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THE INFLUENCE OF NATURAL ANTIOXIDANTS ADMINISTERED DURING FEEDING OR PROCESSING ON THE OXIDATIVE QUALITY OF CURED PORK

By

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ABSTRACT

THE INFLUENCE OF NATURAL ANTIOXIDANTS ADMINISTERED DURING FEEDING AND PROCESSING ON THE OXIDATIVE QUALITY OF CURED PORK

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The effects of natural antioxidants on lipid stability in uncured and cured pork were assessed. In the initial study, the effect of dietary natural antioxidants on lipid stability in raw pork, frozen pork and cooked pork was evaluated. In the second study, the effects of natural antioxidants on cured meat color development, lipid oxidation and cholesterol oxidation in dry-cured pork were determined.

Dietary α-tocopherol improved lipid stability ($p<0.05$) in raw pork, frozen pork and cooked pork during storage; however, dietary oleoresin rosemary (OR) and oleoresin sage had no protective effect ($p>0.05$). In the dry-cured pork study, there were significantly ($p<0.05$) higher Hunter a-values in nitrite-treated samples than nitrite-free samples. Dietary OR had no antioxidant effect on lipid and cholesterol stability, while dietary α-tocopherol reduced lipid and cholesterol oxidation in dry-cured pork. Nitrite increased both lipid and cholesterol stability in dry cured-pork and its antioxidant effect was greater than dietary α-tocopherol.

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INTRODUCTION

Lipid oxidation is one of the primary mechanisms of quality deterioration in meat products, and leads to loss in flavor, color, texture, and nutritive value (Pearson et al., 1983). A variety of aldehydes, ketones and organic acids arise from the breakdown of lipid hydroperoxides and contribute to the sensory properties of meats after cooking (Mottram et al., 1987). It is known that unsaturated fatty acids are especially susceptible to lipid oxidation (Allen and Foegeding, 1981). Phospholipids are the primary contributors to lipid oxidation and warmed-over flavor (WOF) development in cooked meat (Igene and Pearson, 1979; Pearson and Gray, 1983). Although cooked meat is more susceptible to lipid oxidation than uncooked meat (Igene and Pearson, 1979; Pearson and Gray, 1983), any degree of oxidation occurring in raw materials can accelerate the development of WOF or oxidized off-flavors in cooked products.

Recently, detection of the presence of cholesterol oxidation products (COPS) in foodstuffs has raised significant health concerns (Addis and Park, 1989). Cholesterol oxides may be atherogenic (Kumar and Singhal, 1991), cytotoxic (Taylor et al., 1979), and may interrupt sterol metabolism (Chen et al., 1974; Peng et al., 1979; Kumar and Singhal, 1991).

Salt is an important additive in meat processing. It

provides flavor and inhibits growth of microorganisms in meat products. However, it is a prooxidant under certain circumstances (Lea, 1937) and enhances nonheme-iron-catalyzed lipid oxidation (Kanner et al., 1991).

In order to resolve the problem of lipid oxidation, food manufacturers employ synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) to foods. However, consumers are concerned about the safety of synthetic chemical compounds. A recent study has suggested toxicity of synthetic antioxidants (Ommen et al., 1992). This concern has led to interest in preparing antioxidants from natural foodstuffs by extraction and purification. Researchers have shown that natural antioxidants can be good alteratives to synthetic chemicals because they have similar antioxidant activities (Houlihan et al., 1985; Resurrection and Reynolds, 1989).

Natural antioxidants available to meat processors include vitamin E and rosemary extracts. Natural antioxidants can be incorporated into meat not only during processing but also through dietary feeding prior to slaughter of the animal (Buckley and Connolly, 1980; Chen et al., 1984a). Little work has been done to explore the effects of dietary antioxidants other than α-tocopherol during feeding and during processing in dry-cured pork. The effects of these antioxidants on color, lipid oxidation and cholesterol oxidation in dry-cured pork need further study.

The overall goal of this project was to assess the effects of vitamin E and oleoresin rosemary (OR) on the stability of lipids, including cholesterol, in dry-cured pork loins. Specific objectives were: (1) to monitor the effects of natural antioxidants (α-tocopherol, oleoresin rosemary and oleoresin sage) on lipid stability in raw and cooked pork during short- and long-term storage; (2) to determine the fate of nitrite and changes in color when natural antioxidants were present in cured pork (either through dietary supplementation or by mixing with the dry cure ingredients); and, (3) to compare the effectiveness of vitamin E, oleoresin rosemary and nitrite in preventing lipid or cholesterol oxidation during processing and refrigerated storage of dry-cured pork.

REVIEW OF LITERATURE

Lipids in Muscle Structure

Lipids vary in quantity and composition within avian, aquatic and mammalian muscle foods. The lipid portion of these foods is associated with numerous characteristics including flavor, color stability, texture, juiciness, protein stability and frozen storage shelf life.

Lipids are found in intermuscular, intramuscular and adipose tissues. Neutral lipids and phospholipids make up animal fats. Allen et al. (1967) reported that there were 79% neutral lipids and 21% phospholipids in pork L. dorsi. Neutral lipids are composed principally of glycerol esters of straight chain carboxylic acids having an even number of carbon atoms. A single glycerol molecule binds with one, two or three fatty acids to form mono-, di- or triglycerides. It is well known that most animal neutral lipids are mixed triglycerides. Neutral lipids are present as microscopic droplets within the muscle cell, or in adipocytes (Moody and Cassens, 1968). They provide fatty acids for energy metabolism in living muscle and contribute to the characteristics of meat.

In addition to differences in the lipid content between muscles, the fatty acid composition of muscle also has distinct differences among species. The fatty acids found in triglycerides and other muscle lipids differ in the carbon

chain length and in the type of bonding between the carbon atoms. Most fatty acids found in muscle contain an even number of carbon atoms. Branched chain and odd-number carbon fatty acids have been found at low levels in mutton and beef. Saturated fatty acids and monounsaturated fatty acids are dominant in meat. The unsaturated fatty acids found in meat are those with one or more double bonds. The most common unsaturated acids are oleic, linoleic and linolenic acids (Dugan, 1987).

Phospholipids from animal tissues are almost exclusively present in biological cellular membranes because of their role in the structure and function of the muscle cell. They play an important role in governing the quality of meat during cooking and processing (Keller and Kinsella, 1973). Pearson and Gray (1983) reported that phospholipids are primary contributors to lipid oxidation and warmed-over flavor development in cooked meat.

