

LIPID OXIDATION

I. INTRODUCTION

Oxidation of lipids is a major cause of deterioration in the quality of meat and meat products - affects many characteristics such as flavor, color, texture and nutritive value.

Rapid development of rancid flavors during storage is a major problem facing meat industry, especially with increased demand for precooked meat items.

Warmed-over flavor defined as rapid onset of rancidity in cooked meats during refrigerated storage.

II. FATTY ACIDS AND OXIDATIVE RANCIDITY

1. WHAT IS OXIDATIVE RANCIDITY

Oxidative rancidity is a chemical change that results in unpleasant odors and taste in a fat.

WOF first recognized by Tims and Watts (1958) who defined it as rapid onset of rancidity in cooked meats during refrigerated storage.

Oxidized flavors are readily detectable after 48 hours in **cooked** meats.

Slow development of rancidity encountered in **raw or fatty tissues** after prolonged freezer storage (Pearson and Gray, 1983).

Rapid development in **raw** meat that has been ground and exposed to air (Greene, 1969; Sato and Hegarty, 1971) - or any process involving the disruption of muscle membranes enhances the development of WOF (cooking, grinding, restructuring).

2. BIOCHEMICAL ASPECTS OF LIPID OXIDATION

Oxidative stability of meats is related to the degree of saturation of the lipid fraction - chicken is the most susceptible, followed by pork, beef and lamb (Wilson et al., 1976; Pearson et al., 1977).

Oxidation takes place in two different fractions -

1. **Triacylglycerols** in the fat depots - subcutaneous, intermuscular, intramuscular.

Largely composed of straight-chain, even-numbered carbon fatty acids, which contain 16 or 18 carbon atoms (Dugan, 1971).

Sheep contain highest amount of saturated fatty acids.

2. **Phospholipids** in muscle which are altered slightly by dietary changes (Pearson et al., 1977).

Contain much larger proportion of C 20 and C22 unsaturated fatty acids.

Polyunsaturation of the phospholipid fraction is about 15 times greater than that of triacylglycerol fraction.

Significant variation in total phospholipid content among species and from muscle to muscle location in the same animal. Poultry and fish muscles are known to be higher in phospholipids than red meats.

Wilson et al. (1976) suggested that phospholipids play a major role in development of WOF in turkey, chicken, beef and lamb, but that total lipids are the major contributor to WOF in pork.

Igene and Pearson (1979) showed that by adding phospholipids, triacylglycerols and total lipids back to a model meat system of lipid-extracted muscle fibers that phospholipids were the major contributors to WOF. Triacylglycerols were found to enhance the development of WOF only when combined with the phospholipids in total lipids.

Oxygen attacks the **double** bond in **fatty acids** to form **peroxide** linkages - therefore, phospholipids which contain a high content of unsaturated fatty acids, mainly linoleic and arachidonic acids are more susceptible to oxidation. **Phospholipids** from **beef** are 15% more **unsaturated** than **triacylglycerols**. **Chicken - phospholipid** fraction in **16** (white) to **22%** (dark) unsaturated versus **3** (white) to **1.5%** (dark) from **triacylglycerols**.

Phospholipids may be more susceptible to oxidation because of close physical association in membranes with tissue catalysts of oxidation.

Also why tissue disruption enhances development of rancidity.

3. CATALYSIS OF LIPID OXIDATION

Lead or copper catalyzes rancidity, as well as hemoglobin, myoglobin, and cytochromes

In the 1950's and 1960's, myoglobin was viewed as major catalyst of lipid oxidation (Tappel, 1962).

In 1970's, Pearson's lab (Love, 1972; Igene, 1978) revealed that nonheme iron, rather than heme iron, was the active catalyst responsible for the rapid oxidation of cooked meat.

We still do not know what the source of primary catalysits which initiate lipid oxidation in raw muscle tissue. Is lipid oxidation in meat systems catalyzed by enzymic or nonenzymic oxidation systems or a combination of both?

4. OXIDATIVE MECHANISMS AND REACTIONS

Autoxidation - referred to as rat of oxidation increases as the reaction proceeds.

