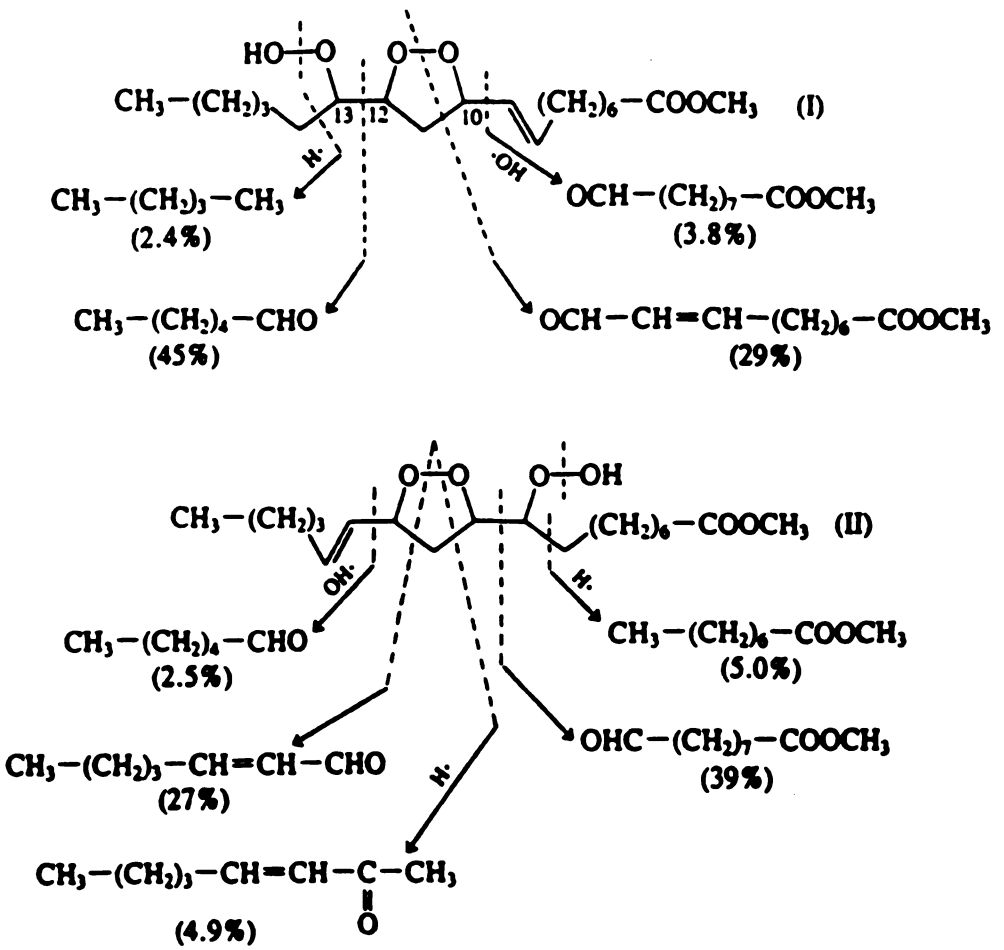

Figure 4. Reaction schemes proposed for the thermal decomposition of methyl 13-hydroperoxy-10,12-epidioxy-trans-8-octadecenoate (I) and methyl 9-hydroperoxy-10,12-epidioxy-13-octadecenoate (II). The percentage proportion of the volatile products in the experiment at 210°C is given in parentheses.

B. Lipid Oxidation in Processed Meats

During processing, meat undergoes particle size reduction, mixing, heat treatment, drying or combinations of two or more of these treatments. Salt, which is a well known prooxidant (Neer and Mandigo, 1977; Huffman et al., 1981; Chen et al., 1984; King and Earl 1988) is also added during processing. These treatments generally destroy meat structure and increase the permeability to oxygen or oxygen dissolved in the food. They also increase the amount of available phospholipids and heme pigments by disrupting the membranes and structure (Erickson et al., 1971; Sato and Hegarty, 1971). During heat processing amino compounds can react with sugar and produce Maillard reaction products which have a reported antioxidant effect (Sato et al., 1973; Einerson and Reineccius, 1977; Lingnert and Eriksson, 1980). Heat also completely inactivates enzyme catalyzed lipid oxidation.

Major Factors That Affect Lipid Oxidation

The rate of lipid oxidation is increased mainly by increasing the rate of initiation reactions which are influenced by factors that increase free radical formation in the food system. In lipids, heavy metals and their derivatives are the most important catalysts of lipid oxidation reactions. The effect on lipid oxidation of any given factor depends upon the reaction conditions.

A. Heme Compounds as Catalysts of Lipid Oxidation

In early studies, lipid oxidation has been attributed to heme catalysts such as hemoglobin, myoglobin and cytochrome (Barron and Lyman, 1938; Haurowitz et al., 1941; Watts and Peng, 1947; Tappel, 1952). Watts and Peng (1947) examined the catalytic activity of hog muscle extract and concluded that myoglobin and hemoglobin were the catalysts responsible for fat oxidation. Tappel (1952) also reported hemoprotein to be the major catalyst of lipid oxidation in beef, chicken, turkey, and fish muscle.

In 1956 Gibson and Ingram studied the mechanism of hematin or hemoglobin by electron spin resonance and reported that the electron is removed from the electron cloud of the porphyrin ring and not from the central metal ion. It is the spin state and not the valency state of iron in the heme derivative that is responsible for catalyzing lipid autoxidation.

In 1962 Tappel proposed a probable heme catalyzed lipid oxidation mechanism. He postulated that the major function of heme in catalyzing lipid oxidation was the catalytic decomposition of hydroperoxides to generate free radicals. During lipid oxidation, the heme compound and lipid hydroperoxide will form an intermediate compound. This compound undergoes homolytic scission of the peroxide bond to generate free radicals. In 1962 Lundberg presented a similar hematin catalyzed oxidation mechanism (Figure 5). In this mechanism, there would be no change in the valence of heme iron.

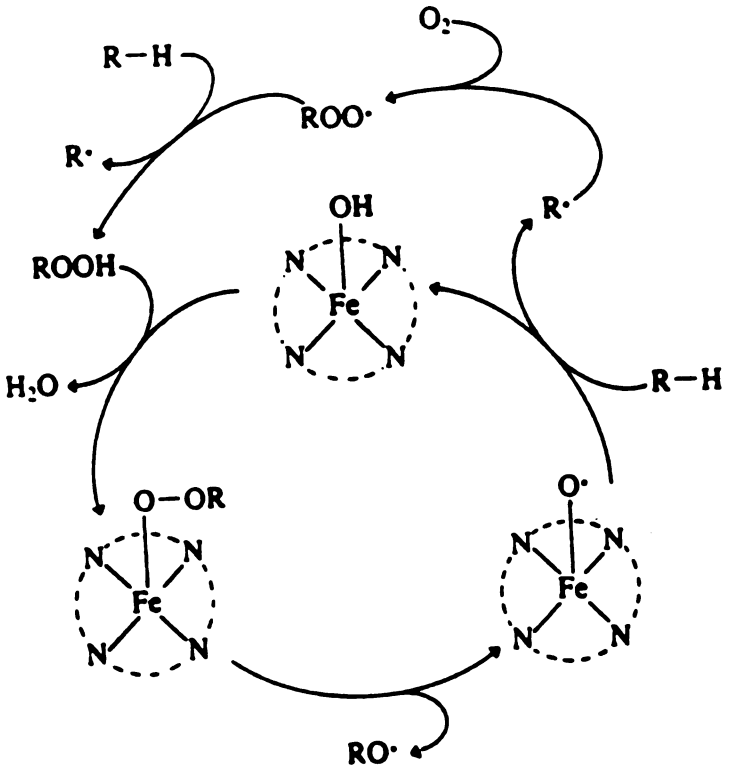

Figure 5. The mechanism of hematin catalyzed oxidation

B. Role of Non Heme Iron

Wills (1966) attempted to assess the relative activity of hemoprotein and non heme iron as catalysts of lipid oxidation in various animal tissues incubated in air. Wills concluded that both heme and non heme iron were capable of promoting the oxidation of unsaturated fatty acids. Catalysis by non heme iron was reported to be pH sensitive, with iron more active at acid pH values. The pH had less effect on hemoprotein catalyzed lipid oxidation. Liu (1970a,b) and Liu and Watts (1970) presented evidence that confirmed Wills' observation that both heme and non heme iron could function as prooxidants in meats.

Sato and Hegarty (1971) first cast doubt on the role of heme pigments in the catalysis of lipid oxidation in meat. They extracted meat with water to remove the heme pigments, added back various heme fractions or pigments and found that no catalysis of lipid oxidation occurred after cooking. Only the diffusate showed significant catalytic activity, indicating that low molecular weight compounds are active prooxidants. They suggested that non heme iron is the major prooxidant in cooked meat. Love and Pearson (1974) added purified metmyoglobin (MetMb) and ferrous iron back to the water extracted muscle model system and indicated that ferrous iron was effective as a prooxidant in cooked meat,

whereas MetMb at concentrations from 1 to 10 mg/g of meat failed to catalyze the oxidation of lipids. This confirmed the observations of Sato and Hegarty (1971). Igene et al. (1979a) reported that non heme iron plays a major role in lipid oxidation of cooked muscle foods. The percent of bound heme iron in fresh meat pigment extract was slightly over 90% while the level of free non heme iron was less than 10%. During the cooking procedure, the heme iron was destroyed by heat and released about 27% of the total iron which accounts for the increased rate of lipid oxidation on the cooked meat. They also reported that hydrogen peroxide destroyed the heme molecule and released approximately 60.0% of the total iron in the pigment extract.

Harel and Kanner (1985a,b) suggest that ferric heme pigments may only be effective catalysts in the presence of hydrogen peroxide. Rhee (1988) has claimed that this catalytic effect may be due, at least in part, to release of iron from the heme environment by hydrogen peroxide. Recently, in studies using the enzymatic microsome model systems, Kanner et al. (1988a,b) have concluded that lipid oxidation in minced turkey muscle is affected by "free" metal ions at the concentrations found in turkey and chicken muscle (0.2 to 2.5 ug/g).

Uri (1956) proposed a possible mechanism for the iron ion to act as a catalyst in muscle foods. The ferric and ferrous forms of iron allow it to act as an electron donor and acceptor, which give it important catalytic properties.

In the presence of oxygen, ferrous ions produce the superoxide anion radical which dismutates to hydrogen peroxide. Ferrous ions further reduce hydrogen peroxide to hydroxyl radicals. The hydroxyl radical can attack and oxidize any bioorganic compounds present in live cells. Recently, Kanner et al. (1988a) demonstrated that hydroxyl radicals are generated during the iron reduction oxidation cycle by ascorbic acid.

There are still many contradicting and confusing results that have been obtained in work with model systems. In 1975 Lee et al. concluded that hemoproteins were the predominant and possibly the sole catalysts of lipid oxidation in mechanically deboned chicken. Sato and Hegarty (1971), Love and Pearson (1974) and Igene et al. (1979a) reported that non heme iron is the major prooxidant in cooked meat and that heme compounds had little effect on the rate of oxidation in cooked meat. Decker and Schanus (1986) investigated linoleate oxidation catalyzed by an aqueous extract of chicken drumstick muscle and reported that both heme and non heme catalysts may be involved in catalysis of lipid oxidation in raw chicken dark meat. Johns et al. (1989) used the same water extract model system as Sato and Hegarty (1971), Love and Pearson (1974) and Igene and Pearson (1979a) but with a small modification: (1) the ratio of water washed muscle and distilled water and (2) Cooking methods. They reported hemoglobin was a powerful catalyst, but all forms of inorganic iron appeared to have little

prooxidant activity. This was in direct contrast to results reported by Sato and Hegarty (1971), Love and Pearson (1974) and Tichivangana and Morrissey (1985). Johns et al. (1989) thought this was because of two reasons. First, In Sato and Hegarty's (1971) study they directly mixed hemoglobin and muscle fibers. This could create high concentrations that could be within the inhibitory range. Fox and Benedict (1987) also suggested that poor distribution of hemoprotein may explain the results of Sato and Hegarty (1971) and Love and Pearson (1974) who found that heme pigments had no effect on the rate of lipid oxidation in cooked meat. Second, in Sato and Hegarty's study, samples were only stored for 2 days which may not have been long enough to overcome the induction period required for heme catalysis. Kanner et al (1988b). thought Sato and Hegarty (1971), and other researchers (Liu and Watts, 1970; Igene et al., 1979a; Shahidi et al., 1986) utilized a high concentration of EDTA (2 % EDTA) in raw and heated muscle tissues to inhibit lipid oxidation when evaluating the role of non heme iron or heme proteins in the catalysis of this reaction. This concentration could inhibit not only free iron catalysis but also inactivate hemoproteins.

Important Antioxidants and Their Properties

Antioxidants are substances that can suppress lipid oxidation. Their effectiveness depends on activity and

structure of the antioxidant, the rate of chain initiation, and the antioxidant concentration. According to their antioxidant mechanism, they are classified as: A. free radical terminators; B. oxygen scavengers; C. chelating agents; and D. important component with antioxidant activity.