The Mechanism of Lipid Oxidation

Lipid oxidation is a complex process involving the reaction of unsaturated fatty acids with oxygen to generate free radicals and lipid hydroperoxides. The mechanism of lipid peroxidation was proposed by Farmer (1946) as follows:

Initiation	$LH + O_2 \xrightarrow{\text{initiator}} L. + .OOH$
Propagation	$L. + O_2 \longrightarrow LOO.$

LOO. + LH ---------> LOOH + L.

LOOH --------------> LO. + .OH

Termination L. + L. -----------> L-L

L. + LOO. ---------> LOOL

LOO. + LOO. -------> LOOL + O_2

where LH=unsaturated fatty acid

.OOH=hydroperoxyl radical

.OH=hydroxyl radical

L.=alkyl radical

LO.=alkoxyl radical

LOO.=peroxyl radical

LOOH=hydroperoxide

Per Farmer's theory, the initiation reaction between unsaturated lipid (LH) and dioxygen is possible. However, Heaton and Uri (1961) showed that this reaction was endothermic (ΔH=35 kcal) and hence could not proceed without the involvement of energy to abstract a proton from an unsaturated lipid. However, a metal catalyst could initiate this reaction and form an alkyl free radical (L.). At the propagation stage, the alkyl radical would react with oxygen to form the peroxyl radical (LOO.). Subsequently, the peroxyl radical may abstract one hydrogen atom from another unsaturated lipid to form a hydroperoxide (LOOH) and another alkyl radical. The alkyl radical could be regenerated in the chain reaction as a cycle, whereas the hydroperoxides could be decomposed to alkoxyl radical (LO.) and hydroxyl radical

(.OH). Alternatively, at the termination stage, a free radical could react with another free radical to form nonradical products (LOOL or L-L) which stop lipid oxidation.

Lipid Oxidation in Meat

Lipid oxidation is one of the primary mechanisms of quality deterioration in meat during storage (Pearson et al., 1977). The term "warmed-over flavor" introduced by Tims and Watts (1958), describes the rapid onset of rancidity in cooked meat during refrigerated storage and often is used synonymously with lipid oxidation. Lipid oxidation leads to the formation of short-chain aldehydes, ketones and fatty acids, as well as to the development of some polymers (Frankel, 1962). The oxidation of muscle lipids involves peroxidation of unsaturated fatty acids, in particular, polyunsaturated fatty acids (PUFA) (Allen and Foegeding, 1981). Many PUFA are associated with phospholipids. Phospholipids are the primary contributors to lipid oxidation and WOF development in cooked meat (Igene and Pearson, 1979; Pearson and Gray, 1983). The oxidative deterioration of meat lipids can directly affect the quality characteristics such as flavor, color, texture, nutritive value and safety (Pearson et al., 1983).

(A) Lipid Oxidation in Uncooked Meat

Cooked meat is more susceptible to lipid oxidation than uncooked meat (Igene and Pearson, 1979; Pearson and Gray, 1983) because cooking destroys the porphyrin ring structure of the heme pigment and releases heme iron which can catalyze lipid oxidation. However, oxidative changes in lipids may occur in uncooked meat when it is subjected to size reduction (such as grinding, flaking and chucking), freeze-thawing, temperature changes in handling/distribution, and/or prolonged storage. Lipid oxidation in uncooked meat occurs via both nonenzymatic and enzymatic mechanisms.

(1) Nonenzymatic Catalysis

The meat pigment myoglobin has been implicated in many studies as playing an important role in the catalysis of lipid oxidation in uncooked red meat (Green, 1969; Govindarajan et al., 1977; Verma et al., 1984; Rhee et al., 1986; Rhee and Ziprin, 1987). Tichivangana and Morrissey (1985) reported that purified metmyoglobin had no catalytic activity on lipid oxidation when added to water-extracted beef or lamb muscle fibers containing lipids. Liu (1970) demonstrated that linoleate oxidation was catalyzed by metmyoglobin (MetMb), Fe^{+2}-EDTA (1:1) and raw beef homogenate. He concluded that the catalytic activity of raw beef homogenate was due to both heme and nonheme iron.

Kanner and Harel (1985) and Harel and Kanner (1985a; 1985b) have extensively studied muscle membranal lipid oxidation initiated by hydrogen peroxide-activated metmyoglobin. They proposed the following sequence of events to explain the initiation and propagation of lipid oxidation in a meat system: (1) oxidation of oxymyoglobin generates metmyoglobin and hydrogen peroxide; (2) activation of metmyoglobin by hydrogen peroxide generates a ferryl species, called the porphyrin cation radical, in which iron has an oxidation number of four ($P^+ - Fe^{4+} = O$); and (3) initiation of lipid oxidation by the porphyrin cation radical proceeds through two-electron reduction of the catalyst:

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

$$L. + O_2 \rightarrow LOO.$$

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

$$P - Fe^{4+} = O + LOOH \rightarrow P - Fe^{3+} + LOO. + OH^-$$

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

These investigators found that little or no lipid oxidation occurred in the sarcoplasmic fraction of turkey dark muscle in the presence of either H_2O_2 or MetMb alone, while lipid oxidation occurred in the presence of H_2O_2 plus MetMb. This study indicated that heme iron alone was not totally responsible for lipid oxidation in muscle tissues. It was partly due to iron released from MetMb.

Rhee et al. (1987) studied the effect of MetMb plus H_2O_2 on lipid oxidation in a model meat system where MetMb-H_2O_2 was

added to water-extracted beef muscle residue. They found that MetMb-H_2O_2 at molar ratios ranging from 1:0.1 to 1:2 catalyzed the oxidation of beef muscle lipids in both raw and cooked systems. They also found that MetMb alone had little catalytic activity, and the catalytic activity of MetMb-H_2O_2 was highest at the molar ratio of 1:0.25 in the raw residue system and at the molar ratio of 1:1.5 or 1:2 in the cooked system. They concluded that the lipid oxidizing activity of MetMb-H_2O_2 in a meat system may be due primarily to MetMb activated by H_2O_2 and secondarily to heme iron released from MetMb-activated by H_2O_2. These conclusions were based on the observations that: (1) the optimum amount of H_2O_2 for lipid oxidation was far below the amount of H_2O_2 causing the greatest release of nonheme iron and (2) the relationship between the 2-thiobarbituric acid reactive substances (TBARS) value and the nonheme iron concentration was not linear.