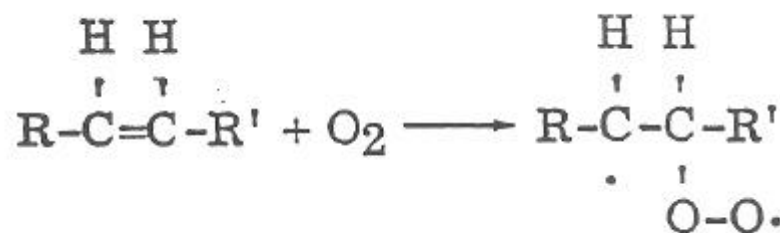
Unless mediated by other oxidants or enzyme systems, oxidation proceeds through a free-radical chain reaction mechanism involving three stages:

1. Initiation - formation of free radicals
2. Propagation - free-radical chain reaction
3. Termination - formation of nonradical products

Free radicals are produced during peroxide formation - hydroperoxides are the major initial reaction products of fatty acids with oxygen.

Subsequent reactions control both the rate of reaction and the nature of products formed.

Initiation - an unsaturated hydrocarbon loses a hydrogen to form a radical, $RH - R^\bullet + H^\bullet$, and oxygen adds at the double bond to form a diradical:



Oxygen in the singlet state can apparently interpose between a labile hydrogen to form a hydroperoxide directly -- $RH + O_2 \rightarrow ROOH$

Direct formation of hydroperoxides is not necessarily a free-radical chain mechanism, although it can initiate chain processes

Initiation reaction is of great interest since it relates both to the site of attack and to the energy requirement

Energy requirement for radical production by rupture of a CH bond is about 80 kcal. Less energy is necessary for addition of oxygen to form diradical at double bond, but both requirements appear to be so excessive that numerous energy-reducing postulates exist for involvement of metal activation, enzyme catalysis or photooxidation

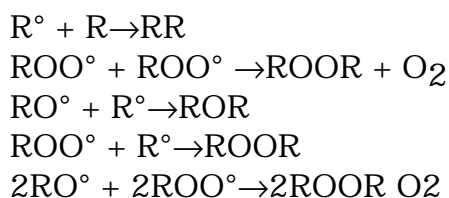
Each initiation process produces two free radicals, each of which participate in the chain reaction mechanism.

Oxygen has to be in singlet state, but normally exists in triplet state. Conversion of oxygen to singlet state can be accomplished by photosensitization in the presence of suitable sensitizers, such as chlorophyll, or heme pigments myoglobin or hemoglobin or by their derivatives.

Propagation - the chain reaction is continued by $R^\circ + O_2 \rightarrow ROO^\circ$ and $ROO^\circ + RH \rightarrow ROOH + R^\circ$ to form peroxy radicals, hydroperoxides and new hydrocarbon radicals

The new radical formed then contributes to the chain by reacting with another oxygen molecule.

Termination - occurs when two radicals interact



Free radicals can damage living tissues unless antioxidants, ie. tocopherols or vitamin E, are present to react with free radicals

When no radicals are available for further reaction with oxygen, necessary for a new initiation reaction to occur if oxidation is to continue

Antioxidants can be added to meat systems, such as BHA, BHT, citric acid, extractives of rosemary, or phosphates to react with free radicals

5. DECOMPOSITION REACTIONS AS FUNCTION OF OXIDATIVE MECHANISMS

A. Hydroperoxide decomposition

Hydroperoxides are readily decomposed by high-energy radiation, thermal energy, metal catalysis, or enzyme activity

Hydroperoxides decompose to form additional radicals which add to the chain process

This proliferation of radicals causes acceleration of oxidation without requiring new initiation events.

When concentration of **hydroperoxide is low**, decomposition can occur as $\text{ROOH} \rightarrow \text{RO}^\circ + \text{}^\circ\text{OH}$, a monomolecular decomposition

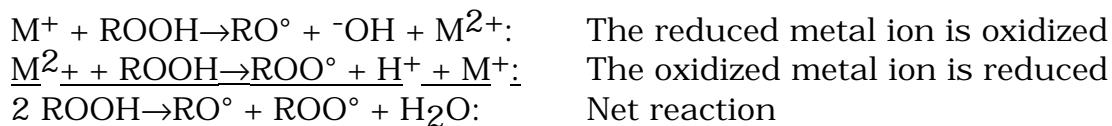
When concentration of **hydroperoxide is high**, decomposition can occur as $2 \text{ROOH} \rightarrow \text{RO}^\circ + \text{ROO}^\circ + \text{H}_2\text{O}$, a bimolecular decomposition

RO° radicals can participate in the chain propagation stage, although more energetic ROO° radicals predominate

Hydroperoxides tend to associate by hydrogen bonding when temperatures are low or when the concentration is high