A. Free Radical Terminators

Most free radical terminators are phenolic compounds. They can break the free radical chain reaction by donating a hydrogen atom (Bickell et al., 1953).

ROO. + AH (antioxidant) ---> ROOH + A. ....(15)

RO. + AH (antioxidant) ---> ROH + A. ....(16)

The radical A. may be stabilized by recombination into dimers, other products or form a stable resonance hybrid (Figure. 6).

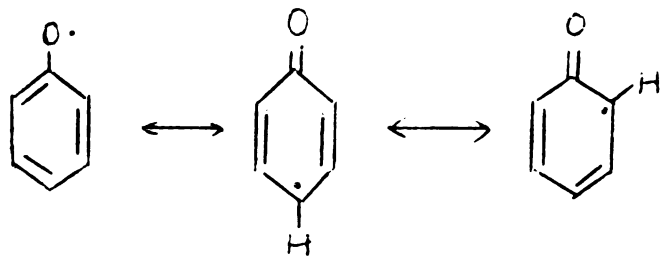

Figure 6. Stable resonance hybrid of the antioxidant free radical

As indicated previously, hydroperoxide decomposition (which will produce more free radicals) is the main initiation reaction of lipid oxidation and hydroperoxide

radical recombination is the main termination reaction of lipid oxidation. By reacting with the R. or ROO., free radical terminator antioxidants can prevent formation of hydroperoxide and accelerate the termination of the chain reaction. Therefore, these types of antioxidants are one of the most effective autoxidation inhibitors.

Most phenolic antioxidants have an optimum concentration. Exceeding the optimum concentration results in decreased stability of the substrate. The inversion of activity is more apparent in the case of tocopherol. It is because that at very high antioxidant levels the side reactions of the antioxidant (equation 17 and 18) may become predominant so that the inhibitor can act as a promoter of lipid oxidation (Lundberg et al., 1947).

AH (antioxidant) + O_2 ---->A. + HOO. .....(17)

AH (antioxidant) + ROOH --> RO. + H_2O + A. .....(18)

B. Oxygen Scavengers

Oxygen is an elementary substrate in lipid oxidation which can only be completely prevented by the total exclusion of oxygen or other oxidizing substances from the system. The other way to inhibit lipid oxidation is to eliminate oxygen by using oxygen scavengers. Generally an oxygen scavenger is a reducing agent like ascorbic acid which can react with oxygen.

C. Chelating Agents

Metal is a very important prooxidant in food. Chelating agents can chelate metal and form a stable complex. Generally a chelating agent is an organic acid and has a multiple negative charge or carboxylic group.

D. Important Compounds with Antioxidant Activity

(1) T-butylhydroxyanisole (BHA)

BHA is an isomeric mixture of 4-methoxy-3-t-butylphenol and 4-methoxy-2-t-butylphenol. The 3-isomer reacts more readily with oxidizing fat than the 2-isomer. Because the 2-isomer has a synergistic effect with the 3-isomer, the BHA isomeric mixture is better than pure 3-isomer. It is insoluble in water but is soluble in fats and oils. Usually it's dissolved in propylene glycol. Like most phenolic antioxidants, BHA is ineffective or only slightly effective in vegetable oils containing appreciable amounts of naturally occurring tocopherol antioxidants.

(2) Tertiary butylhydroquinone (TBHQ)

TBHQ is a white crystalline solid slightly soluble in water. Generally TBHQ is more active than BHT and BHA and other phenolic antioxidants in fried products (Kirleis and Stine, 1975: Buck, 1981). Because of its good antioxidant activity in fried food, TBHQ was recommended as the most suitable antioxidant for frying shortenings.

(3) 3,5-di-t-butyl-4-hydroxytoluene (BHT)

BHT is a white crystalline solid with good solubility in oil and good stability under different processing temperatures. Because of this trait, it may be used for the stabilization of fats in baked products.

(4) Ascorbic acid (Vitamin C)

Ascorbic acid is a white, odorless crystalline solid with a strongly acidic taste, and exists in nature both as the reduced and the oxidized form, dehydroascorbic acid. Stability in solution depends on pH, copper and iron content, exposure to oxygen and temperature.

Ascorbic acid is an oxygen scavenger and is readily oxidized in the presence of oxygen, particularly in alkaline solutions. The first step in the oxidation of ascorbic acid is the removal of the hydrogens from the hydroxyl attached to the enediol carbons, forming dehydroascorbic acid. Dehydroascorbic acid is readily hydrolyzed in aqueous solution to diketogulonic acid. Above pH 4 and in the presence of air, it readily oxidizes to give threonic acid; and in alkaline solutions it undergoes complete degradation to carbon dioxide.

Ascorbic acid and isoascorbic acid have been known for a long time to be inhibitors of fat oxidation (Olcott, 1941; Golumbic, 1941). The action of the antioxidant in such cases may be explained by oxygen binding. Besides acting as an oxygen scavenger, ascorbic acid may be active in

deactivating metals (Morris et al., 1950) and preventing enzymatic browning of fruits and vegetables.

In water/fat systems ascorbic acid does not always behave as an antioxidant. In certain cases, particularly in the presence of copper, ascorbic acid may act as a prooxidant or cause undesirable side reactions (Gelpi et al., 1955). Allen and Wood (1970) explained that in the presence of copper during ascorbic acid oxidation to dehydroascorbic acid free hydroxyl radicals (HO.) were produced. Dehydroascorbic acid is also a prooxidant under these conditions. Ascorbic acid is very sensitive to copper ions in buffered (pH = 5.5-4.7) emulsions of linoleic acid and becomes a prooxidant. As a consequence, a combination of ascorbic acid and citric acid or EDTA was recommended for preventing lipid oxidation, which would form chelates with heavy metals.

(5) Polyphosphates

Polyphosphates have been suggested as antioxidants for fat in heterogeneous systems (Lehmann and Watts, 1951; Watts, 1950) since they are known to be metal sequestering agents. Chang and Watts (1949) reported that polyphosphates were effective antioxidants in artificial aqueous fat systems within the pH range of normal meat.

(6) Tocopherol (Vitamin E)

Mixed tocopherol concentrate is a brownish red to red, nearly odorless, clear viscous oil. Tocopherols have several different forms (alpha, beta,....) present in all vegetable oils and in small amounts in animal fats. They are considered to be the major natural antioxidants of vegetable oils. Mixed tocopherols contain a hydroxylated dihydrochroman ring and a phytol side chain and they differ from one another in the number of methyl substituents. The relative efficiency of different tocopherol depends on the nature of the substrate, the temperature and possibly the concentration (Parkhurst et al., 1968). In practice, the use of tocopherol in vegetable oil is not generally beneficial because several forms are naturally present at adequate concentration. Therefore, the use of tocopherol is limited to products containing animal fats.

Antioxidant Properties of Rosemary

Chipault et al. (1952) measured the antioxidative effect of 32 kinds of spices and reported that the spice, rosemary, belonging to the family Labiatae has an especially strong antioxidative activity in a variety of fats (Chipault et al., 1952, 1955; Gerhardt and Blat, 1984). Chipault et al (1952, 1955) demonstrated that rosemary has antioxidant activities in different lipid systems. However, in most applications, rosemary as a "raw material" creates more

problems for the food manufacturer than it solves. For example, the food will need a large amount of rosemary to achieve the antioxidant effect and rosemary is difficult to disperse. Because rosemary extracts do not have the above problems, scientists have developed several processes to extract antioxidants from rosemary spice.

The use of rosemary spice extract as a natural antioxidant was first reported by Rac and Ostric (1955). Berner and Jacobson (1973) and Chang et al (1977) patented processes for the extraction of rosemary and sage followed by a vacuum steam distillation of the extract in an edible oil to obtain an odorless and flavorless natural antioxidant. They reported that rosemary extract is as effective as Tenox VI (BHA/citric acid/propylene glycol) when used in animal fat and is superior to this commercial antioxidant in vegetable oil. They also showed that 0.02% rosemary appeared to retard the development of reversion flavor in soybean oil and improved the flavor stability of potato chips. More recently, Bracco et al (1981) also reported the use of double step, falling film molecular distillation to obtain an active antioxidant from rosemary extract.

A. Antioxidant Compounds in Rosemary Extract

The crude extract of Rosemarinus officinalis L. is composed of at least 45 compounds. Sixteen of them belonging to classes of flavonoids (Brieskorn et al., 1964;

Brieskorn and Domling, 1969) diterpenes, steroids, triterpenes and hydrocarbons (Brieskorn et al., 1964). They were identified by infrared, ultraviolet, mass and nuclear magnetic resonance spectrometry (Wu et al. 1982).

In 1964, Brieskorn et al. isolated the most important antioxidant compound, carnosol, from rosemary leaves. Wu et al (1982). using different isolation methods, identified carnosol as the most active antioxidant constituent found in rosemary leaves. They reported that when carnosol was added to lard, its antioxidative efficacy was comparable to BHT. Bracco et al (1981). suggested that the antioxidant activity of rosemary extract must be primarily related to its carnosic acid and carnosol content.

Inatani et al (1982). isolated another antioxidant compound, rosmanol, from the leaves of the same plant. Rosmanol which has an antioxidative activity four times stronger than that of BHA, was also a phenolic diterpene and possessed a structure closely related to that of carnosol. In a subsequent study, Inatani et al (1983). reported that rosmanol was an effective antioxidant in several fat substrates. The antioxidant activity of rosmanol is similar to that of carnosol. Nakatani and Inatani (1984) isolated a new type of phenolic diterpene lactone, named rosmadial, from the leaves of rosemary and determined its structure.

Houlihan et al. (1984) isolated and identified a diphenolic diterpene compound, named rosmaridiphenol. When tested in lard, the antioxidant activity of this compound

was superior to BHA and approached the effectiveness of BHT. Later, they isolated another antioxidant compound, named rosmariquinone, from rosemary leaves. Pure rosmariquinone crystals were needle like red crystals. The antioxidant activity of rosmariquinone was superior to BHA, but it was slightly less effective than that of BHT (Houlihan et al., 1985). In addition to these specific compounds, several investigators have reported preparing various rosemary extracts which contained antioxidant activity.

The antioxidant activity of flavonols is apparently due to their ability to act as a free radical acceptor or to complex metal ions (Kelley and Watts, 1957). BHA and BHT function as antioxidants by terminating free radical chain type reactions which lead to lipid deterioration while citric acid functions via quenching of heavy metal catalyzed lipid oxidation (Richardson and Dahl, 1982). The antioxidant properties of oleoresin rosemary in sausage are attributed to its variety of isoprenoid quinone (Wu et al., 1982; Houlihan et al., 1984) which are capable of terminating free radical reactions and quenching reactive oxygen species (Richardson and Dahl, 1982). The antioxidative activity of phenol type antioxidants is due to free radical acceptors.

B. Inhibition of Lipid Oxidation by Rosemary Extract in Meat Products

The effectiveness of antioxidants depends not only upon the antioxidant but also upon the nature or composition of the substrate. When the same type of antioxidant is used with different samples of the same type of fat as substrates, different antioxidant results are experienced. These are due, at least in part, to slight differences in the content of the antioxidants and prooxidants naturally present or introduced during processing of the fat (Chipault et al., 1952). Previous studies (Chipault et al., 1952, 1955) have shown that the antioxidant effectiveness of a spice largely depends on the type of substrate in which they are used.

In early lipid oxidation studies, lard, baked piecrusts and fat emulsions were usually used as the substrate. Chipault et al. (1952, 1955) first used ground pork as a new substrate to study the antioxidant properties of spices in foods. They reported that sage and rosemary are the most effective antioxidants in this substrate and that these spices might contain powerful thermal stable phenolic antioxidants.

MacNeil et al (1973) used rosemary spice extract, polyphosphate and BHA plus citric acid in quality maintenance of deboned poultry meat and found that 0.05% rosemary extract and BHA plus citric acid were the most effective antioxidants followed closely by polyphosphate and

0.01% rosemary. They also found a 0.01% rosemary extract could improve the flavor of the mechanically deboned poultry meat.

In a study of the antioxidant properties of oleoresin rosemary in turkey sausage, Barbut et al. (1985) reported that the addition of 20 part per million oleoresin rosemary to 25% mechanically deboned and 75% hand deboned poultry sausage effectively reduced oxidative deterioration of lipid fractions when the sausages were stored at 4 C. From a commercial standpoint, incorporation of oleoresin rosemary in labile meat products can substantially suppress lipid oxidation and increase shelf life at refrigerated temperatures.

Synergism between Phosphates and Antioxidants

Several investigators have reported that phosphates can effectively retard oxidative spoilage of fruit, vegetable and cooked meat products, either alone or in combination with other approved antioxidants. Tims and Watts (1958) studied the effect of phosphates on Thiobarbituric acid (TBA) values of refrigerated cooked pork and found that phosphates could prevent cooked meat from oxidation. They also found a synergistic effect of ascorbic acid and tripolyphosphates on TBA values of cooked pork. Because tripolyphosphates are known to be metal sequestering agents, this may explain their antioxidant activity.