(2) Enzymatic Catalysis

Researchers have shown that there are enzymatic systems in the microsomal fraction of chicken skeletal muscle (Lin and Hultin, 1976; Player and Hultin, 1977) and in fish muscle microsomes (McDonald et al., 1979; Hultin, 1980; Slabyi and Hultin, 1982). The enzymatic systems can catalyze the oxidation of microsomal lipids in the presence of cofactors. Rhee (1988) summarized enzymatic catalysis by stating that lipid oxidation is dependent on nicotinamide adenine

dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), adenosine 5'-diphosphate (ADP), ascorbate, and Fe^{+2} or Fe^{+3}. Rhee et al. (1984) reported that lipid oxidation was more rapid with NADPH than with NADH in a beef muscle microsomal system. However, in fish microsomes, NADH was much more efficient than NADPH (McDonald et al., 1979; Slabyi and Hultin, 1982).

In addition to microsomal lipid peroxidation systems, mitochondrial enzymatic lipid peroxidation systems may also play an important role in raw muscle. Luo and Hultin (1986) demonstrated that an enzymatic lipid peroxidation system was present in fish (trout) muscle mitochondria, and they reported that the mitochondrial system was similar to the microsomal system in fish muscle in terms of cofactor requirements. However, this enzymatic system has not been isolated from beef, chicken and pork muscle mitochondria.

German and Kinsella (1985) studied mechanisms underlying the initiation of lipid oxidation in fish, using a crude aqueous extract from trout skin tissue as the source of enzyme and exogenous radioactive fatty acids as substrates. They concluded, on the basis of the type of monohydroxy compounds produced from the oxidation reaction and responses to lipoxygenase inhibitors, that the trout skin extract contained lipoxygenase and that the skin lipoxygenase released postmortem may constitute a significant source of initiating radicals leading to subsequent lipid oxidation in fish.

Grossman et al. (1988) provided evidence that lipoxygenase-type enzymes were present in chicken muscle by examining the oxidation products of [^{14}C]arachidonic acid. They suggested that 15-lipoxygenase was present in chicken muscle and may be responsible for some of the oxidative changes occurring in fatty acids on frozen storage of chicken meat.

These studies indicate that enzyme systems can initiate lipid oxidation in raw muscle tissues. Microsomal and mitochondrial enzyme systems, as well as lipoxygenase, may be involved in initiation of lipid oxidation in skeletal muscles.

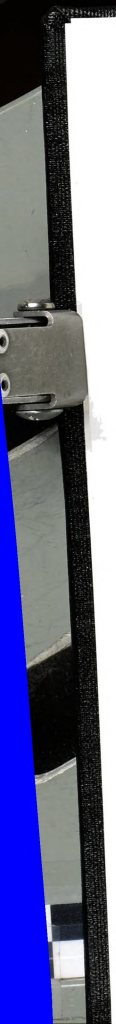
(B) Lipid Oxidation in Cooked Meat

Before 1970, researchers reported that MetMb was the major catalyst of lipid oxidation in cooked red meat (Younathan and Watts, 1959). It was not until 1970 that both heme iron and nonheme iron were suggested to play an important role in lipid oxidation in meat (Liu and Watts, 1970). This suggestion was based on the observation that lipid oxidation still occurred in cooked beef muscle whose heme pigments were degraded by treatment with 30% H_2O_2, but the degree of lipid oxidation was much more than untreated cooked meat. Decker and Schanus (1986) investigated linoleate oxidation catalyzed by an aqueous extract of chicken drumstick muscle and reported that both heme and nonheme iron may be involved in catalysis of lipid oxidation in raw chicken dark meat.

Sato and Hegarty (1971), Love and Pearson (1974) and Igene

et al. (1979) proposed that nonheme iron played a major role in accelerating lipid oxidation in cooked meat. This proposal was based on the findings that purified MetMb, added to water-extracted beef muscle, did not accelerate oxidation of beef muscle lipids upon heating and refrigeration, whereas the nonheme iron added to the muscle accelerated oxidation. However, Johns et al. (1989) added hemoglobin and inorganic iron to washed muscle fibers and concluded that hemoglobin was a powerful catalyst. All forms of inorganic iron had little prooxidant activity. These results are in direct contrast to results reported by Sato and Hegarty (1971) and Love and Pearson (1974). Johns et al. (1989) concluded that these results were due, at least in part, to the difficulty of evenly dispersing the catalysts in the washed fibers, and that H_2O_2, formed by oxidation of oxypigments, may be necessary for ferric heme pigments to be effective catalysts.

Liu (1991) studied a water extracted model system similar to Johns et al. (1989). He verified the data reported by Johns et al. (1989) and concluded that inorganic iron had a prooxidant activity only during early storage (day 1). After day 1, inorganic iron did not give a significant ($p>0.05$) response. Monahan et al. (1993) also reported that heme protein had a greater prooxidant effect than inorganic iron in a raw and heated pork water extracted model system. These observations were made when the prooxidants were present at concentrations approaching those in red meat. They



suggested that the apparently contradictory results obtained from studies with muscle model systems may be due to differences in (1) muscle species used in the preparation of water-washed muscle residue, (2) sample storage time during which oxidation was monitored, and (3) levels of prooxidants incorporated into model systems.

It has been proposed that the increased rate of lipid oxidation in cooked meat compared to uncooked meat is due to the release of iron from heme pigments during cooking (Igene et al., 1979; Chen et al., 1984b). Igene et al. (1979) also concluded that heme pigments served as a source of free iron, being readily broken down during the cooking process. The observation of increasing amounts of nonheme iron as a consequence of heating was verified by Schricker et al. (1982), Schricker and Miller (1983) and Rhee and Ziprin (1987).

The rate of heating and final temperature both can influence the release of iron from meat pigments (Schricker and Miller, 1983; Chen et al., 1984b). Schricker and Miller (1983) reported that microwave cooking of meat released less iron than did roasting and braising. Chen et al. (1984b) also found that slow heating released more iron compared to fast heating.

Cholesterol in Foods

Cholesterol (cholest-5-en-3β-ol) is a non-polar simple lipid (Figure 1). It is the precursor for all of the steroid hormones, vitamin D and the bile acids. The variation of cholesterol content in foods depends on the species of comparison. Feeley et al. (1972) concluded from a review of literature (Pihl, 1952; Del Vecchio et al., 1955; Tu et al., 1967) that lean tissue and adipose tissue from beef, lamb, pork and veal had approximately the same percentages of cholesterol. Researchers (Church and Church, 1975; Rhee et al., 1982) reported that red meat, poultry and fish contain appreciable amounts of cholesterol and that lard and other animal fats contain slightly higher amounts than the muscle foods. Cholesterol levels in lean tissue range from 60 to 70 mg/100 g, and from 70 to 75 mg/100 g in adipose tissue.