Metallic prooxidants contribute to formation of additional radicals by acting as hydroperoxide decomposers

A metal, capable of existing in two valence states function as:



In a system containing multivalent metal ions, Cu^+ and Cu^{2+} or Fe^{2+} and Fe^{3+} , hydroperoxides decompose readily with formation of both RO° and ROO° radicals as the metal ions undergo oxidation-reduction

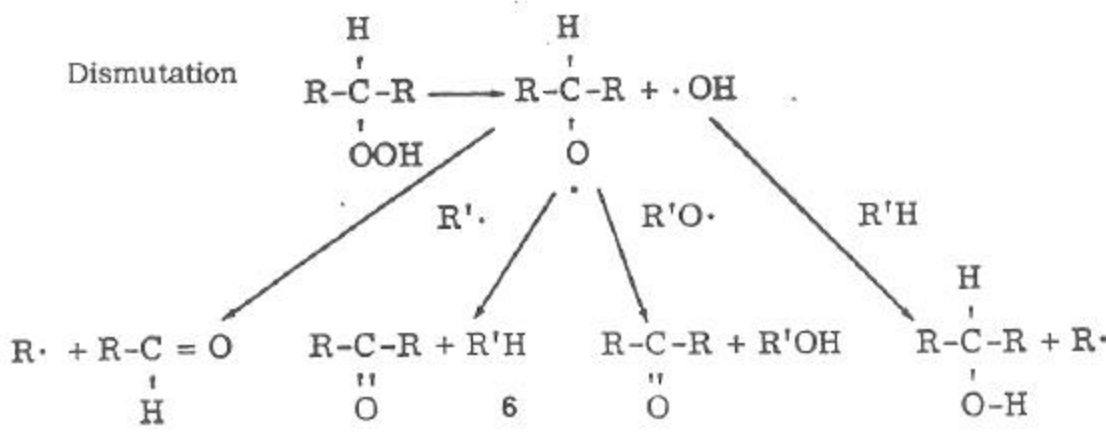
Heme compounds containing Fe have been implicated as oxidation catalysts either as direct initiators of peroxidation or as peroxide decomposers.

Heme concentration exists where an antioxidant effect is evident

Heme compounds present at two to four times the amount most effective for oxidation and no oxidation occurs.

Stable complexes of lipid peroxides and heme compounds apparently form and these presumably inhibit oxidation.

When hydroperoxide decompose to form RO^\bullet radicals, the radicals are in turn capable of a series of reactions leading to several products which can be isolated from oxidizing lipid systems



Some of these products are radicals and can continue in the chain propagation process.

Others, such as hydroxy acids, keto acids, and aldehydes, are commonly found in oxidizing lipid systems.

Aldehydes (many are short chain) and short-chain acids derived by further oxidation of aldehydes, are largely responsible for off flavors and odor characteristic of rancid foods

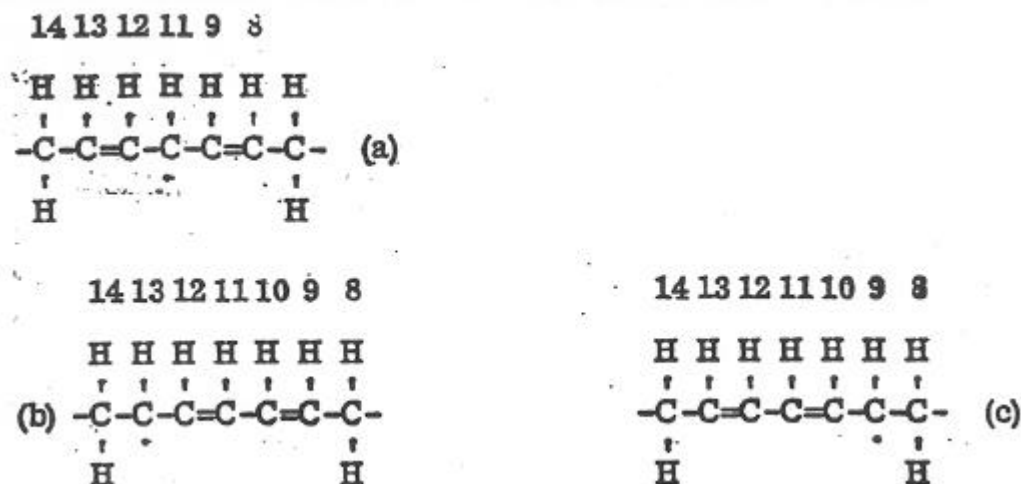
B. Oxidation of monoenoic acids - one double bond