Eckey (1935) first reported that combinations of tocopherols and phosphates exhibited antioxidant activity in dry fats. He also reported increased stabilization when phosphates were used in bleaching fats or oils (Eckey, 1934). Calkins (1947) studied the synergistic action of phosphates with tocopherol and other quinone like phenolic compounds as antioxidant systems in fats and oils. They reported that the stability of their test system was dramatically increased as the level of phosphate increased from 0.009% to 0.09% in the presence of 0.02% quinone.

Lehmann and Watts (1951) previously found that EDTA or polyphosphates, both metal chelators, could act as antioxidants in synergism with ascorbic acid in aqueous fat systems. Chang and Watts (1950) demonstrated that phosphates and ascorbic acid reacted synergistically to retard hemoglobin catalysis of fats. In meat, oxidative enzymes (Tappel, 1952; 1954), hematin compounds (Haurowitz et al., 1941), traces of metals or metal ions, in particular, iron, copper and nickel (Ingold, 1963) heat, light and salt (Chen et al., 1984) generally influence the rate of lipid oxidation. Sequestrants or chelators combine with metal ions to form complexes which, depending on their stability, immobilize the metal ions in the system and, therefore, delay the onset of oxidation. It is well recognized that a synergistic effect exists when phenolic antioxidants are used together with citric acid or alkaline phosphates. This is attributed to the metal chelating function of the organic acids.

Measurements of Lipid Oxidation

A. 2-Thiobarbituric Acid (TBA) Test

The TBA test has been the most widely used test for measuring the extent of oxidative deterioration of lipids in muscle foods (Gray, 1978; Rhee, 1978). The principle of the test is that unsaturated fatty acid oxidation leads to formation of various oxidation products. One such product is malonaldehyde, which has been extensively measured by reacting it with 2-thiobarbituric acid to create a color pigment. The concentration of pigment can be converted to the so called "TBA values" which have been used to estimate rancidity development in muscle foods (Tarladgis et al., 1960).

There are three ways to do the TBA analysis on muscle foods (Rhee, 1978). It can be performed: (1) directly on the food product, followed by extraction of the colored complex (2) on an extract of the food (Witte et al., 1970; Vyneke, 1975); or (3) on a portion of the steam distillate of the food (Tarladgis et al., 1960). The distillation method is the most popular method for measuring the TBA values in muscle foods. It has been used to follow lipid deterioration during the cooking of beef, pork, and poultry (Huang and Greene, 1978; Igene et al., 1979b; Keller and Kinsella, 1973) as well as to study lipid deterioration in

relation to frozen storage and warmed over flavor of beef and poultry (Igene and Pearson, 1979; Igene et al., 1980; Sato and Hegarty, 1971).

Although the distillate method of Tarladgis et al. (1960) is the most popular method, it may not be the most accurate or reproducible method. It is found that food extracts TBA values are more consistent than distillation method TBA values. Witte et al. (1970) and Vyneke (1975) reported that the TBA values of muscle foods determined by the distillate method were twice as large as those determined on muscle food extracts. Other researchers also found that not only malonaldehyde but also other compounds like alka-2,4-dienals contribute to the TBA values (Jacobson et al., 1964; Marcuse and Johansson, 1973).

There are several possibilities for the differences between the distillation method and filtration method. Witte et al. (1970) and Siu and Draper (1978) suggested that the filtration method may have resulted in less extraction of malonaldehyde, since no heat was involved in the method. Heat may have promoted release of malonaldehyde from carbonyl compounds or produced more malonaldehyde from thermal decomposition of hydroperoxides. Rhee (1978) found that for muscle foods, such as fish, containing high levels of polyunsaturated fatty acids, any modification of the distillate method which reduces the chance of oxidation during blending and distillation lowers the TBA values.

Other investigators have modified the method. Moerck

and Ball (1974) added 20% BHA, 6% PG, and 4% citric acid in propylene glycol at the distillation stage to prevent oxidation of chicken meat during that step. Ke et al. (1977) added 100 mg each of PG and EDTA at the distillation stage to prevent further oxidation of mackerel samples. The addition of PG and EDTA probably did lower the TBA values of fish samples.

There are several factors which affect the TBA analysis. Zipser et al. (1964) reported that nitrite apparently reacts with malonaldehyde and will lower the TBA values. They modified the TBA method by adding sulfanilamide at the blending stage of the distillate method to prevent nitrite interference.

Another factor that may affect any TBA test is the microorganisms present in the food. Smith and Alford (1968) reported that certain bacteria such as those in the Pseudomonas genus can completely destroy alka-2,4-dienals which are the major contributors to the TBA absorbing species at 532 nm. Brown et al. (1979) reported that grass produced beef had higher levels of polyunsaturated fatty acids and lower TBA values than grain produced beef. The lower TBA value was thought to be because grass produced beef had an altered rumen microbial population when compared to grain fed beef.

Patton (1974) demonstrated that absorbance at 452 nm could occur by TBA reactions with aldehydes that were not oxidation products. They reported that fatty acids with

three or more double bonds must be present in the food fat for the TBA values at 532 nm to be correlated with the oxidized flavor.

Although malonaldehyde is a secondary product of lipid oxidation, it does not necessarily mean that the TBA values of food continue to increase throughout storage. TBA values have been observed to decline during frozen storage of cooked meat and fishery products. This could be due to malonaldehyde reacting with amino acids or protein in foods (Tarladgis et al., 1960; Benedict et al., 1975) or further degradation of malonaldehyde.

B. Hexanal Measurement

Hexanal is one of the major secondary products formed during oxidation of linoleic acid (Frankel et al., 1982). Ellis et al. (1968) and Badings (1970) agree that hexanal is the major aldehyde formed at ambient temperature. Cross and Ziegler (1965) examined the volatile carbonyl compounds of both cured and uncured meats and found that hexanal was present to a much greater extent in uncured ham than in cured ham. They assumed that hexanal was derived by oxidative cleavage of unsaturated fatty acid residues, probably linoleate. Shahidi et al. (1987b) used the hexanal content as an indicator of oxidative stability and flavor acceptability in cooked ground pork. They reported that the hexanal content, sensory scores, and TBA values of treated meats were linearly interrelated. Hexanal and

sensory values are more indicative of warmed over flavor (St. Angelo et al., 1987), while TBA values are not specific for WOF but are indicative only of oxidation (Sato and Hegarty, 1971; Sato et al., 1973; Pearson et al., 1977).

C. Sensory Evaluation

The rapid development of psychology, physiology, sociology, and statistics enabled the development of sensory evaluation from converging lines of knowledge (Peryam, 1990). Sensory evaluation can answer many of the questions a company asks when developing new consumer products. It is also the best indicator of food acceptability (Pearson et al., 1983). Sensory analysis has expanded to become a dynamic force in the food industries (Chambers, 1990).

There are theoretical and practical reasons for investigating the relationships between instrumental and sensory flavor data. From a practical point of view the relations between instrumental and sensory may be used in such a way that panel work can be complemented or supplemented with instrumental methods in routine quality control or in product and process development work. In 1985 Igene et al. reported high correlation between TBA values and trained panel scores for warmed over flavor in cooked chicken white and dark meat. Many researchers (Zipser et al.,1964, Smith et al., 1987, Shahidi et al., 1987b) also reported high correlation coefficients (r = 0.5-0.986) between TBA values and relative sensory scores. Lai (1989)

and Stoick (1989) reported a statistically significant but moderate correlation coefficient between TBA values and sensory scores of chicken nuggets and restructured beef steaks in refrigerated and frozen studies. Lai (1989) thought that the variability among literature values may be due to the different methods and the number of factors involved in the experiments. Because of inconsistencies of terminology and sensory scales used for warmed over flavor in sensory evaluation, a wide variation of sensory data exists.

D. Peroxide Value

The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Tsai et al. (1978) reported peroxide value (PV) appeared to be closely related to TBA values. Jeremiah (1980) reported significant relationships between PV and flavor rancidity scores for the fresh pork samples. Because of the transitory nature of the peroxide and its sensitivity to temperature changes, measurement of PV is useful only when extensive decomposition of hydroperoxides begins (Pearson et al., 1977).

There are many analytical procedures for the measurement of the PV. Iodometric methods are the most common methods for measuring PV. They are based on the measurement of the iodine produced from potassium iodide by the peroxides present in the oil. The results and

suitability of the test depend on the experimental conditions and the reducing agent employed. Because the large variation in results using different methods, the method used in the determination should also be accompanied with PV data.

MATERIALS AND METHODS

Materials

Two studies were done to accomplish the objectives stated in the introduction. The first study included various non meat additives including OR in a restructured pork steak. This study was further divided into refrigerated (4°C) and frozen (-30°C) storage treatments. The second study used a meat fiber model system with selected non meat additives.

A. Ingredients and Additives for Restructured Pork Steaks

Pork shoulders were obtained uncured from Peet Packing Co. (Cheasing, MI) and processed within 24 hr of purchase. Salt was obtained from International Salt Co. (Clark Summit, PA). Sodium tripolyphosphate (STPP) was purchased from Stauffer Chemical Co. (Westport, CT). Citrate was supplied by Fisher Scientific Company. TBHQ was donated by Eastman Chemical Product Inc. (Kingsport, TN). The oleoresin rosemary and sage were prepared by Kalsec Inc. (Kalamazoo, MI). All of the additives were food grade. All chemicals and solvents used in both studies were reagent grade. The

nylon film (Koch Supplies Inc. St. Kansas City, MO) used as overwrap in both studies has a water vapor transmission rate of 0.041 ml/m^2/day/mmHg and oxygen transmission rate of 0.124 ml/m^2/day/mmHg at 22.7^{o}C, 50% RH. However, it is recognized that this information is useful only in treatments in which steaks were vacuum packaged.

B. Ingredients and Additives for the Meat Fiber Model System

Uncured pork leg was obtained from the M.S.U. Meat laboratory and stored at - 30^{o}C. Major muscles utilized included semimimbranosus, semitendinosus, gracilis, adductor, biceps femoris, vastus intermedius, vastus lateralis, vastus medialis and rectus femoris. Horse hemoglobin (Hb) was purchased from Sigma Chemical Company (St, Louis, MO). Ferrous sulfate anhydrate was purchased from Fisher Scientific Company. All chemicals used in this study were reagent grade.

Methods

A. Preparation of Restructured Pork Steaks

Uncured pork shoulders were trimmed to 12% fat content. The fat content was estimated both visually and using a Hobart F101 Fat Tester. The shoulders were chunked through a kidney plate one time using a Hobart grinder (Figure 7). The meat was randomly divided into 10 portions for 10 different additive treatments (Table 1). Every portion

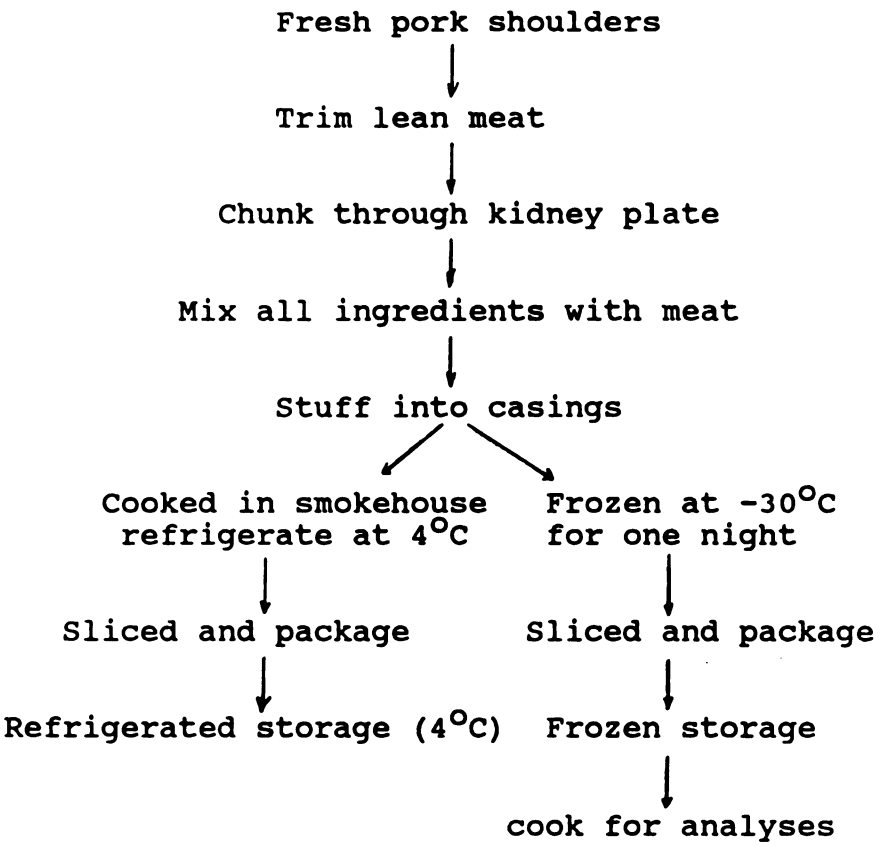

Fig 7. Flow chart for manufacturing restructured pork steaks

Table 1. Experimental treatments used to determine the effectiveness of oleoresin rosemary (OR) as an antioxidant in restructured pork steaks[a]

1.	salt 0.75%
2.	salt 0.75% + STPP 0.5%
3.	salt 0.75% + citrate 0.01%
4.	salt 0.75% + STPP 0.5% + OR (WM) 0.05%[bc]
5.	salt 0.75% + STPP 0.5% + OR (WM) 0.10%
6.	salt 0.75% + STPP 0.5% + OR (W) 0.10%
7.	salt 0.75% + STPP 0.5% + TBHQ 0.02%
8.	salt 0.75% + citrate 0.01% + OR (WM) 0.10%
9.	salt 0.75% + sage 0.05% + STPP 0.5%
10.	treatment #7 vacuum packaged

[a] STPP = sodium tripolyphosphate; OR = oleoresin rosemary WM = water soluble type; W = oil soluble type; TBHQ = tertiary butylhydroquinone

[b] The percentage of all ingredients are based on total weight of meat except for antioxidant concentrations which are based on the fat content (12%).