Mechanism of Cholesterol Oxidation

Cholesterol oxidation has been recognized and studied for the past 90 years. During the 1960's, a systematic study of cholesterol autoxidation was undertaken (Smith, 1981). Cholesterol has one double bond between the 5th and 6th carbon atoms. Therefore, it is susceptible to oxidation via the free radical process in the same manner as PUFA and their esters.

A major mechanism of cholesterol oxidation is where cholesterol is initially attacked on the C_7 of the B ring by

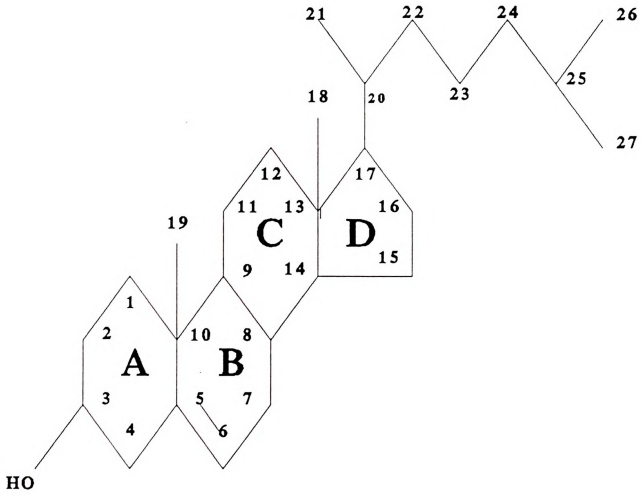


Fig 1. Structure of Cholesterol

oxygen to form the peroxy free radical. The peroxy radical abstracts one hydrogen to form 7α- or 7β-hydroperoxides. The β-epimers are more thermodynamically stable than α-epimers (Smith, 1981). Thermal decomposition of the 7-hydroperoxides yields 7-ketocholesterol or cholest-5-ene-3β,7-diols by way of dehydration. These four cholesterol oxidation products are called primary COPS.

Epoxidation of 7α-hydroxycholesterol or 7β-hydroxycholesterol generates α-epoxidecholesterol or β-epoxidecholesterol. The further hydration of these two epoxides forms 5α-cholesterol-3β,5,6β-triol. These three cholesterol oxidation products are called secondary COPS (Smith, 1981).

In a minor, independent mechanism of cholesterol oxidation, the 3β-alcohol forms cholest-5-en-3-one which will form alcohol 6α- or 6β-hydroxycholest-4-en-3-ones, cholest-4-ene-3,6-dione or 5α-cholestane-3,6-dione after rearrangement, oxygenation and decomposition. Finally, oxidation of the side chain forms 20-, 24-, 25- or 26-hydroperoxides and their decomposition products by attack of oxygen and attraction of one hydrogen. These products are called tertiary COPS (Smith, 1981).

Cholesterol Oxidation Products in Foods

In recent years, oxysterol formation and their presence in foodstuffs have raised concerns (Smith, 1987; Maerker, 1987) because COPS may produce adverse biological effects in

animals. Cholesterol oxides have been detected in egg products (Missler et al., 1985), heated tallow (Bascoul et al., 1986; Park and Addis, 1986), dairy products (Nourooz-Zadeh and Appelqvist, 1988), and meat products (Higley et al., 1986; Park and Addis, 1987; Pie et al., 1991; Monahan et al., 1992b). However, it is difficult to quantify all of the cholesterol oxides present in foods because of the complex nature of the foods and the interconversion of cholesterol oxides during purification procedures (Chicoye et al., 1968; Smith, 1981; Park and Addis, 1986).

Pie et al. (1991) demonstrated the effect of cooking time, cooking method and freezer storage on the oxidation of cholesterol in beef and pork. They found that cholesterol was oxidized in meat samples during household cooking and the rate of oxidation differed according to the cooking time and cooking temperature. They also observed that there was a greater increase in primary cholesterol oxides than in secondary cholesterol oxides, and all cholesterol oxides increased in meat samples stored for 3 months at -20°C in both raw and cooked meat.

Monahan et al. (1992b) studied the effects of dietary treatment on cholesterol oxidation in pork. They found that dietary α-tocopherol significantly reduced cholesterol oxidation. They also found that the rate of formation of cholesterol oxides was low in raw samples compared to that in cooked samples. They concluded that lipid oxidation and COPS

formation were positively correlated in cooked pork.

Engeseth et al. (1993) studied the effects of dietary α-tocopherol on cholesterol oxide development in raw and cooked veal muscles stored at 4°C. Dietary α-tocopherol was effective in controlling the development of cholesterol oxides in both raw and cooked muscles during storage. They also found that cholesterol oxide development was greater in cooked samples. These results are consistent with previous findings (Sato and Hegarty, 1971; Monahan et al., 1992b) and can be explained by the harsh cooking conditions which lead to the disruption of membranes and subsequent exposure of lipids to oxidative catalysts (Rhee, 1988).

Biological Effects of Cholesterol Oxidation Products

Recently, the possible oxidation of cholesterol in foods has raised concerns due to the undesirable biological effects of COPS (Addis and Park, 1989). These cholesterol oxides may be atherogenic (Taylor et al., 1979; Kumar and Singhal, 1991), cytotoxic (Taylor et al., 1979), and may interrupt sterol metabolism (Chen et al., 1974; Peng et al., 1979; Kumar and Singhal, 1991).

Smith and Van Lier (1970) isolated and identified traces of 12 known COPS from atheromata removed from human aortas. It was postulated that humans may ingest foods that contain COPS. These products are absorbed into the cholesterol pool of the body and become a portion of aortic cholesterol

deposits (Taylor et al., 1979). Kumar and Singhal (1991) reviewed the literature and summarized that cholesterol oxides, rather than cholesterol, can initiate the formation of atherosclerotic plaque. They suggested that cholestan-3β,5α,6β-triol and 25-hydroxy-cholesterol were the most potent atherogenic agents.

Cytotoxic effects of COPS in rabbits have been demonstrated by Taylor et al. (1979). They reported that 25-hydroxycholesterol and cholestane-3β,5α,6β-triol were probably responsible for the biological activity.