Oxidation results in formation of hydroperoxides

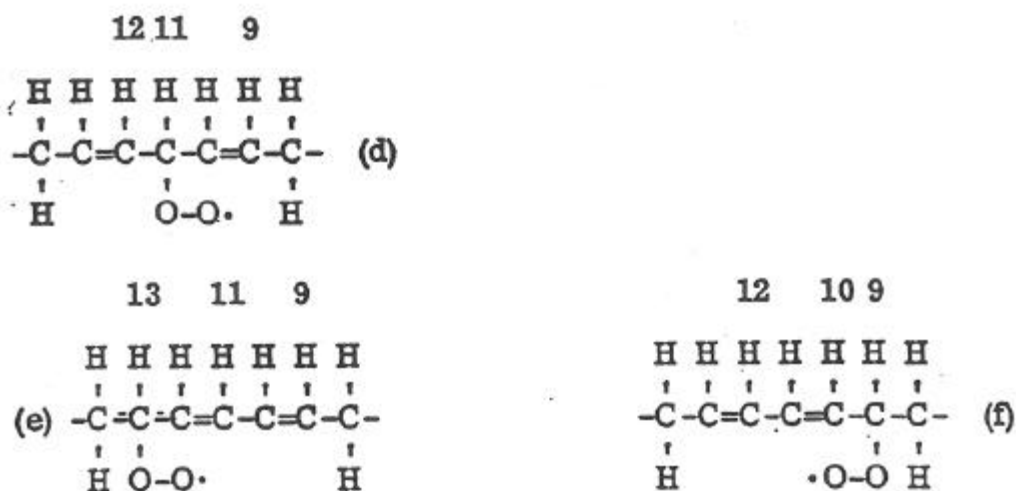
A hydrogen ion is removed during initiation from either of the a C to the double bond - thus two radicals are possible, each of which can assume two forms through resonance

Addition of oxygen at each radical site followed by the addition of a hydrogen free radical results in hydroperoxides at four different positions, two at the carbons involved in the original double bond and two at the a carbons.

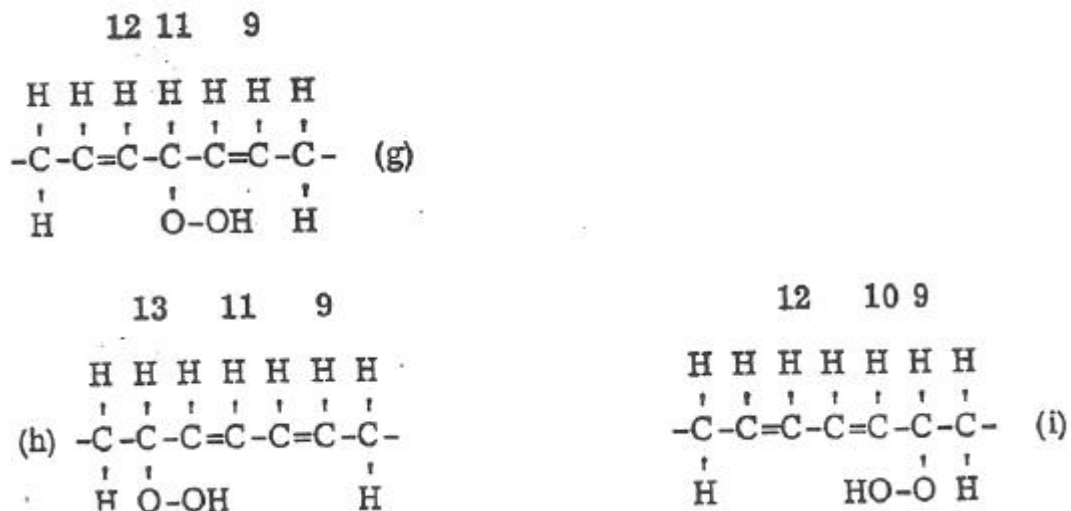
Hydrogen is abstracted from C-11 to form three isomeric radicals (a), (b), and (c):



Addition of oxygen forms peroxy radicals (d), (e), and (f):



Each peroxy radical can abstract a H° from an unoxidized molecule to form isomeric hydroperoxides (g), (h), and (i):



D. Oxidation of saturated acids

Oxidize only when exposed to conditions of severe stress.