[c] OR (WM) relative antioxidant activity = 60 %; OR (W) relative antioxidant activity = 70 %

included 2.5 kg of meat for the frozen and 3 kg of meat for the refrigerated treatments of the study. Each portion of chunked meat was mixed with the designated ingredients (Table 1) in a Keebler mixer (Keebler Inc., Chicago, IL) for 12 min. The ingredients were added to the meat within the first 30 sec of mixing. Care was taken so meat was removed from the dead corners of the mixer. Each treatment was stuffed into 8.8 cm diameter fibrous, moisture proof casings (Viskase Corp., Chicago, IL).

The pork rolls for the refrigerated treatments were cooked immediately in the smokehouse to an internal temperature of 74.4^{o}C according to a designed computer program (appendix A) for approximately 4.5 hr. After cooking the pork rolls were chilled to 4 C overnight. The pork rolls were sliced into 1.2 cm thick steaks on a Hobart Food Slicer model 410 (Hobart MFG, Co., Troy, OH). Pork steak treatments 1-9 were placed on retail meat trays, placed in nylon film pouches and stored at 4^{o}C. Treatment 10 was vacuum packaged as a frozen stability study reference for sensory evaluation. Lipid oxidation was evaluated at 0, 1, 2, 4, 6, and 8 days after cooking for TBA values and 0, 1, and 4 days for sensory attributes.

The pork rolls for the frozen treatments were frozen at -30^{o}C overnight and sliced into 1 cm thick pork steaks. Each treatment was placed on a tray, wrapped as described above and stored at -30^{o}C. Treatment 10 was again vacuum packaged and frozen as reference for sensory evaluation.

Before analyses, samples were thawed in the refrigerator for one night and cooked in a convection oven to an internal temperature of 70°C. Each sample was analyzed as soon after cooking as possible. The temperature was monitored by copper constantan thermocouples placed in the center of the individual steaks. TBA tests and sensory evaluations were carried out after 2, 4, and 8 months of storage. Hexanal contents were measured at 2, 4 and 8 months only in selected treatments; treatment 1 (control), treatment 2 (treatment with phosphate only) and treatment 5 (treatment with the highest OR concentration).

B. Preparation of the Meat Fiber Model System

Water washed muscle fibers (WF) were prepared according to Love and Pearson (1974) as shown in Figure 8 from uncured pork leg. All visible fat and connective tissue was removed from the muscle before grinding through a 3 mm plate using an electric grinder (Model A-200, Kitchenaid Division, Hobart Co. Troy, OH). A 1000 g sample of ground pork was extracted 10 times with 4 volumes of water at 4°C. The water and meat were stirred with a glass rod every 10 min. The final slurry was centrifuged at 2000Xg for 10 min after holding overnight. The resulting WF were vacuum packaged in nylon pouches and stored at -30°C (usually less than 1 week). The moisture and fat content of raw WF was analyzed according to AOAC procedures (1984).

The WF were divided into 12 treatments which were

formulated as described in Table 2. Water washed muscle fibers were mixed with ingredients and cooked immediately in a boiling water bath to an internal temperature of 70°C and then stored in a refrigerated cooler (4°C) immediately. Lipid oxidation was measured by TBA test at 0, 1, 4, and 7 day.

C. Assessment of Lipid Oxidation

Three methods were used to assess lipid oxidation (1) TBA method; (2) Sensory evaluation; (3) Hexanal analyses; and (4) Statistical analysis.

(1) TBA method

The distillation method of Tarladgis et al. (1960) was utilized as modified by Crackel et al., (1988). Two pieces of pork steak (80 g) were ground one time through a 3 mm plate. A 40 g portion of ground pork was mixed with 20 g of antioxidant solution (1g PG/ 1g EDTA/ 40 ml alcohol/ 160 ml distilled water) and 20 g distilled water in a beaker and homogenized (Tekmar company, Cincinnati, Ohio) for 30 sec. A 20 g aliquot was transferred with 87.5 ml of distilled water into a 500 ml Kjeldahl flask. Additionally 2.5 ml of HCl solution (4N), 5 drops of antifoam (Sigma Chemical Co., St. Louis, MO) and 2 boiling chips were added to the Kjeldahl flask. This mixture was then distilled at the second highest setting on a Kjeldahl distillation apparatus until 50 ml of distillate was collected. A 5 ml aliquot of

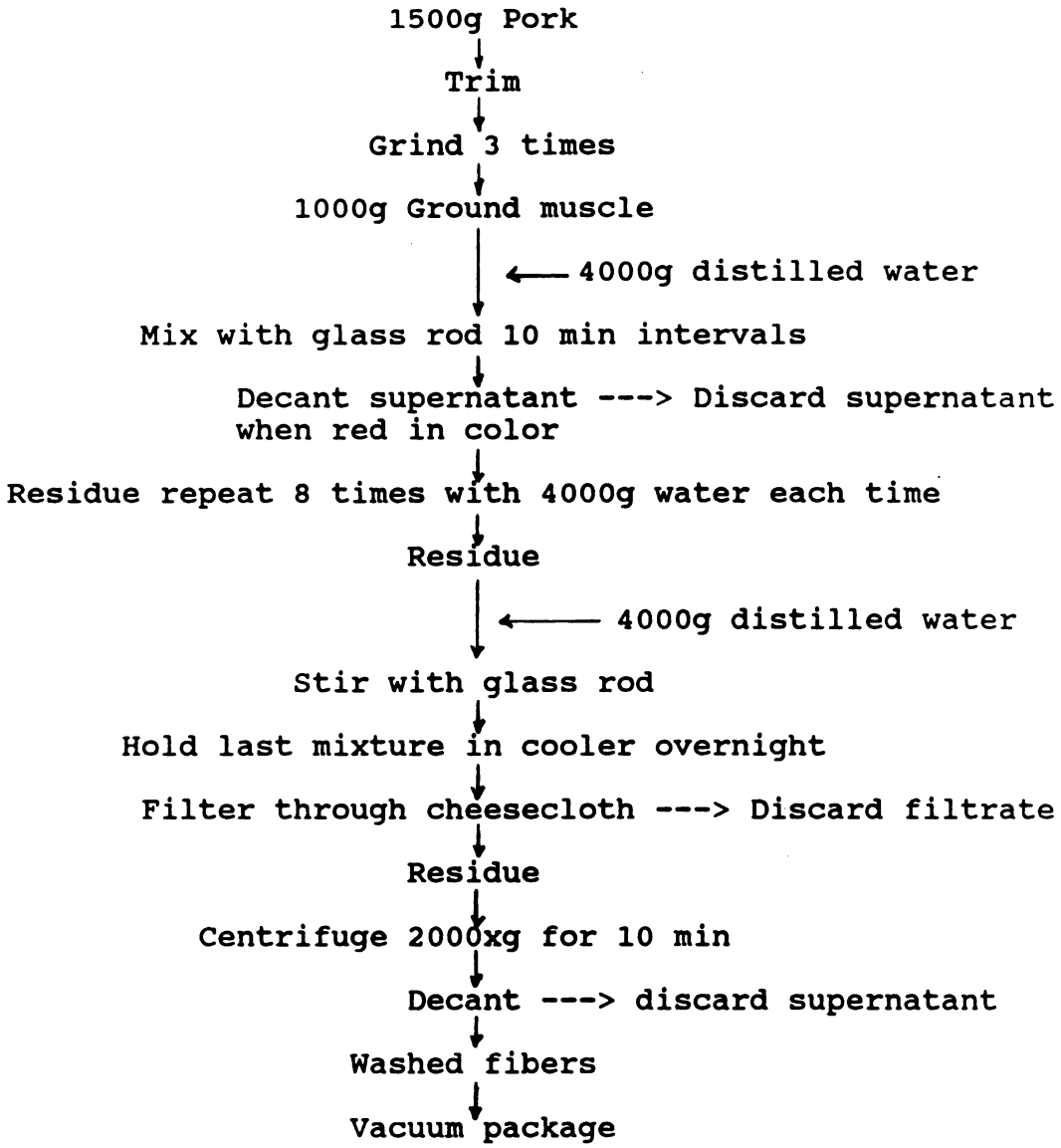

Figure 8. Flow chart for preparation of water washed muscle fibers (WF)

Table 2. Experimental design for treatments studying the synergism of oleoresin rosemary (OR) and sodium tripolyphosphate (STPP) in a meat fiber model system

1.	WF[a]
2.	WF + OR (WM) 0.1%[b]
3.	WF + STPP 0.5%
4.	WF + OR (WM) 0.1% + STPP 0.5%
5.	WF + Fe (1 mg/kg WF)
6.	WF + Fe (1 mg/kg WF) + OR (WM) 0.1%
7.	WF + Fe (1 mg/kg WF) + STPP 0.5%
8.	WF + Fe (1 mg/kg WF) + OR (WM) 0.1% + STPP 0.5%
9.	WF + Hb (16.5 mg Fe/kg WF)
10.	WF + Hb (16.5 mg Fe/kg WF) + OR (WM) 0.1%
11.	WF + Hb (16.5 mg Fe/Kg WF) + STPP 0.5%
12.	WF + Hb (16.5 mg Fe/kg WF) + OR (WM) 0.1% + STPP 0.5%

[a] WF = water extract muscle fiber; OR (WM) =water soluble oleoresin rosemary with 60 % relative antioxidant activity STPP = Sodium tripolyphosphate; Hb = horse hemoglobin

[b] The percentage of STPP was based on total weight of WF. Antioxidant concentration was based on the fat content (3.7%), Ferrous Sulfate anhydrate concentration was based on 1 mg ferrous ion per kilogram of WF and Hb was based on 16.5 mg of iron per kilogram of WF. (Love and Pearson, 1974)

the distillate was mixed with 5 ml of TBA reagent (0.02 M) in a test tube and its cap closed. The stoppered test tubes were heated for 35 min in a boiling water bath and cooled for 10 min under tap water. Absorbance of each sample was read on a Beckman model ACTA CIII spectrophotometer (Beckman Instruments, Inc., San Ramon, CA) at 532 nm. A standard curve was derived by using pure tetramethoxypropane (TMP). Absorbance readings obtained were multiplied by 6.2 to obtain a TBA value (mg malonaldehyde/kg of meat) (Crackel et al., 1988).

(2) Sensory evaluation

Sensory evaluation for oxidized flavor was carried out by 15 experienced panelists who had been trained and selected by triangle test for more than 40 hr in beef and chicken oxidized flavor studies. At each setting, all panelists were presented with three different coded samples representing the different treatments and one reference sample. The environmental conditions of the tasting facility included partitioned booths with fluorescent light which did not influence the appearance of the steaks being tasted. All of the samples were evaluated on a 6 point scale (Appendix B) with 0 described as absent of oxidized flavor and 5 very strong oxidized flavor. Each treatment containing 2 samples (4g/sample) per panelist was reheated in petri dishes by microwave for 15 sec and served hot.

(3) Hexanal analysis

A vacuum collection/solvent extraction method which was developed by Stoick (1989) was used to analyze for hexanal. For the purge-and-trap procedure, samples (15g) were placed in a 500 ml flat bottom flask to which 4 ml of aqueous 2-heptanone solution (internal standard, 16.6 ng 2-heptanone/ml solution) and 36 ml of distilled water were also added. The flask was connected to a condenser. The exit end of the condenser was connected to one end of a glass trap that was packed with 200 mg (60/80 mesh) of Tenax (Tekmar company, Cincinnati, OH) sandwiched between two plugs of volatile free glass wool (Vercellotti et al., 1987). The traps were conditioned at 210^{o}C under a stream of nitrogen (20-30 ml/min) for 16-20 hr then capped and stored in a desiccator until used. A 0.35 kg/cm^{2} vacuum was applied to the other end of the glass trap. The sample was stirred and incubated at 60^{o}C for 1.5 hr. The volatiles were desorbed from the Tenax cartridge by charging 3 ml of 2-methylbutane and centrifuged in Ice Clinical centrifuge (Damon/Ice Division, Needham, Mass) at approximately 50xg for 2 min to retention the volatiles. The extract was flushed by nitrogen to 0.5 ml and a 2 ul sample was immediately injected onto the GC column using a prechilled 10 ul syringe.