Chen et al. (1974) noted that selected oxygenated sterols influenced cholesterol biosynthesis by inhibiting the enzyme, 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (HMG-CoA reductase). They observed that 5-cholesten-3β,25-diol was a potent inhibitor of HMG-CoA reductase, whereas other COPS inhibited HMG-CoA reductase less. The inhibition of cholesterol synthesis is associated with atherosclerosis because the COPS cause membrane fragility which result in improper membrane growth and subsequent cell death. Peng et al. (1979) presented the same theories but postulated an alternative mechanism. They proposed that the replacement of cholesterol in membranes by COPS easily occurred as a result of the presence of both polar and nonpolar functional groups on the same molecule and resulted in cellular malfunction. They also reported that cholestan-3β,5α,6β-triol had a greater toxic effect than 5-cholesten-3β,25-diol.

The Role of Salt as a Prooxidant in Meat Products

Salt is added to muscle foods for a variety of purposes, including flavor and the inhibition of growth of micro-organisms. However, it has been reported that salt will act as a prooxidant (Lea, 1937; Chang and Watts, 1950; Tappel, 1952; Banks, 1961; Ellis et al., 1968; Powers and Mast, 1980; Kanner and Kinsella, 1983) or as an antioxidant (Chang and Watts, 1950; Mabrouk and Dugan, 1960). Rhee et al. (1983) reported that salt activated lipid oxidation at low concentrations but inhibited lipid oxidation at concentrations greater than 2% in ground pork. Ellis et al. (1968) postulated that salt may activate a component in lean meat resulting in a change in the oxidation characteristics of adipose tissue. The mechanism by which salt function as prooxidant is poorly understood (Rhee et al., 1983; Anon, 1988; Hultin, 1988).

Salt will accelerate rancidity in frozen pork (Dubois and Tressler, 1943; Watts and Peng, 1947), in cured pork (White, 1941), and in fish (Banks, 1937). Shomer et al. (1987) reported that increasing the concentration of salt enhanced lipid oxidation in raw minced muscle, especially after freeze-thawing. Kanner et al. (1991) suggested that salt acted to displace non-heme iron ions from binding sites on the muscle portion. There is a small amount of free iron in muscle tissue. These free iron ions interact with muscle protein. The interaction prevents the iron from affecting

membranous lipids and acting as catalyzers of lipid peroxidation. Osinchak et al. (1991) demonstrated that salt was a potent prooxidant in a model system comprising the soluble fraction of mackerel muscle cells. They also reported that the prooxidant effect of salt was due to the chloride ion and not to the sodium ion because the chloride ion was a good binder of Fe^{+3}. Consequently, chloride ion binding with more Fe^{+3} also prevented the interaction of Fe^{+3} with proteins, thus enhancing lipid oxidation.

The Role of Nitrite in Meat Products

The roles of nitrite in cured meat are: (1) to develop the cured-meat color of lean tissues, (2) to contribute to the characteristic flavor of cured meat, (3) to inhibit the growth of food poisoning and spoilage microorganisms, and (4) to retard the development of rancidity. In this review, the role of nitrite in cured meat color development and as an antioxidant will be discussed in detail.

(A) Development of Cured-Meat Color

The mechanism of development of cured color in meat was summarized by Kramlich et al. (1973):

Nitrite --------> NO + H_2O

NO + Mb --------> NOMMb

NOMMb --------> NOMb
(reduce)

NOMb + Heat (or + Smoke) ----------> NO-hemochrome

NOMb = nitrosylmyoglobin; NOMMb = nitrosylmetmyoglobin

When nitrite is added to a meat system, it breaks down to form nitric oxide which reacts with myoglobin in meat to form nitrosylmetmyoglobin (brown color). Then, nitrosylmetmyoglobin is reduced to nitrosylmyoglobin (red color). Reduction of nitrosylmetmyoglobin involves addition of an electron to ferric ion of heme converting it to ferrous ion. This reduction might be accomplished either naturally in meat, or by a reductant included in the cure. The reducing activity of ascorbic acid accelerates nitrosylmetmyoglobin reduction by donating electrons to the ferric ion of the heme. Sulfhydryl groups released during heat processing of cured meat also are very strong reducing compounds, and can contribute significantly to reduction of metmyoglobin or nitrosylmetmyoglobin. After heating and/or smoking, nitrosylmyoglobin will form dintrosylhemochrome (pink color) which is the typical cured meat color. Tarladgis (1962) and Lee and Cassens et al. (1976) reported that the structure of cured meat pigment depended upon the state of the protein. If the myoglobin was undenatured, the heme bound one molecule of nitric oxide to form nitrosylmetmyoglobin pigment. If the myoglobin was denatured as in cooking, the heme bound two molecules of nitric oxide to form dinitrosylhemochrome.

(B) Development of Cured-Meat Flavor

The effect of nitrite in modifying fresh meat flavor was first documented by Brooks et al. (1940). Cho and Bratlzer (1970) suggested that nitrite reacted with components in muscle tissues and produces the characteristic cured-meat flavor. However, the mechanism of flavor development in cured meat is not clearly understood. Gray et al. (1981) summarized previous studies and concluded that carbonyl compounds in the volatile fraction may be responsible for the characteristic cured-meat flavor. Shahidi et al. (1986) reported that major contributors to uncured and cured meat flavor were volatile compounds including acids, alcohols, aldehydes, heterocyclics, hydrocarbons, ketones and sulfides. Rubin and Shahidi (1988) reported that the nature of cured-meat flavor is due to suppression of lipid oxidation by nitrite.

(C) Inhibition of Microorganism

Nitrite can inhibit food poisoning and spoilage microorganisms (Townsend and Olson, 1987). Researchers have verified the effectiveness of nitrite in inhibiting the outgrowth of Clostridium botulinum in cured meat products (Duncan and Foster 1968; Johnson et al., 1969; Cuppett et al., 1985). Duncan and Foster (1968) and Johnson et al. (1969) indicated that nitrite does not inhibit the true spore germination but inhibits the outgrowth and division of the

cells. Nevertheless, commercial experience has shown that the following combination of practices is highly effective in producing safe cured meat (Townsend and Olson, 1987): adding sodium nitrite at an initial concentration of 75 to 150 ppm, with a residual concentration of 20 ppm or more, attaining a sodium chloride concentration of 1.5 to 2.0% and heating the product to 71°C.