Hydroperoxide group generally forms on *B* carbon to the carboxyl or ester group

Hydroperoxide then changes to a keto group, giving rise to a **B** keto acid

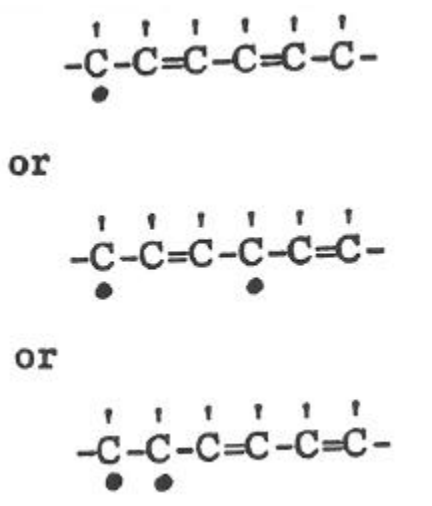
E. Oxidation of conjugated double-bond systems

Conjugated = multiple bonds which alternate with a single bond between the double bonds.

Oxidizes with considerable ease by oxidation of the terminal carbons in the conjugated system

Conjugated triene oxidizes 1, 6, but is possible to have 1, 4 or 1, 2 oxidation in the same system

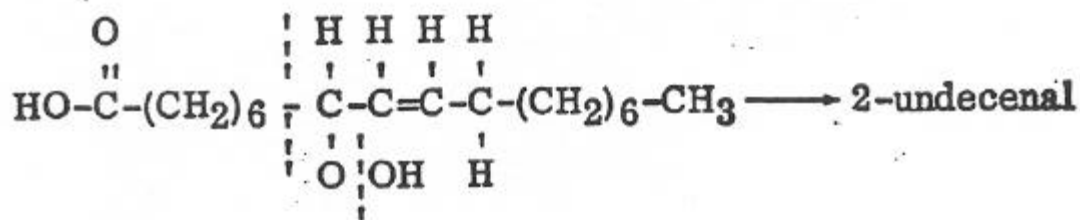
These reactive radical species then form **polyperoxides, cyclic peroxides,** and **peroxide polymers** to further complicate the oxidation picture.



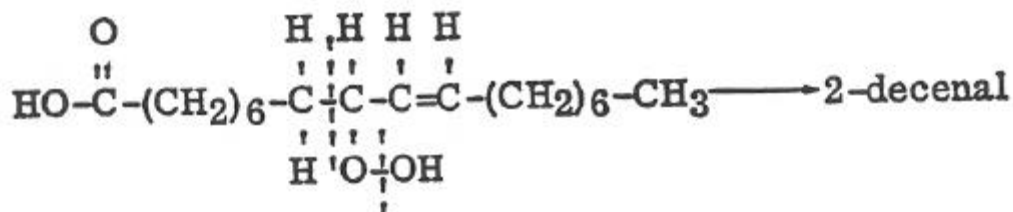
F. Aldehydes as secondary oxidation products

Dominant aldehydes isolated from oxidatized fats apparently arise from oleate or linoleate hydroperoxides by scission and dismutation

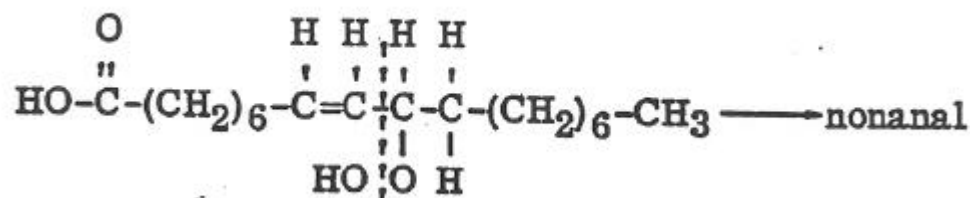
Typical aldehydes from oleate hydroperoxides are listed:



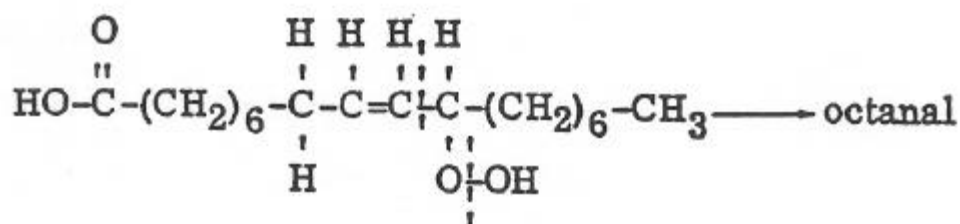
8-Hydroperoxide



9-Hydroperoxide



10-Hydroperoxide



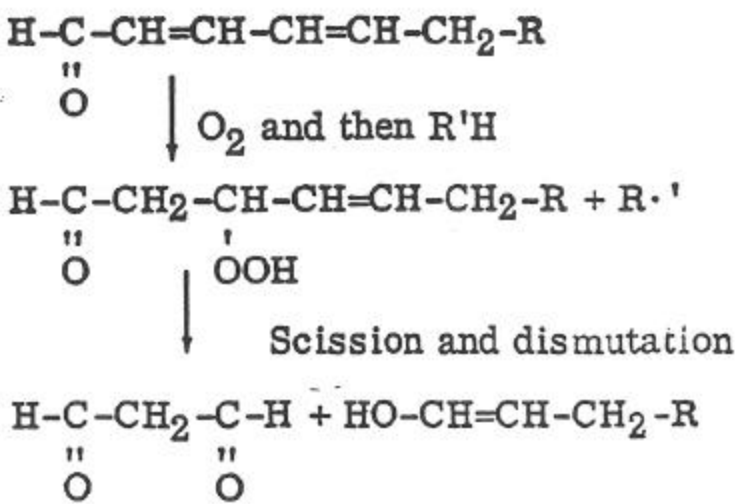
11-Hydroperoxide

Scission of bond between two carbons, one of which possesses a hydroperoxide group, may result in aldehyde formation at each carbon.

The fragment attached to the carboxyl group or esterified to glycerol is relatively nonvolatile, while the other fragment is more volatile and is readily identifiable since it has only the one functional group

Two section locations are designated **A or B**

Malonaldehyde measured in oxidizing systems containing linoleate may be derived from further oxidation of 2-enals or 2, 4-dienals.



H. Other secondary oxidation products

The number of **aldehydes**, **alcohols**, and **acids** found in an oxidizing system is compounded by secondary and tertiary oxidations in the system

Primary alcohols do not oxidize appreciably at ambient temperatures, but substantial conversion to the corresponding acids occurs at 50°C

Secondary alcohols convert to the corresponding **ketone**

Aldehydes presumably are converted to **acids**

If the **aldehydes** are enals or dienals, these may oxidize further to provide yet shorter chain compounds.

6. EVALUATION OF STATE OF OXIDATION

Measurement of peroxide value to determine which extensive decomposition of hydroperoxides begins

Measurement of carbonyl compounds is useful provided secondary reactions and volatilization have not occurred to a significant extent

The pattern of oxidation may be changed by the reaction of aldehydes with amino groups from proteins, amino acids, and amino lipids, or by the nature of the stress on the system

This causes type A or B scission and different carbonyl species to form

Measurement of acid-type carbonyls is not revealing is used alone

A composite of peroxide, TBA, carbonyl type, and acid determinations provides the best indication of the state of oxidation, yet the data are so variable that no oxidized system has been very well characterized or defined

TBA values - most common measurement

III. ANTIOXIDANTS

Chemicals used to control or retard WOF development or oxidative reactions.

A. MECHANISMS OF ANTIOXIDANT ACTION

Two main mechanisms for inhibition of oxidative reactions:

1. Interrupt the free-radical chain mechanism
2. Function as being preferentially oxidized - poor protection

An antioxidant AH reacts with radicals as follows:



Shows that antioxidants interfere with free-radical, chain oxidation and that reaction products of antioxidant molecules and oxidized lipid molecules may appear among the final products.

B. NITRITES

Effective inhibitor of WOF development in cooked meats

At 2000 mg/kg nitrite completely eliminates WOF (Sato and Hegarty, 1971) and delays development at 50 mg/kg.

Mechanism by which nitrite prevents or retards oxidation of meat lipids has not been clarified - suggests more than one mechanism involved

Proposed mechanisms include:

1. Formation of a stable complex between the heme pigments and nitrite, thereby preventing the release of nonheme iron and its subsequent catalysis of lipid oxidation (Igene et al., 1985; Morrissey and Tichivangana, 1985)
2. Stabilization of unsaturated lipids in the membranes which are normally disrupted and exposed to oxygen by cooking or grinding
3. Formation of inactive "chelates" between nitrite and metal ions such as ferrous ions, thus rendering them unavailable for catalysis of oxidation reactions (MacDonald et al., 1980; Igene et al., 1985; Morrissey and Tichivangana, 1985).
4. Formation of nitric oxide myoglobin which has antioxidant properties per se (Kanner et al., 1980; Morrissey and Tichivangana, 1985).