A HP 5890 gas chromatography equipped with a 50m X 0.32 mm id carbowax 20M fused silica capillary column (Hewlett-Packard Co., Avondale, PA) was used. The flow rate of the

carrier gas (He) was 0.5 ml/min with a split ratio of 3:1. The oven temperature was held at 40°C for 10 min and was increased at a rate of 3°C/min from 40°C to 80°C, followed by a rate of 10°C/min from 80°C to 200°C for 5 min. Injector and detector temperatures were 225°C and 275°C, respectively. Hexanal was identified by comparing its relative retention time with a standard and was corrected to 100 % recovery based on an internal standard.

D. Statistical Analysis

Three replicate experiments were done for the two studies. The experiments were designed as a three factor (treatment*time*replication) complete randomized model with balanced data. Means, standard errors, sum of square and mean square errors were calculated using the MSTAT-C microcomputer statistical program (Michigan State University, 1989).

The Bonferroni t-test was used to make contrasts between specific treatments. Tukey's test was used to determine the efficiency of the antioxidant. Interactions of main effects and correlation between sensory scores and results from chemical analyses were interpreted according to Gill (1978).

RESULTS AND DISCUSSION

Evaluation of Restructured Pork Steaks and Water Washed Muscle Fibers

A. Proximate Analysis

The proximate composition of the restructured pork steaks is listed in Table 3. The average moisture and protein content of the restructured pork steaks were 67 % and 17 %, respectively. These values are consistent with Huffman et al. (1981) and Chastain et al. (1982) who reported 67-69 % moisture and 16-17 % protein in restructured pork steaks. The average lipid content of the restructured pork steaks was 13 % which is quite close to the target lipid content of 12 %.

The proximate composition of the water washed muscle fibers (WF) are also presented in Table 3. The average fat content of the WF was similar to previously reported values of water washed beef muscle fibers (Igene and Pearson, 1979b). This value was also consistent with Schweigert (1987) who indicated that carefully selected and trimmed lean muscle may have as little as 3 to 5 % fat. The moisture content of the WF was 2 % higher than that reported by Igene and Pearson (1979b) but 1.5% lower than that

Table 3. Chemical composition of raw restructured pork steaks and washed pork fibers [a]

	Moisture (%)[a]	Fat (%)[b]	Protein (%)[c]
Restructured pork steaks	67.5 ± 0.2	12.8 ± 0.5	16.7 ± 3.5
Washed pork fibers	81.8 ± 0.3	3.6 ± 0.1	8.6 ± 0.8

[a] Mean±standard error of the mean.
[b] Values represent means of three replications which were analyzed in 5 samples.
[c] Values represent means of three replications which were analyzed in triplicate.

Table 4. Smokehouse yield of restructured pork steaks

Treatment[a]	% yield [bc]
1. Salt	81 ± 4.9
2. Salt + STPP	96 ± 0.1
3. Salt + Citrate	77 ± 8.2
4. Salt + STPP + OR (WM 0.05%)	96 ± 0.1
5. Salt + STPP + OR (WM 0.10%)	95 ± 0.6
6. Salt + STPP + OR (W 0.10%)	95 ± 0.1
7. Salt + STPP + TBHQ	92 ± 2.8
8. Salt + Citrate + OR (WM 0.10%)	80 ± 4.2
9. Salt + sage + STPP	94 ± 0.5

[a] STPP: Sodium tripolyphosphate, OR: Oleoresin rosemary, WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.
[b] Values represent means of two replications which were analyzed in duplicate.
[c] Mean ±standard error of the mean.

attained by Stachiw (1988). This may due to sample variation or different processing methods. The average protein content of the WF was 9 % which was similar to what Stachiw (1988) reported in washed ham muscle (8-9 % protein).

B. Cooking Yield of Restructured Pork Steaks during Refrigerated Storage

The cooking yields of the different treatments of restructured pork steaks are presented in Table 4. Treatments containing sodium tripolyphosphate (STPP) had a significantly ($p<0.01$) higher yield than those without STPP. These results are similar to previously reported studies (Shults et al., 1972; and Matlock et al., 1984). The mechanism of the action of alkaline phosphates in improving water binding appears to be: (1) raising the pH of the meat; and (2) solubilization of muscle proteins (Swift and Ellis, 1956; Shults et al., 1972). Treatments containing STPP had more consistent yield (i.e. less variation) than those treatments which did not contain STPP (Table 4). This may be due to higher water binding capacity. Therefore, cooking loss and variation during cooking was less. Treatments containing citrate had similar cooking yields when compared to the salt control because the amount of citrate was low and assumed not to influence pH or muscle protein.

Evaluation of Antioxidant Efficiency in Restructured Pork Steaks during Refrigerated Storage

A. TBA Values and Sensory Scores

TBA values for restructured pork steaks during refrigerated storage are presented in Table 5 and Figure 9. The average TBA value of the raw pork shoulders was 0.28 which indicates that the raw pork shoulders were not oxidized. It is suspected that mistakes were made in the TBA analysis procedure for the replicate 2. Therefore, TBA data from only replication 1 and 3 TBA data are presented for the refrigerated study. Sensory scores for various treatments of restructured pork steaks during refrigerated storage are presented in Table 6. Analysis of variance demonstrated significant interaction between treatments and time of refrigerated storage (appendix C). Specific contrasts between treatments were made using Bonferroni t-test at each level of storage time for both TBA values and sensory scores. These results are presented in Table 7.

The TBA values for all treatments increased over time (Table 5 and Figure 9). Treatments described as control (with salt), citrate only and OR/citrate had the highest TBA values and oxidized most rapidly. There were no significant differences in TBA values among these three treatments. Treatments with STPP and STPP plus antioxidant had lower TBA values. In the corresponding sensory scores (Table 6) a similar trend exists. Control (with salt), citrate only and

OR and citrate treatments had the highest oxidized flavor.

There were highly significant differences ($p<0.01$) in TBA and sensory values between control and any treatment containing STPP (Table 7). This indicates that STPP has a protective effect on cooked restructured pork steaks held under these conditions. These results are in agreement with those reported previously (Shahidi et al., 1986, 1987a; Lai, 1989; and Stoick, 1989). The antioxidant role of polyphosphates is suspected to be due to their ability to sequester heavy metals (Watts, 1950; Tims and Watts, 1958), particularly the ferrous ion, which is the major prooxidant in meat systems (Love and Pearson, 1974; Igene et al., 1979a).

Citrate did not significantly retard lipid oxidation in this study (Table 7) even though the TBA values are lower than the control on day 0 and day 1 (Table 5). This is agrees with the results of Olcott and Mattill (1936) who demonstrated that citric acid was ineffective in lard. Shahidi et al. (1986) reported that citric acid (0.05%) and monoglyceride citrate (0.05%) had only a slight effect during the first few days when storing cooked ground pork. The variation may be due to different food materials studied and the experimental errors of each research group. Another possible explanation is that citrate (0.01%) is such a weak antioxidant (Sheu, 1988) that it is not efficient enough to retard lipid oxidation in foods that are labile to oxidation or retard lipid oxidation under conditions that are labile

Table 5. TBA[a] values of cooked restructured pork steaks during refrigerated storage at 4°C [b]

Treatment[c]	Storage time (Days)					
	0[d]	1	2	4	6	8
1.Salt	1.71	4.33	5.15	7.41	8.92	11.46
2.Salt+STPP	0.36	0.54	1.27	0.98	1.04	0.69
3.Salt+Citrate	0.82	3.61	5.85	7.53	6.20	10.69
4.Salt+STPP+OR (WM 0.05 %)	0.34	0.56	O.85	1.01	1.64	1.31
5.Salt+STPP+OR (WM 0.10 %)	0.17	0.38	0.62	0.61	1.21	1.84
6.Salt+STPP+OR (W 0.10 %)	0.25	0.32	0.65	0.61	0.77	1.33
7.Salt+STPP+TBHQ	0.20	0.44	0.41	0.49	0.46	0.38
8.Salt+Citrate+ OR (WM 0.10 %)	0.88	3.34	5.64	7.16	8.82	10.45
9.Salt+STPP+Sage	0.25	0.80	0.67	0.87	0.89	0.80
MSE[e]	0.11	0.20	0.63	0.24	0.56	0.93

[a] mg malonaldehyde per kg meat.
[b] All values represent means of two replications
[c] STPP:Sodium tripolyphosphate, OR: Oleoresin rosemary, WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.
[d] Storage time designated as 0 day represents steak analyses immediately after formulating, cooking, chilling overnight, slicing, and packaging.
[e] MSE: mean square error over the same storage time

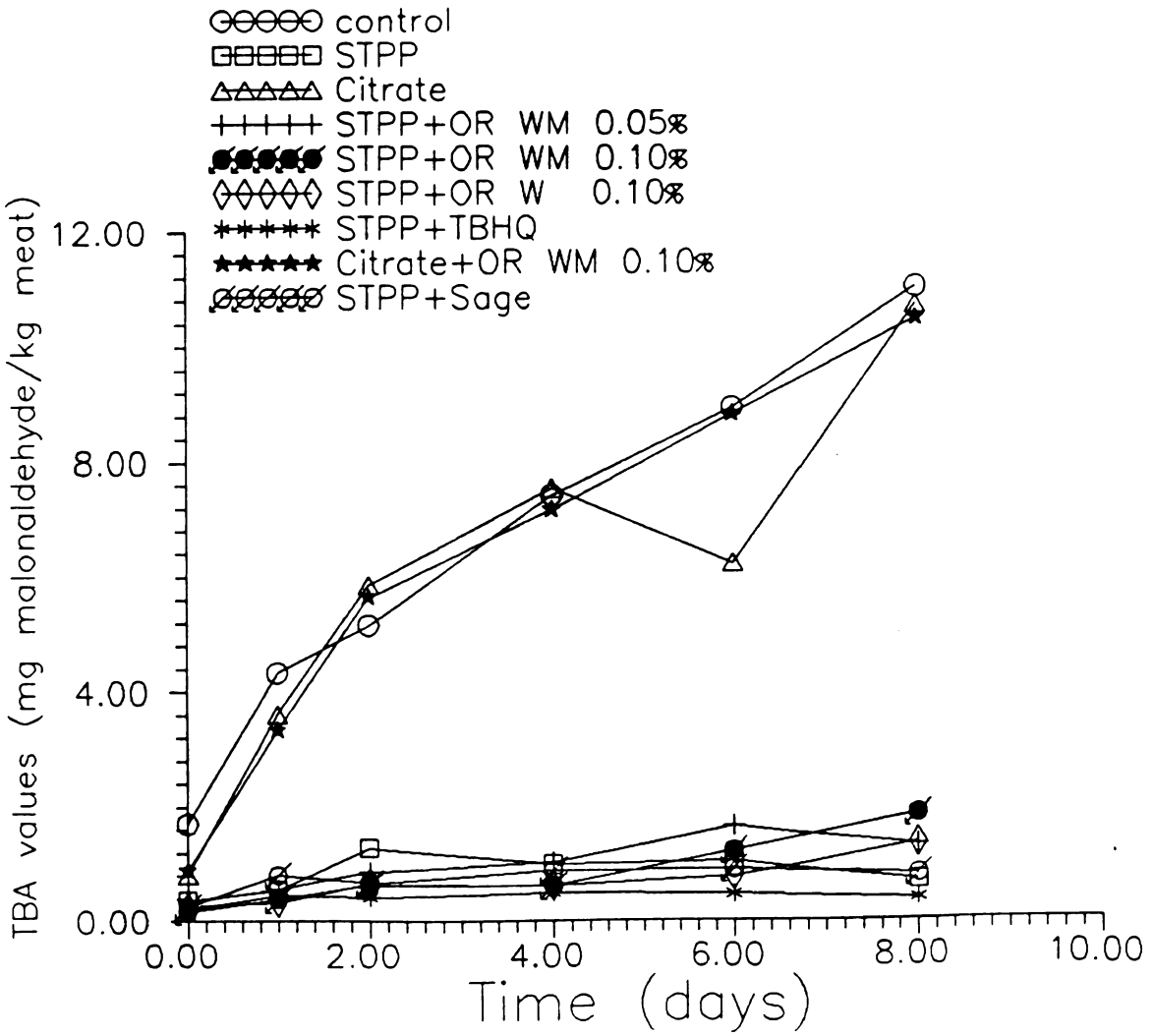

Fig 9. TBA values of cooked restructured pork steaks stored at $4^{o}C$

Table 6. Sensory values[a] for cooked restructured pork steaks during refrigerated storage [b]

	Storage time (Days)		
treatment [c]	day 0[d]	day 1	day 4
1.Salt	1.9	2.6	3.1
2.Salt+STPP	0.9	1.3	1.5
3.Salt+Citrate	1.9	3.2	3.2
4.Salt+STPP+OR (WM 0.05%)	0.8	1.2	1.6
5.Salt+STPP+OR (WM 0.10%)	0.7	1.1	2.0
6.Salt+STPP+OR (W 0.10%)	0.6	0.9	1.1
7.Salt+STPP+TBHQ	0.7	0.8	1.5
8.Salt+Citrate+OR (WM 0.10%)	1.4	2.1	3.0
9.Salt+STPP+Sage	0.8	1.4	1.6
MSE[d]	0.1	0.1	0.1

[a] Scale: 0 = absent, 5 = very strong.
[b] All values represent the average of 3 replications.
[c] STPP:Sodium tripolyphosphate, OR: Oleoresin rosemary, WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.
[d] Storage time designated as 0 day represents steak analyses immediately after formulating, cooking, chilling overnight, slicing, and packaging.
[e] MSE: Mean square error over the same storage time.