(D) Antioxidant Activity of Nitrite

Nitrite is an antioxidant in cured meat. Zipser et al. (1964) proposed that nitrite reacts with heme-containing proteins to form catalytically inactive species. Nitrite also reacts with other constituents of muscle, such as nonheme proteins, low-molecular-weight peptides and amino acids. Furthermore, nitrite can react with unsaturated fatty acids in adipose tissue. Sato and Hegarty (1971) reported that nitrite added at the level of 2000 mg/kg significantly inhibited thiobarbituric acid (TBA) values in the cooked ground beef. Nitrite added at a level of 50 mg/kg lowered TBA values in cooked ground beef. In addition, they suggested that nitrite may inhibit natural prooxidants present in muscle or stabilize the lipid components of the membranes. Fooladi et al. (1979) also reported that nitrite added at the level of 156 mg/kg could prevent WOF in cooked beef, pork and chicken. McDonald et al. (1980b) investigated the effect of various levels of nitrite (50, 200 and 500 mg/kg) on lipid oxidation

in cooked hams and demonstrated that there was a significant reduction in TBA values at all nitrite levels studied. Morrissey and Tichivangana (1985) reported that nitrite as low as 20 mg/kg can significantly inhibit lipid oxidation in fish, chicken, pork and beef products. Cuppett et al. (1989) reported that addition of nitrite to smoked whitefish significantly reduced lipid oxidation.

The Mechanism of Nitrite as an Antioxidant

How nitrite functions as an antioxidant is not well understood. Possible mechanisms are: (1) formation of a stable complex with heme pigments, thereby preventing the release of heme iron and the subsequent catalysis of lipid oxidation (Igene et al., 1985; Morrissey and Tichivangana, 1985), (2) stabilization of membrane lipids which are normally disrupted and exposed to oxygen by cooking and grinding (Igene et al., 1985), (3) formation of an inactive "chelate" between nitrite and various metal ions, resulting in the reduction of the prooxidant activity of metal catalysts (McDonald et al., 1980a; Igene et al., 1985; Morrissey and Tichivangana, 1985, and (4) formation of nitric oxide myoglobin, which has antioxidant properties (Kanner, et al., 1980; Morrissey and Tichivangana, 1985).

(A) Stabilization of Heme Pigments

Zipser et al. (1964) first proposed that nitrite could form stable complexes with the iron porphyrin in heated meat systems, therefore, inhibiting heme-catalyzed lipid oxidation. When nitrite is added to meat, it reacts with heme pigments to form nitrosyl heme pigments before cooking or dinitrosyl heme pigment after cooking. This reaction greatly increases the stability of heme pigment in a cured meat system and indirectly reduces heme-catalyzed lipid oxidation. Morrissey and Tichivangana (1985) demonstrated the effect of nitrite on the stability of heme pigments in an aqueous extract of beef muscle. They indicated that cooking may enhance the release of heme iron from heme pigments and concluded that heme pigments treated with nitrite released less heme iron than those without nitrite after cooking. Freybler et al. (1991) demonstrated that nitrite can stabilize heme pigments and prevent the release of iron in the presence of hydrogen peroxide and/or heat. Therefore, nitrite can stabilize the heme pigments and reduce the amount of heme iron released from the heme pigments thus indirectly reducing lipid oxidation.

(B) "Chelation" of Trace Metal Ions

McDonald et al. (1980a) proposed that nitrite could bind ferrous ions in a cured meat system and inhibit nonheme-iron-catalyzed lipid oxidation. In their model system of linoleic

acid, phosphate buffer and Tween 20, they found that nitrite at a level smaller than 25 mg/kg could decrease the oxidation of linoleic acid. Morrissey and Tichivangana (1985) also proposed that nitrite forms inactive "chelates" or complexes with nonheme iron, copper, and cobalt, thus reducing the catalytic activity of these ions and subsequent lipid oxidation. They used a model system of water-extracted muscle fibers with various prooxidants (ferrous ion, copper and cobalt), and found that nitrite could significantly inhibit the catalytic activity of these prooxidants, thus retarding lipid oxidation. Apte and Morrissey (1987) studied the complexing of nitrite with metal ions by adding nitrite to pork and fish systems before heating, after heating, 24 hr after heating or 48 hr after heating. They found that nitrite was an effective antioxidant when added to meat systems before heating and even when added after heating. In the latter systems, the effect of nitrite could not be explained by the fact that nitrite stabilizes the heme pigments. Therefore, Apte and Morrissey (1987) concluded that nitrite must react with free iron, thereby preventing iron-catalyzed lipid oxidation.

(C) Formation of Nitrite-Derived Antioxidants

Kanner et al. (1980) reported that nitrosyl heme pigment could act as an antioxidant in cured meat. They proposed that the antioxidant activity of the nitrosyl heme pigment was due

to its ability to quench the free radicals. This process caused the nitrosylmyoglobin to dissociate leaving metmyoglobin in the system. The metmyoglobin may act as a hydroperoxide decomposer and also quench free radicals formation if the concentration of hydroperoxide was low enough to be decomposed by metmyoglobin.

Other compounds derived from nitrite with antioxidant activity have been identified. Kanner (1979) reported that S-nitrosocysteine could inhibit lipid oxidation in an aqueous linoleate model system and in a turkey meat system. Kanner et al. (1984) also found that both hemin nitroxide and cysteine-iron-nitroxide have antioxidant activity. They believed that the functions of these compounds were similar to those of nitrosyl heme pigments.

(D) Stabilization of Lipids

Woolford et al. (1976) found that nitrite was associated with the lipid fraction in bacon. Pearson et al. (1977) suggested that nitrite could stabilize membranal lipids and inhibit lipid oxidation. Goutefongea et al. (1977) demonstrated that the degree of binding of sodium nitrite with lipids was positively related to the degree of unsaturation of the lipids. Hotchkiss et al. (1985) reported that a lipid-bound nitrite compound existed in bacon and suggested that oxides of nitrogen formed from added nitrite reacted with unsaturated lipids during the curing process to form lipid

bound-nitrite compounds. These compounds were capable of nitrosating amines and could not be extracted or purged from bacon fat.

Zubillaga and Maerker (1987) studied the antioxidant activity of polar lipids extracted from nitrite-treated meat. They found that the antioxidant activity of the extracted polar lipids was 1.5 to 3 times greater than the activity of polar lipids isolated from untreated meat. The antioxidant activity of the polar lipids from cured meat was stable during storage.

Ross et al. (1987) studied the reaction of dinitrogen trioxide with methyl oleate, methyl linoleate and methyl linolenate and demonstrated that these compounds can form reaction products which were capable of nitrosating amines. They used high pressure liquid chromatography (HPLC) and infrared spectrophotometry (IR) to verify the reaction of dinitrogen trioxide with unsaturated lipids to form nitro-nitroso derivatives. Freybler et al. (1991) studied the reaction of phospholipids and polyunsaturated fatty acid ethyl esters with dinitrogen trioxide and found that these reactions can stabilize lipids. These studies demonstrated that nitrite could react with unsaturated lipids in cured meat and stabilize the lipids, thus inhibiting lipid oxidation.