Of the four mechanisms, it is believed that stabilization of the porphyrin ring and preventing release of Fe^{2+} during cooking process appears to be the most important (Igene et al., 1985).

C. CHELATING AGENTS

1. PHOSPHATES

Added to meat systems to increase water holding capacity and yield of finished product

Delays and prevents lipid oxidation (Tims and Watts, 2958; Sato and Hegarty, 1971)

Pyrophosphates, tripolyphosphates and hexametaphosphates all offer protection; whereas, orthophosphates do not

Mode of action; chelate the heavy metal ions. Exact mechanism no known.

2. EDTA

EDTA - ethylenediamine tetracetic acid - prevents Fe²⁺ -catalyzed oxidation in raw beef (Liu and Watts, 1970) and in cooked ground beef systems at a concentration of 2.5 mg/g (Sato and Hegarty, 1971).

Igene et al., (1979b) reported that the addition of 2% EDTA to meat pigments extracted from cooked meat significantly reduced the catalytic activity

It was concluded that EDTA effectively chelated free iron and thereby significantly reduced lipid oxidation in cooked meat.

Not approved for use in commercial meat products.

3. CITRIC ACID

Found to be less effective than EDTA or phosphates.

Level of 1000 mg/kg in hams was effective, but at 5 mg/g in cooked ground beef, citric acid was not effective.

D. ASCORBATE

At levels greater than 1000 mg/kg, ascorbic acid is an effective inhibitor of oxidation; whereas, at low levels (< 100 mg/kg) ascorbic acid has been shown to catalyze WOF (Tims and Watts, 1958; Sato and Hagarty, 1971)

Sato et al. (1973) suggested that high levels shifted the balance between ferrous and ferric iron or else acted as an oxygen scavenger.

Kanner et al. (1986) recently demonstrated that iron in the presence of ascorbic acid stimulates membranal lipid peroxidation in muscles, presumably through the involvement of hydroxyl radicals.

Synergistic relationship between ascorbic acid and phosphates in inhibiting lipid oxidation in meats was demonstrated by Tims and Watts (1958) and Sato and Hegarty (1971).

Theorized by Sato and Hegarty that ascorbic acid functions by keeping a part of the iron in the reduced state.

E. SYNTHETIC PHENOLIC ANTIOXIDANTS

Most effective antioxidants function by interrupting the free radical chain mechanism of lipid oxidation (Dugan, 1976).

BHT - butylated hydroxytoluene and BHA - butylated hydroxyanisole and other synthetic phenolic antioxidants are known to retard lipid oxidation - synergistic (increased functionality when used together)

Greene et al. (1969) showed that BHA and propyl gallate retarded oxidation

F. NATURAL ANTIOXIDANTS

Flavonoids, a major group of plant phenols, have been found to have antioxidant properties.

Glandless cottonseed has been shown to have antioxidant properties (Rhee and Ziprin)

Rosemary contains a number of compounds possessing antioxidant activity - carnosol, rosmanol, rosmariquinone and rosmaridiphenol.

Rosmariquinone and rosmaridiphenol found to be better antioxidants than BHA (Houlihan et al., 1984, 1985).

Barbut et al. (1985) demonstrated that rosemary oleoresin added to turkey breakfast sausage at 20 mg/kg produced antioxidative effect comparable to commercial BHA/BHT/citric acid.

Feeding alpha-tocopherol to pigs to incorporate into the cellular membrane was examined by Buckley et al., (1988) and increased stability of fresh pork products was reported.

G. MAILLARD REACTION PRODUCTS

Maillard reaction which occurs as meat products are cooked have been shown to have antioxidant capacities (Zipser and Watts, 1961).

Therefore, establishing the theory that well cooked meat has less oxidative development than meat cooked to lower degrees of doneness.

H. SMOKING OF MEATS

Phenols, phenol aldehydes and phenolic acids (Seher, 1967) which are components of smoke, have been shown to have antioxidant characteristics - 2, 6-dimethoxyphenol and 2, 6-dimethoxy-4-ethylphenol have been shown to be effective.