Table 7. Significance levels of designed contrasts for treatment effects on TBA values and sensory scores of cooked restructured pork steaks during refrigerated storage

treatment[a] contrasts		Day					
		0	1	2	4	6	8
1.Control vs STPP	TBA	0.01	0.01	0.01	0.01	0.01	0.01
	Sensory	0.01	0.01		0.01		
2.Control vs Citrate	TBA	ns	ns	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns	ns	ns
3.STPP vs Citrate	TBA	ns	0.01	0.01	0.01	0.01	0.01
	Sensory	0.01	0.01		0.01		
4.STPP vs STPP/OR	TBA	ns	ns	ns	ns	ns	ns
	Sensory	ns	ns		ns		
5.STPP/Antioxidant vs STPP/TBHQ	TBA	ns	ns	ns	ns	ns	ns
	Sensory	ns	ns		ns		
6.STPP vs STPP/Sage	TBA	ns	ns	ns	ns	ns	ns
	Sensory	ns	ns		ns		
7.Citrate vs STPP/TBHQ	TBA	ns	0.01	0.01	0.01	0.01	0.01
	Sensory	0.01	0.01		0.01		
8.STPP/OR WM 0.05% vs STPP/OR WM 0.1%	TBA	ns	ns	ns	ns	ns	ns
	Sensory	ns	ns		ns		

[a] STPP:Sodium tripolyphosphate, OR: Oleoresin rosemary, WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.

[b] ns: not significant

to accelerate lipid oxidation such as cooking and high temperature storage. When comparing antioxidant efficiency of treatments containing citrate, STPP and STPP/TBHQ (Table 7), it is observed that STPP and STPP/TBHQ have a better antioxidative effect than citrate when used alone. This may support Sheu's (1988) conclusion that citrate is not an effective antioxidant. There were no significant differences between STPP and STPP/TBHQ (Table 7).

The addition of OR as well as sage to treatments with STPP did not have additional measurable benefits in retarding lipid oxidation (Table 7). Sheu (1988) reported that a synergistic effect between OR and STPP existed in precooked pork patties. However, the addition of OR to treatments containing STPP resulted in significantly lower TBA values ($p<0.05$) after 32 days of storage. In another study examining the antioxidant effect of OR in chicken nuggets, Lai (1989) reported no interaction between OR and STPP and therefore, Lai (1989) was not able to demonstrate a synergistic effect between OR and STPP.

The addition of different concentrations of OR with STPP did not result in significant differences (Table 7). This is in agreement with Lai's (1989) study. It was also found that there were no significant differences between OR (W)/STPP and OR (WM)/STPP treatments as measured by TBA or sensory testing (Table 7).

Evaluation of Antioxidant Efficiency in Restructured Pork Steaks during Frozen Storage

A. TBA Values and Sensory Scores

TBA values and sensory scores of restructured pork steaks stored at - 30°C are presented in Table 8 and 9, respectively. The TBA values are also graphed in Figure 10. Analyses of variance (appendix D) indicated time and treatment effects were significant ($p<0.01$). However, no time and treatment interaction was found to be significant. This was contrary to similar reports by Lai (1989) and Stoick (1989) who used similar antioxidants in chicken nuggets and restructured beef steaks respectively. These results may be due to different sources of raw materials, specie effects or differences in cooking methods. Because there were no time and treatment interactions, the treatment effects were analyzed over the mean of all sampling periods. Specific contrasts were made using the Bonferroni t-test and are presented in Table 10.

The TBA values and sensory scores of all treatments increased over frozen storage time except for the 8 month sensory scores which either decreased or leveled off (Table 8 and 9). This phenomenon was also noted in the cooked, refrigerated study and is similar to results reported by Poste et al. (1986). Igene and Pearson (1979) also found that samples containing high total lipids exhibited the highest TBA values, but did not consistently show pronounced

Table 8. TBA[a] values of restructured pork steaks during frozen storage at -30°C[b]

Treatment[c]	Storage time (months)			
	0[d]	2	4	8
1.Salt	1.27	1.21	1.25	1.87
2.Salt+STPP	0.25	0.28	0.41	0.58
3.Salt+Citrate	1.01	1.16	0.96	1.36
4.Salt+STPP+OR (WM 0.05%)	0.24	0.31	0.34	0.49
5.Salt+STPP+OR (WM 0.10%)	0.12	0.25	0.26	0.48
6.Salt+STPP+OR (W 0.10%)	0.17	0.36	0.35	0.61
7.Salt+STPP+TBHQ	0.14	0.27	0.28	0.38
8.Salt+Citrate+OR (WM 0.10%)	0.61	0.50	0.56	0.99
9.Salt+Sage+STPP	0.16	0.29	0.31	0.60
MSE[e]Storage time	0.14	0.07	0.02	0.01

[a] mg malonaldehyde per kg meat
[b] All values represent the average of three replications
[c] STPP: Sodium tripolyphosphate, OR: Oleoresin rosemary WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.
[d] Storage time designated as 0 day represents steak analyses immediately after formulating, cooking, chilling overnight, slicing, and packaging.
[e] MSE: Mean square error of the same storage time.

Table 9. Sensory[a] values of restructured pork steaks during frozen storage at -30°C[b]

Treatment[c]	Storage time (months)			
	0[d]	2	4	8
1.Salt	1.91	2.67	3.08	2.71
2.Salt+STPP	0.85	1.34	1.81	1.78
3.Salt+Citrate	1.93	2.09	2.50	2.86
4.Salt+STPP+OR (WM 0.05%)	0.76	1.55	1.70	1.58
5.Salt+STPP+OR (WM 0.10%)	0.67	1.28	1.83	1.62
6.Salt+STPP+OR (W 0.10%)	0.56	1.38	1.58	1.40
7.Salt+STPP+TBHQ	0.66	1.21	1.71	1.82
8.Salt+Citrate+OR (WM 0.10%)	1.39	1.69	1.99	2.40
9.Salt+STPP+Sage	0.76	1.32	1.69	1.99
MSE[e] Storage time	0.09	0.07	0.09	0.05

[a] Scale: 0 = absent, 5 = very strong oxidized flavor
[b] All values represent means of three replications
[c] STPP: Sodium tripolyphosphate, OR: Oleoresin rosemary WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.
[d] Storage time designated as 0 day represents steak analyses immediately after formulating, cooking, chilling overnight, slicing, and packaging.
[e] MSE: Mean square error of the same storage time

warmed over flavor (WOF). The discrepancy in trends after 8 month between the TBA values and the sensory scores in this study may be due to interference from rancid aroma. Rancid aromas gradually mask the initial WOF perceived by the panelists (Poste et al., 1986). In the later stages of this study some panelists had difficulty discriminating between rancid and warmed over flavors. Control (treatment with salt), treatment with citrate only and treatment with citrate/OR demonstrated the highest ($p<0.01$) TBA values (Table 8) and sensory scores (Figure 9). Treatments containing STPP and antioxidant had lower TBA values and sensory scores when compared to the control (Table 10). A similar trend was observed during cooked refrigerated storage treatments (Tables 5 and 6) except that citrate had no antioxidant effectiveness.

The STPP/OR treatments were not significantly different ($p<0.05$) from the citrate/OR treatment during frozen storage. This may be due to citrate being a mild antioxidant (Sheu, 1988; Shahidi et al., 1986). Citrate may only have antioxidant efficiency at the mild lipid oxidation conditions or during the first few days of more severe lipid oxidation conditions. During frozen storage, citrate and low temperatures slowed lipid oxidation. In the refrigerated study, samples were cooked prior to storage. Cooking may provide energy for initiation and thus accelerate lipid oxidation (Igene et al., 1979b). Heat may also destroy some of the citrate. During the cooking stage

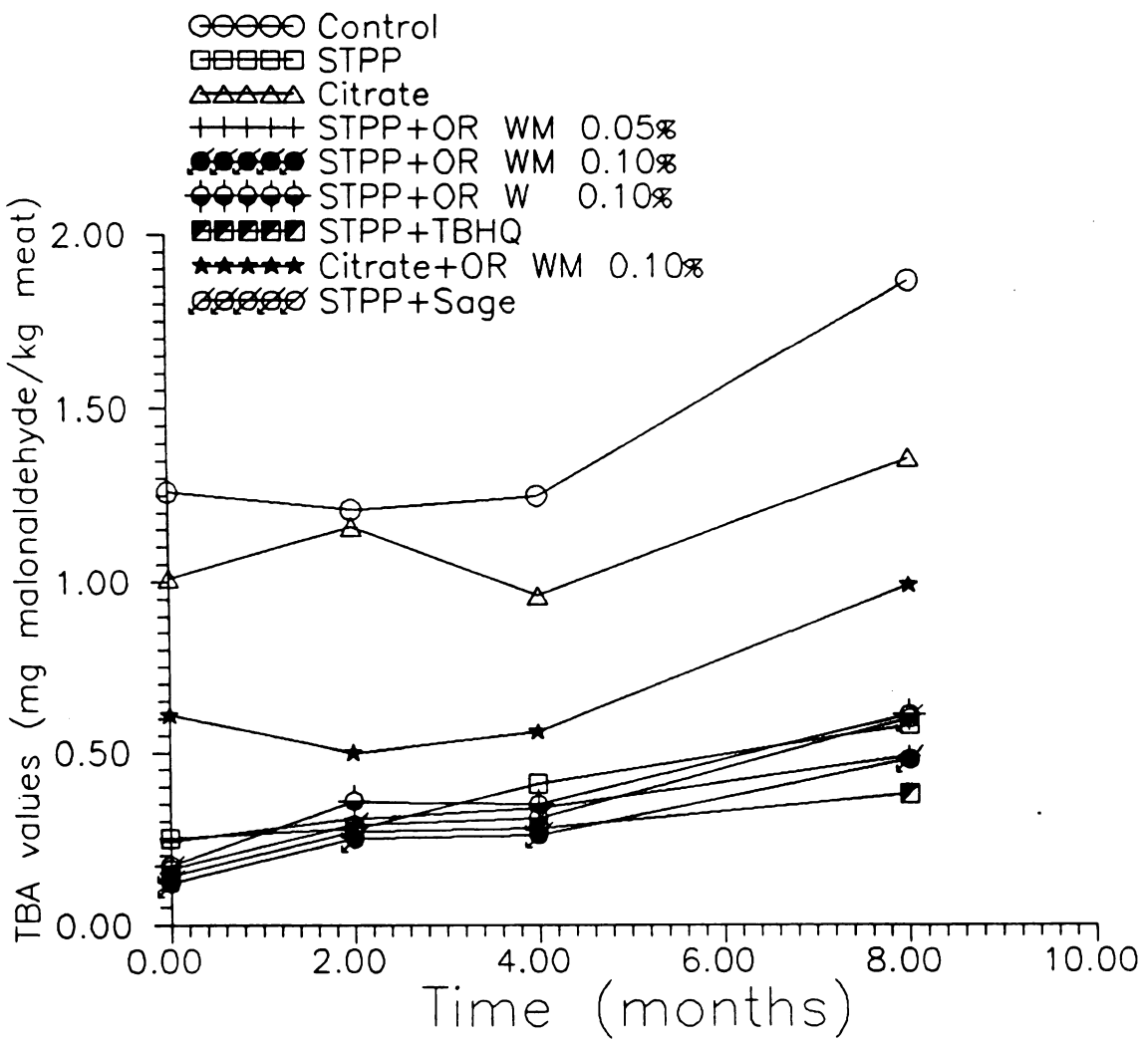

Fig 10. TBA values of restructured pork steaks stored at -30°C

Table 10. Significance levels of designed contrasts for treatment effects on TBA values and sensory scores averaged over time of restructured pork steaks during frozen storage at -30°C

Contrast[a]	Test	significance level[b]
1.Control vs STPP	TBA	0.01
	Sensory	0.01
2.Control vs Citrate	TBA	0.01
	Sensory	0.05
3.STPP vs Citrate	TBA	0.01
	Sensory	0.01
4.STPP vs STPP/OR	TBA	n.s.
	Sensory	n.s.
5.STPP/Antioxidant vs STPP/TBHQ	TBA	n.s.
	Sensory	n.s.
6.STPP vs STPP/Sage	TBA	n.s.
	Sensory	n.s.
7.Citrate vs Citrate/TBHQ	TBA	0.01
	Sensory	0.01
8.STPP/OR WM 0.05% vs STPP/OR WM 0.1%	TBA	n.s.
	Sensory	n.s.