Natural Antioxidants

There are many ways to retard lipid oxidation in foods. Food manufacturers apply synthetic antioxidants in foods to retard lipid oxidation and prolong the shelf life. Several synthetic phenolic antioxidants have been used successfully to prevent WOF development in restructured meat products (Chastain et al., 1982; Crackel et al., 1988b). These antioxidants are not permitted in the USDA regulations. However, they are approved for pork sausage. The most commonly used antioxidants at the present time are BHA and BHT. These are added to a variety of processed foods in the market place. However, consumers are interested in the use of natural antioxidants because of the possible toxicity of synthetic antioxidants (Ommen et al., 1992). Ommen et al. (1992) reported that the glutathione conjugates of tert-butyl hydroquinone, a metabolite of BHA, possesses much higher redox potentials than the non-conjugated hydroquinone. The redox cycling activity of the conjugates is increased tenfold when compared to the non-conjugated hydroquinone. When the redox activity is increased, there is a formation of reactive oxygen species and free hydroxyl radicals which react with DNA and ultimately produce carcinogenicity in the rat forestormach (Ommen et al., 1992). The concern has led to preparing antioxidants from natural ingredients by extraction, purification and fractionation, and incorporating them into processed foods.

Alpha-Tocopherol (Vitamin E)

At least eight compounds (α, β, γ, δ-tocopherol and α, β, γ, δ-tocotrienol) have been isolated from plant sources that have vitamin E activity. All have a 6-chromonal ring structure and a side chain. The tocopherol has a phytol side chain and the tocotrienol had a similar structure with double bonds at the 3', 7' and 11' positions of the side chain. Because α-tocopherol has the highest biological activity in foods, it is defined as vitamin E. Alpha-tocopherol is insoluble in water but is soluble in oils, fats, acetone, alcohol, chloroform, ether, and other fat solvents. Tocopherols are stable to heat and alkali in the absence of oxygen and are unaffected by acids up to 100°C. They are slowly oxidized by an oxygen atmosphere.

The Biological Function of Alpha-Tocopherol

Olcott and Mattill (1941) suggested that α-tocopherol functions as an antioxidant in vivo. The common theory for initiation and propagation of free-radical-mediated oxidation and their inhibition by antioxidants is outlined in Figure 2.

Tappel (1962) proposed that vitamin E functions as an antioxidant which protects tissue lipids from free radical attack. This proposal has been detailed and expanded by Molenaar et al. (1980) and by McCay and King (1980). Tocopherol is located primarily in the membrane portion of

Figure 2. Lipid oxidation and antioxidant reactions involving vitamin E.

1. Initiation (formation of a free radical)
   initiator
   LH ----------> L.

2. Reaction of the radical with oxygen
   L. + O_2 ----------> LOO.

3. Propagation
   LOO. + LH ----------> L. + LOOH

4. Antioxidant reaction
   LOO. + E ----------> E. + LOOH

5. Regeneration
   E. + C ----------> E + C.

   semidehydro ascorbate reductase
   C. + NADH ----------> C + NADP

   enzyme ?
   E. + 2GSH ----------> E + GSSG

   glutathione reductase
   GSSG + NADPH ----------> 2GSH + NADP

6. Termination
   E. + E. ----------> E-E

   E. + LOO. ----------> EOOL

LH : Fatty acid
L. : Fatty acid radical
LOO. : Peroxyl radical
E : Tocopherol
E. : Tocopheroxyl radical
LOOH : Hydroperoxide
C : Ascorbic acid
C. : Ascorbyl radical
GSH : reductase glutathione
GSSG : Oxidized glutathione

(Machlin, 1991)

the cell. A number of enzymatic and nonenzymatic free-radical generating reactions occur in cells and tocopherol acts as part of the cell's defense against oxygen-containing radicals. The enzymes superoxide dimutase, catalase, glutathione peroxidase and glutathione reductase also play a protective role. Molenaar et al. (1980) proposed that the chromanal ring of tocopherol was located at the polar surface of the membrane and that the phytol side chain interacts with PUFA of phospholipids in the nonpolar interior of the membrane.

Alpha-tocopherol is an effective membrane radical scavenger because it is able to move very rapidly through the nonpolar portion of the membrane. McCay and King (1980) have proposed that superoxides (O_2.-) interact with hydrogen ions to produce hydrogen peroxide which will be distributed in both the aqueous and membrane phases of the cell. Glutathione peroxidase, which is a selenium-containing enzyme, may destroy hydrogen peroxide in the aqueous phase, thus leaving most of hydrogen peroxide in the membrane. Hydrogen peroxide remaining in the membrane may react with the superoxide anion to form hydroxy radicals, which can react with tocopherol localized in the membrane. If there is enough tocopherol available in the membrane to trap the hydroxyl radicals, the trapping can terminate the initiation of lipid oxidation.

One concern of this antioxidant hypothesis is that one should expect to detect peroxides in tissues that have pathology resulting from a vitamin E deficiency. However, no

unequivocal evidence has been presented for the existence of peroxides in these tissues. It is now realized that adequate levels of glutathione peroxidase in such tissues may destroy peroxides as quickly as they are formed.

There is no evidence of any diminution in the tocopherol content of affected tissues or for the appearance of tocopherol oxidation products. Two suggestions on this point are that tocopherol could be regenerated from tocopherol radicals either by reduced glutathione or by ascorbic acid with the generation of either oxidized glutathione or an ascorbate radical (Pryor et al., 1976; Packer et al., 1979).

Effect of Dietary Alpha-Tocopherol Supplementation on the Oxidative Rancidity of Meat

Application of α-tocopherol to feeds to stabilize the resultant animal products was first suggested by Burr et al. (1946). Duncan et al. (1959) concluded that dietary α-tocopherol could improve the stability of pork. Laksesvela (1960) established that an addition of as little as 37 ppm of α-tocopherol could improve the quality of poultry meat. Dietary vitamin E (α-tocopherol) supplementation has been shown to improve the oxidative stability of muscle from fish (Frigg et al., 1990), chicken (Brekke et al., 1975; Lin et al., 1989), pork (Astrup, 1973; Buckley and Connolly, 1980; Buckley et al., 1988; Monahan et al., 1990) and veal (Shorland et al., 1981; Engeseth et al., 1993).