[a] STPP: Sodium tripolyphosphate, OR: Oleoresin rosemary WM: Oil soluble, W: Water soluble, and TBHQ: Tertiary butylhydroquinone.

[b] n.s. : not significant.

protein is also denatured and free iron released (Igene et al., 1979a). Citrate could chelate with free iron and reduce the concentration of free citrate. In addition, the free citrate concentration (0.01%) in these treatments may be low for effectiveness before cooking. Because of the above three factors (1. cooking before storage, 2. storage temperature and 3. citrate concentration) treatments containing citrate were not different from the control in the refrigerated study.

STPP is a strong antioxidant, not only during cooked refrigerated storage but also during raw frozen storage (Table 10). Treatments containing STPP were less oxidized than the control (Table 10). Treatments with sage, OR, or TBHQ combined with STPP were not significantly different from treatments with STPP only (Table 10). There were also no significant differences among sage/STPP, OR/STPP, and TBHQ/STPP treatments (Table 10). Therefore, there appears to be no additional benefit in retarding lipid oxidation by adding OR or sage in treatments containing STPP (Table 10) using the methods and conditions of this study. This is consistent with conclusions of Lai (1989) and Stoick (1989) who reported that there were no differences between STPP/TBHQ and STPP/OR treatments in chicken nuggets and restructured beef steaks.

Different concentrations (0.05 % and 0.1 % based on fat content) and types (W and WM based upon water or oil solubility) of OR combined with STPP had no significant

effect on lipid oxidation as measured by TBA values and trained sensory scores. Lai (1989) and Stoick (1989) reported that no interaction between STPP and OR existed in the chicken nuggets and restructured beef steaks, respectively. There was an additive effect between STPP and OR but no synergistic effect was demonstrated. This is contrary to work of Sheu (1988) which may be due to sample variation, materials variation and different processing methodology.

B. Hexanal Content

Hexanal concentration of restructured pork steaks during frozen storage are presented in Table 11. Treatments with STPP/OR had lower hexanal content than treatments with STPP after 2 and 4 months of storage but not after 8 months of frozen storage. Both treatments were significantly ($p<0.05$) different from the control during the entire frozen storage period and the control always had the highest hexanal level ($p<0.05$). The treatment effects of STPP and STPP/OR in these analyses are consistent with other measures of lipid oxidation. That is, treatments with STPP were significantly ($p<0.05$) different from the control and addition of OR in the treatment with STPP did not provide additional benefit to retarding lipid oxidation in raw, frozen restructured pork steaks. These results are also in agreement with those of Shahidi et al (1987b) who demonstrated that various antioxidants could decrease hexanal levels.

C. Correlation of TBA Values, Hexanal analyses and Sensory Scores

In this study the correlation coefficients (pooled within times and treatment combinations, appendix H) between TBA values and sensory scores in refrigerated storage and frozen storage were 0.307 and 0.414, respectively. This indicated that change in TBA values accounted for only 10 % and 17 % of the variation in sensory scores. The correlation coefficients (pooled within and time combinations) between hexanal content and TBA values and sensory scores in restructured pork steaks after six months of frozen storage were -0.22 and -0.19, respectively. Our r values are relatively low compared to other results (Igene and Pearson, 1979; Igene et al., 1985; Shahidi et al., 1987b) but were similar to results of Lai (1989) and Stoick (1989). Although correlation coefficients between TBA values and sensory scores calculated by pairing the individual observations of TBA values and sensory scores over treatments and storage time were high (r = 0.975, 0.980), the value of this information is limited (Gill, 1978). These low correlation coefficients indicate that the association between hexanal content, TBA values and sensory scores in restructured pork steaks are not as close in this meat system as experienced by Shahidi et al (1987b) and St. Angelo et al (1988). This may be due to the different methods used to calculate the correlation coefficient (Lai, 1989).

Table 11. Hexanal concentration of raw restructured pork steaks during frozen storage at -30° C

Treatment[b]	Hexanal[a] (part per million) 2 month	4 month	8 month
Control	61±4	44±12	405±290
STPP	10±8	6±5	20±5
STPP/OR 0.1%	1±1	2±3	21

[a] Mean ±standard error of the mean.
[b] control: no additives except salt, STPP: Sodium tripolyphosphate, and OR: Oleoresin rosemary.

Effect of Polyphosphate and Oleoresin Rosemary on Lipid Oxidation in a Meat Fiber Model System

The TBA values for the meat model systems are presented in Table 12 and Figures 11 and 12, respectively. Generally TBA values of all the treatments increased during the 7 day storage time. Treatments with hemoglobin (Hb) oxidized faster than treatments with free iron. Treatments without Hb and free iron (control) exhibited less lipid oxidation as measured by the TBA value. Generally the antioxidant performances of OR and STPP in the model systems were consistent with their performances in the restructured pork steaks. Treatments containing STPP were all significantly less rancid ($p<0.01$) than the controls (Table 13) and OR was

not an effective antioxidant in these meat model systems. The OR was only effective in the model system containing free iron in the early stages of refrigerated storage (Table 13) and did not show an antioxidant effect in the system containing Hb. This may be because the concentration of free iron is higher in the model containing Hb.

Sheu (1988) found that a synergism existed between STPP and OR in precooked pork patties. Many other researchers (Watts, 1950; Lehmann and Watts, 1951) have also reported a synergism between organic acids and phenolic types of antioxidants. Lai (1989) and Stoick (1989) reported that no synergism existed between STPP and OR in the chicken nuggets and restructured beef steaks, using a similar source of oleoresin rosemary and similar methods of analysis. Because there was no interaction between STPP and OR, Lai (1989) postulated that these results may be due to the different meat systems used.

According to the definition of Gill (1978), a synergism exists if two conditions are satisfied: (1) STPP and OR significantly interact; and (2) differences between treatments containing STPP/OR and treatments containing STPP have greater differences than treatments containing OR and control (i.e. treatment 3 - treatment 2 > treatment 1 - treatment 4). In this study, all of the three model systems satisfied condition 1. That is, significant ($p<0.01$) interactions between STPP and OR existed in all of the meat model systems (appendix E,F, and G.) Only the meat model

Table 12. TBA[a] values in meat fiber model systems over refrigerated storage at 4°C[b]

Treatment[c]	Storage time (Days)			
	0[d]	1	4	7
1.OR	1.00	0.96	1.36	2.00
2.STPP	0.39	0.43	0.39	0.54
3.STPP+OR	0.45	0.35	0.41	0.47
4.control	1.25	1.36	1.89	2.76
5.Fe	1.64	2.06	2.88	4.01
6.Fe+OR	1.08	1.37	2.00	3.15
7.Fe+STPP	0.55	0.69	0.53	0.65
8.Fe+STPP+OR	0.45	0.41	0.42	0.54
9.Hb	0.87	1.40	4.64	9.34
10.Hb+OR	0.74	1.20	5.67	10.15
11.Hb+STPP	0.36	0.31	2.59	7.21
12.Hb+STPP+OR	0.35	0.31	0.60	2.21
MSE[e] Storage time	0.02	0.07	0.27	0.67

[a] mg malonaldehyde per kg meat
[b] All values represent means of three replications
[c] STPP: Sodium tripolyphosphate, OR: Oleoresin rosemary, Fe: Ferric iron, and Hb: hemoglobin.
[d] Storage time designated as 0 day represents steak analyses immediately after formulating, cooking, chilling overnight, slicing, and packaging.
[e] MSE: Mean square error of the same storage time.

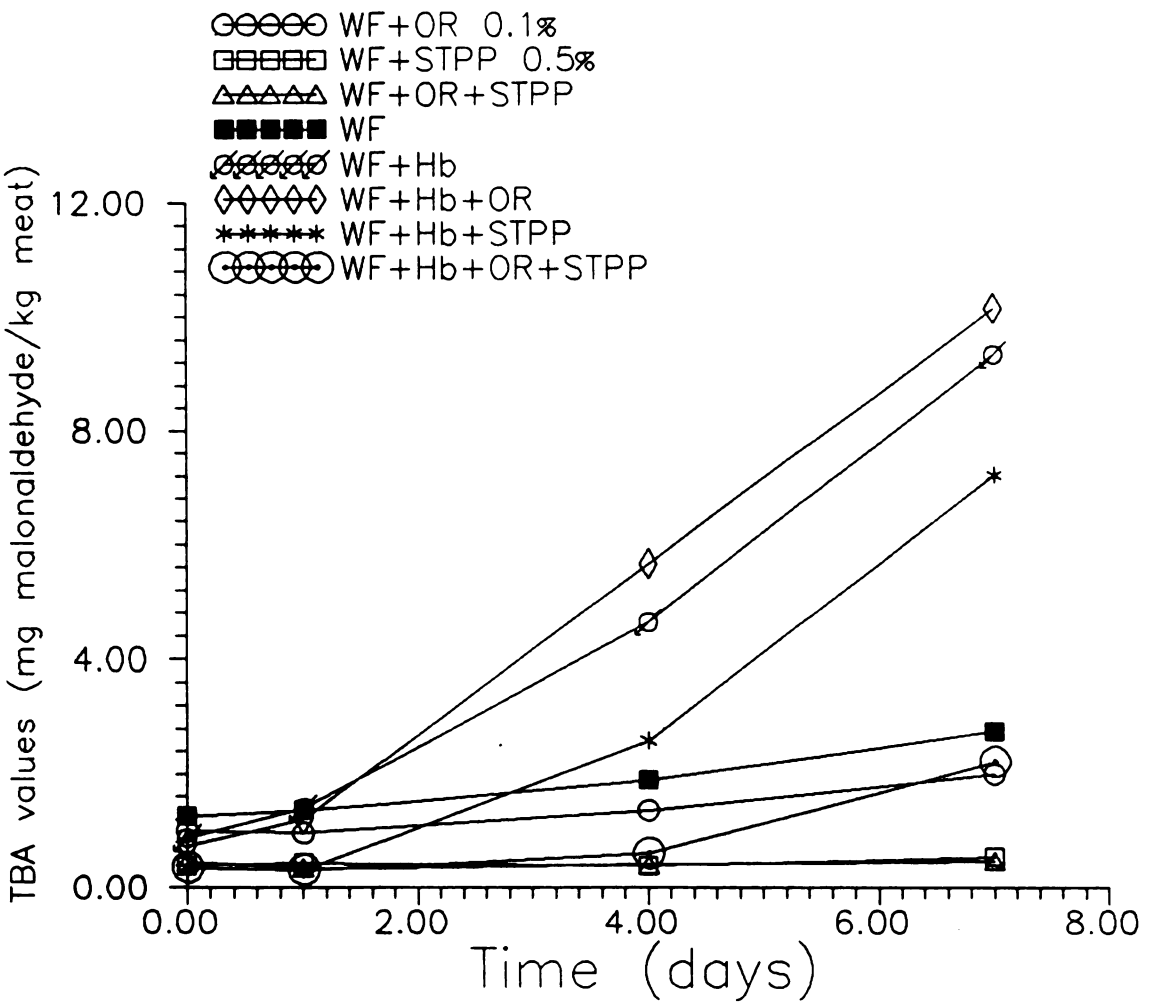

Fig 11. Effects of treatments (controls and with Hb) in a meat fiber model system described by Love and Pearson (1974)

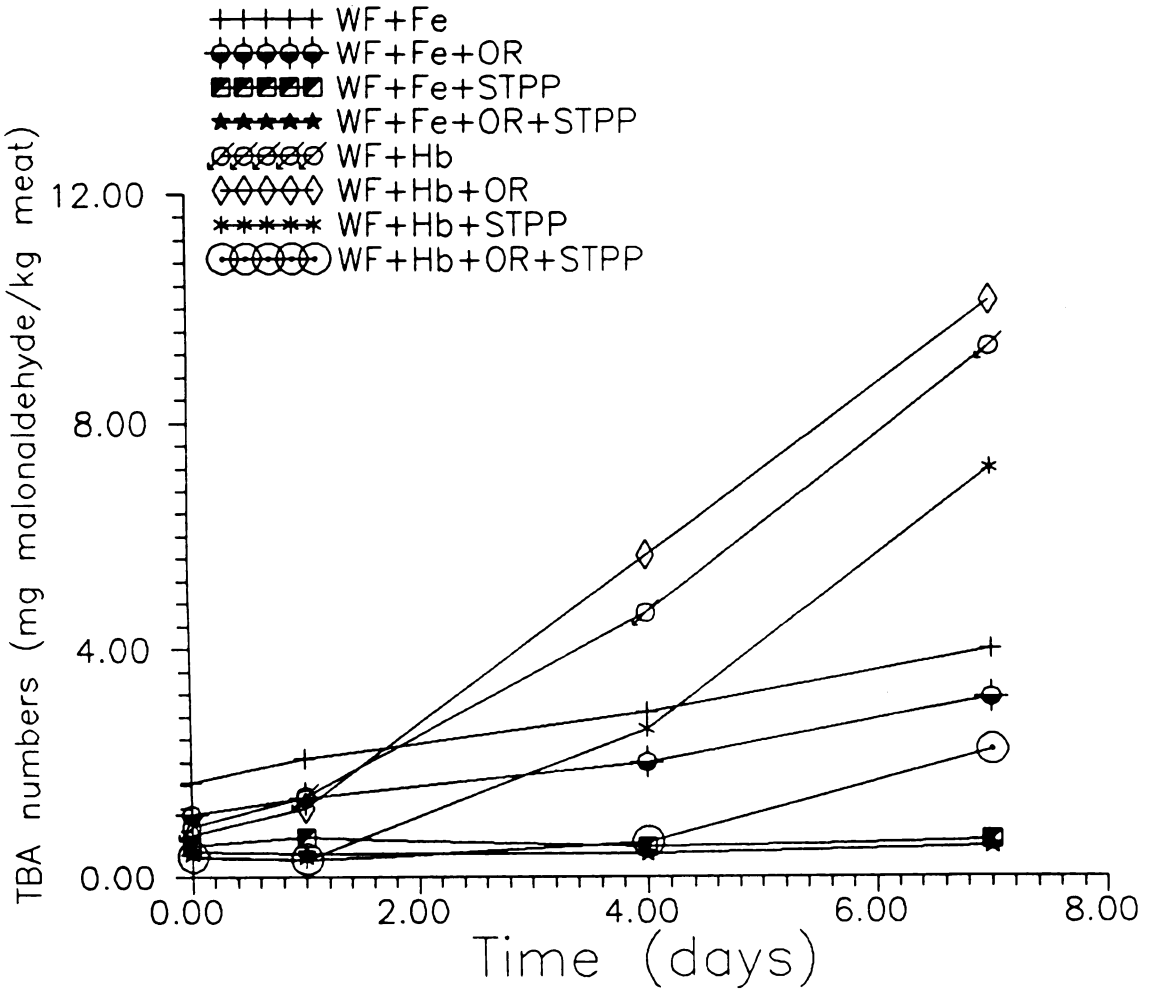

Fig 12. Effects of treatments (with free iron and with Hb) in a meat fiber model system described by Pearson and Love (1974)

Table 13. Significance levels of designed contrasts for treatment effects on TBA values of meat fiber model systems over refrigerated storage at 4°C

Contrast[a]	Storage time (Days)			
	0	1	4	7
1.control vs Fe	ns	0.05	ns	ns
2.control vs Hb	ns	ns	0.01	0.01
3.Fe vs Hb	0.01	0.05	0.01	0.01
4.STPP vs STPP/OR	ns	ns	ns	ns
5.Fe/STPP vs Fe/STPP/OR	ns	ns	ns	ns
6.Hb/STPP vs Hb/STPP/OR	ns	ns	0.01	0.01
7.OR vs control	ns	ns	ns	ns
8.Fe/OR vs control (Fe)	0.01	0.05	ns	ns
9.Hb/OR vs control (Hb)	ns	ns	ns	ns
10.STPP vs control	0.01	0.01	0.01	0.05
11.Fe/STPP vs control (Fe)	0.01	0.01	0.01	0.01
12.Hb/STPP vs control (Hb)	0.01	0.01	0.01	0.05

[a] STPP: Sodium tripolyphosphate, OR: Oleoresin rosemary, Fe: Ferric iron, and Hb: hemoglobin.
[b] n.s.: no significant

system containing Hb satisfied condition 2. Therefore, a synergism exists only in the model systems containing Hb (treatments 9-12, Table 12). No synergism existed in the meat model systems without free iron and Hb (controls) and in the model system with free iron. These results support Lai's (1989) conclusion that the different results may be due to the different meat systems used. Additional research is needed in these model systems containing Hb and free iron to understand why there are different synergistic responses and if there are any interactions between STPP, OR, free iron, Hb and STPP/OR combinations.

Because of the interaction between treatments and time (Appendices E, F, and G) specific contrasts were made using the Bonferroni t-test to identify the possible prooxidant effect of Hb and free iron (Table 13). In the present study Hb was significantly different from the control ($p<0.05$) only at day 4 and day 7 (Table 13). Hb was not significantly different from the control at day 0 and day 1. These results support those of Liu (1970a) who concluded that Hb is a prooxidant but that a short lag period was needed before heme catalysis begins. Because a lag period was found in the present study, this may provide a good explanation why Tichivangana and Morrissey (1985), Sato and Hegarty (1971) and Love and Pearson (1974) observed that Hb is not a strong prooxidant during a 2 day study period.

Although the TBA values of all treatments with free

iron were always higher than the control, these are only significant ($p<0.05$) from the control at day 1 but not at day 0, day 4 and day 7 (Table 13). These data indicate free iron is a prooxidant only during early storage. This may be due to the fact that ferrous ion (Fe^{+2}) is a catalyst but ferric ion (Fe^{+3}) is not a prooxidant (Labuza, 1971). During refrigerated storage, most of the ferrous ions (Fe^{+2}) used in this study are converted to ferric (Fe^{+3}) at the later stage. Therefore, the prooxidant effect is significant only at the early stage. Another possibility is that inorganic iron, either as ferrous or ferric ion, is a very weak catalyst (Wills, 1966; Liu, 1970a) and can only function as a prooxidant at the early stage. A final explanation may be that the observation at day 1 is a mistake (type I error) because the significance level is only $p<0.05$.

Tichivangana and Morrissey (1985) used one concentration of free iron and heme iron in raw and cooked muscle systems. They concluded that free iron had a higher prooxidant activity than that of heme iron. However, it is very difficult to compare the prooxidant effect of free iron and heme iron at only one concentration and come to a general conclusion that free iron has a higher prooxidant effect. Additional research is needed to conclude that free iron has higher prooxidant activity than heme iron in all cases. Different concentrations of free iron and heme iron were used by early researchers (Sato and Hegarty, 1971; Love

and Pearson, 1974) and the prooxidant effect of free iron and heme iron was not in direct proportion to the range of concentrations (Kendrick and Watts, 1969; Hirano and Olcott, 1971). Factors such as pH also contribute to the prooxidant effect of free iron and heme iron (Liu, 1970a) and needs to be studied over an appropriate range of concentration.

SUMMARY AND CONCLUSIONS

The objectives of this study were to determine the antioxidant properties of OR in restructured pork steaks and the possible synergism between STPP and OR in a meat model system. The antioxidative efficiency of OR was studied in cooked restructured pork steaks stored at 4^{o}C for 8 days and -30^{o}C for 8 months and compared with other antioxidants such as sage, citrate and STPP. Lipid oxidation was characterized by TBA, sensory evaluation and hexanal analyses. Two types (water soluble and water insoluble) OR were tested at two levels with STPP. Sage/STPP, citrate/STPP, TBHQ/STPP, citrate only and STPP treatments were used as reference for the antioxidative efficiency of OR.

The possible synergistic effect between OR and STPP was further studied using a meat model system. Lipid oxidation was monitored over time using the TBA method. One level of STPP and OR, and a combined level of STPP/OR were tested in the model system with: 1. ferrous ion only; 2. hemoglobin only; and 3. without a metal catalyst.

It was found that STPP is a good antioxidant in restructured pork steaks. Treatments with STPP had less

($p<0.01$) lipid oxidation and were not different from each other. The OR/STPP treatments did not significantly ($p<0.01$) differ from Sage/STPP treatments. Neither OR nor citrate alone were a strong antioxidant when compared to TBHQ and STPP in restructured pork steaks regardless of refrigeration (2, 4, 6, 8 days) or frozen storage (0, 2, 4, 8 months) treatments. Water soluble OR did not significantly ($p<0.01$) differ from the water insoluble type OR using TBA, hexanal and sensory measures of lipid oxidation.

A synergism existed only in the meat fiber model system with hemoglobin. No synergistic effect was demonstrated in the other meat fiber model system studies. This observation may due to a lack of sensitivity in measurement of lipid oxidation. Lipid oxidation was greatest in the model meat system containing hemoglobin. Therefore, a synergistic effect was demonstrated. The antioxidative performance of STPP and OR in the model meat system was consistent with their performance in restructured pork steaks.

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APPENDIX A.

Cooking cycle for restructured pork rolls

Stage	Time (min)	Dry bulb (°C) temperature	Wet bulb (°C) temperature	Internal (°C) temperature
1	30	37.7	37.7	Less than 26.6
2	70	54.4	52.8	<26.6 -> 37.7
3	70	70.0	68.9	37.7 -> 57.7
4	105	79.4	76.6	57.7 -> 74.4
Shower	30	32.2	32.2	74.4 -> 64.4

APPENDIX B. Sensory evaluation form

SENSORY EVALUATION OF OXIDIZED FLAVOR IN RESTRUCTURED PORK STEAK

NAME _____ DATE _____

(1) You are receiving a series of coded samples and a reference sample, marked R.

(2) Smell and Taste each sample; consider the reference sample to have no oxidized flavor and evaluate the degree of oxidized flavor, i.e. oxidative off-flavor.

(3) Then mark the intensity of the flavors that you detect from the samples.

(4) Also, indicate with yes or no the presence of other non-meat flavors.

THE INTENSITY OF OXIDIZED FLAVOR

Absent: 0
Very Slight: 1
Slight: 2
Moderate: 3
Strong: 4
Very Strong: 5

CODE	INTENSITY OF OXIDIZED FLAVOR	NON-MEAT FLAVOR	COMMENTS
SET #1			
-----	----------	--------	----------
-----	----------	--------	----------
-----	----------	--------	----------
SET #2			
-----	----------	--------	----------
-----	----------	--------	----------
-----	----------	--------	----------
SET #3			
-----	----------	--------	----------
-----	----------	--------	----------
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APPENDIX C.

Analysis of variance of TBA values and sensory scores for refrigerated storage of restructured pork steaks

	Source		Sum of Squares	Mean Square	F Value	Significance Level
TBA value	treatment	(A)	569.722	71.215	67.1481	0.01
	time	(B)	126.030	25.206	23.7665	0.01
	AxB interaction		172.647	4.316	4.0697	0.01
Sensory Score	treatment	(A)	48.226	6.028	56.4187	0.01
	time	(B)	14.520	7.260	67.9481	0.01
	AxB interaction		4.354	0.272	2.5467	0.05

Appendix D.

Analysis of variance of TBA values and sensory scores for the frozen storage of restructured pork steaks

Frozen Storage	Source	Sum of Squares	Mean Square	F Value	Significance Level
TBA test	Treatment (A)	14.831	1.854	36.8218	0.01
	Time (B)	2.578	0.859	17.0676	0.01
	AxB interaction	0.836	0.035	0.6922	n.s.
Sensory Scores	Treatment (A)	22.988	2.873	35.7305	0.01
	Time (B)	16.343	5.448	67.7418	0.01
	AxB interaction	0.885	0.079	0.9767	n.s.

n.s. Not significant

Appendix E.

Analysis of variance for the meat fiber model system without free iron and hemoglobin addition

Source[a]	degree of freedom	Sum of Squares	Mean Square	F Value	Significance Level
Factor A	1	0.753	0.753	41.4665	0.01
Factor B	1	15.744	15.744	867.5570	0.01
AB	1	0.670	0.670	36.9075	0.01

Factor A: oleoresin rosemary (OR)
Factor B: sodium tripolyphosphate (STPP)
n.s. : no significant

Appendix F.

Analysis of variance in the meat fiber model system with hemoglobin addition

Source	degree of freedom	Sum of Squares	Mean Square	F Value	Significance Level
Factor A	1	5.651	5.651	9.2992	0.05
Factor B	1	75.476	75.476	124.1952	0.01
AB	1	13.494	13.494	22.2040	0.01

Factor A: oleoresin rosemary
Factor B: sodium tripolyphosphate
n.s. : no significant

Appendix G.

Analysis of variance in the meat fiber model system with free iron addition

Source	degree of freedom	Sum of Squares	Mean Square	F Value	Significance Level
Factor A	1	2.417	2.417	72.1993	0.01
Factor B	1	36.523	36.523	1091.2071	0.01
AB	1	1.059	1.059	31.6432	0.01

Factor A: oleoresin rosemary
Factor B: sodium tripolyphosphate
n.s. : no significant

APPENDIX H.

Formulas for calculation of correlation coefficients (pooled, within treatments and storage times) between TBA values and sensory scores of restructured pork steaks

X = Sensory scores

Y = TBA values

i = 1,2,.....9 (treatments)
j = 1,2,3,4 (months)
k = 1,2,3 (replications)

Variances (S_x^2) of sensory scores (x) within three replications.

$$S_x^2 = [\sum_{k=1}^{3} X_k^2 - (\sum_{k=1}^{3} X_k)^2/3]/(3-1)$$

Variances (S_y^2) of TBA values (y) within three replications.

$$S_y^2 = [\sum_{k=1}^{3} Y_k^2 - (\sum_{k=1}^{3} Y_k)^2/3]/(3-1)$$

Covariances (Sxy) of sensory scores (x) and TBA values (y) within three replications

$$S_{xy} = [(\sum_{k=1}^{3} X_k Y_k) - (\sum_{k=1}^{3} X_k)(\sum_{k=1}^{3} Y_k)/3]/(3-1)$$

$$r_{xy} = S_{xy}/(S^2_x * S^2)^{0.5}$$