Astrup (1973) reported that supplementation of vitamin E in pigs prior to slaughter could increase the levels of vitamin E in the fat, thus reducing lipid oxidation. He also indicated that the stability of the fat increased with increasing levels of vitamin E in the diet. Shorland et al. (1981) reported that supplemental vitamin E had a significant effect in enhancing the stability of lipids in veal L.dorsi and perinephric tissues. Monahan et al. (1990) reported that dietary α-tocopherol supplementation may offer an effective means of incorporating α-tocopherol into subcellular membranes and stabilizing muscle tissue which contained elevated levels of unsaturated fatty acids. Monahan et al. (1992a) also reported that lipid oxidation was significantly influenced by dietary α-tocopherol supplementation.

Rosemary

It is known that crude extracts from selected leafy materials are stable to oxidation despite their high linolenic acid content. The resistance to oxidation is due to the presence of active naturally occurring antioxidants, probably of the phenolic or polyphenolic compound class.

Extracts of spices have been shown to have varying degrees of antioxidant activity. They have been used to extend the storage time of meat from early times. Chipault et al. (1952) reported that only rosemary and sage, out of 32 common spices, were effective as antioxidants. The use of rosemary 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). However, it was a problem when rosemary was used as a raw material in foods because it was difficult to disperse, or a large amount was needed to achieve the antioxidant effect. Over the years, several approaches have been used to prepare rosemary extracts to resolve these problems (Rac and Ostric, 1955; Berner and Jacobson, 1973; Chang et al., 1977; Bracco et al., 1981). In addition to the production of rosemary extracts, several studies have been aimed at isolating and identifying active antioxidant compounds in rosemary (Brieskorn et al., 1964; Chang et al., 1977).

Compounds in Rosemary Extracts with Antioxidant Activity

The antioxidant properties of compounds extracted from rosemary were apparently related to their phenolic contents, thus their antioxidant action might be similar to synthetic phenolic antioxidants (Bracco et al., 1981). The first important antioxidant isolated from rosmary (Rosemarinus officinalis L.) was a phenolic diterpene named carnosol. Its structure was determined by Brieskorn et al. (1964). Wu et al. (1982) reported that carnosol added to lard had antioxidant activity comparable to BHT. Bracco et al. (1981) suggested that the antioxidant activity of rosemary must be primarily related to its carnosic acid which contained

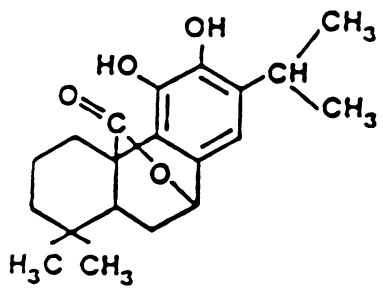
phenolic diterpene structure.

Another compound isolated from rosemary leaves by Inatani et al. (1982) was called rosmanol which is also a phenolic diterpene with a structure similar to that of carnosol. They reported that rosmanol was a good antioxidant with similar activity to carnosol in several fat substrates.

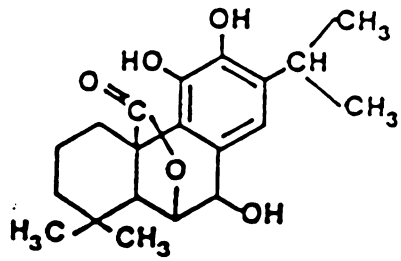
Recently, rosemaridiphenol, isolated from rosemary leaves, was investigated by Houlihan et al. (1984). They reported that the antioxidant activity of rosemaridiphenol surpassed BHA and approached the effectiveness of BHT. Another diterpene, rosmariquinone, was also shown to possess antioxidant activity in prime steam lard at a concentration of 0.02% (Houlihan et al., 1984). The structures of the antioxidant compounds in rosemary extracts are shown in Figure 3.

Application of Oleoresin Rosemary in Food Processing

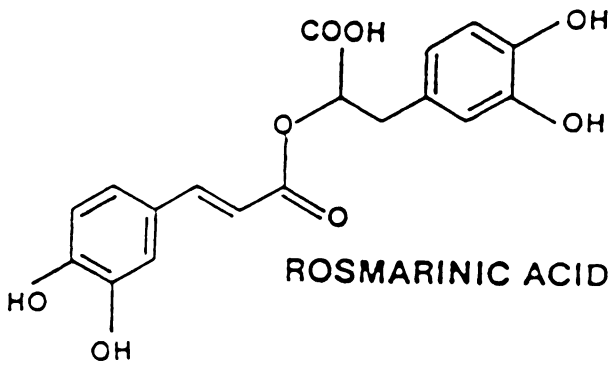
Oleoresin rosemary contains a number of compounds such as rosmarol, carnosol, rosmaridiphenol and rosmariquinone which impart antioxidant activity similar to or greater than BHA (Houlihan et al., 1985). Barbut et al. (1985) incorporated oleoresin rosemary into turkey breakfast sausage and concluded that it was comparable to a commercial blend of BHA/BHT/citric acid in suppressing lipid oxidation. Resurrection and Reynolds (1989) found that oleoresin rosemary was as effective as BHA/BHT in retarding lipid oxidation in chicken/pork

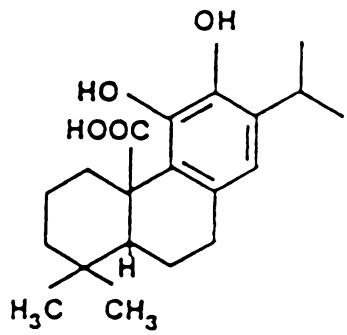


CARNOSOL

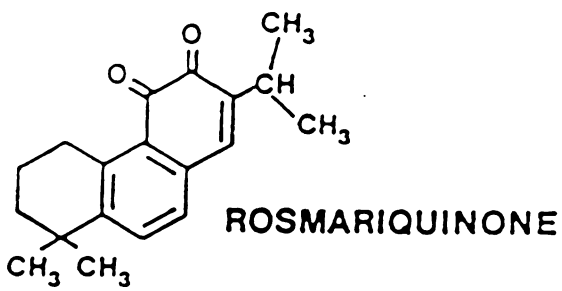


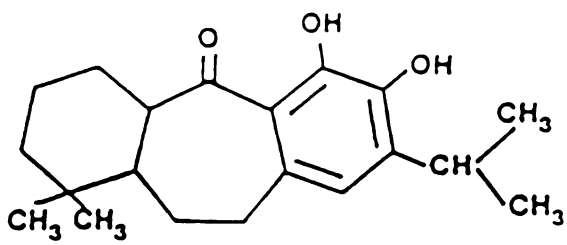
ROSMANOL





CARNOSIC ACID





ROSMARIDIPHENOL

Figure 3. Structures of some antioxidant compounds in rosemary extracts.

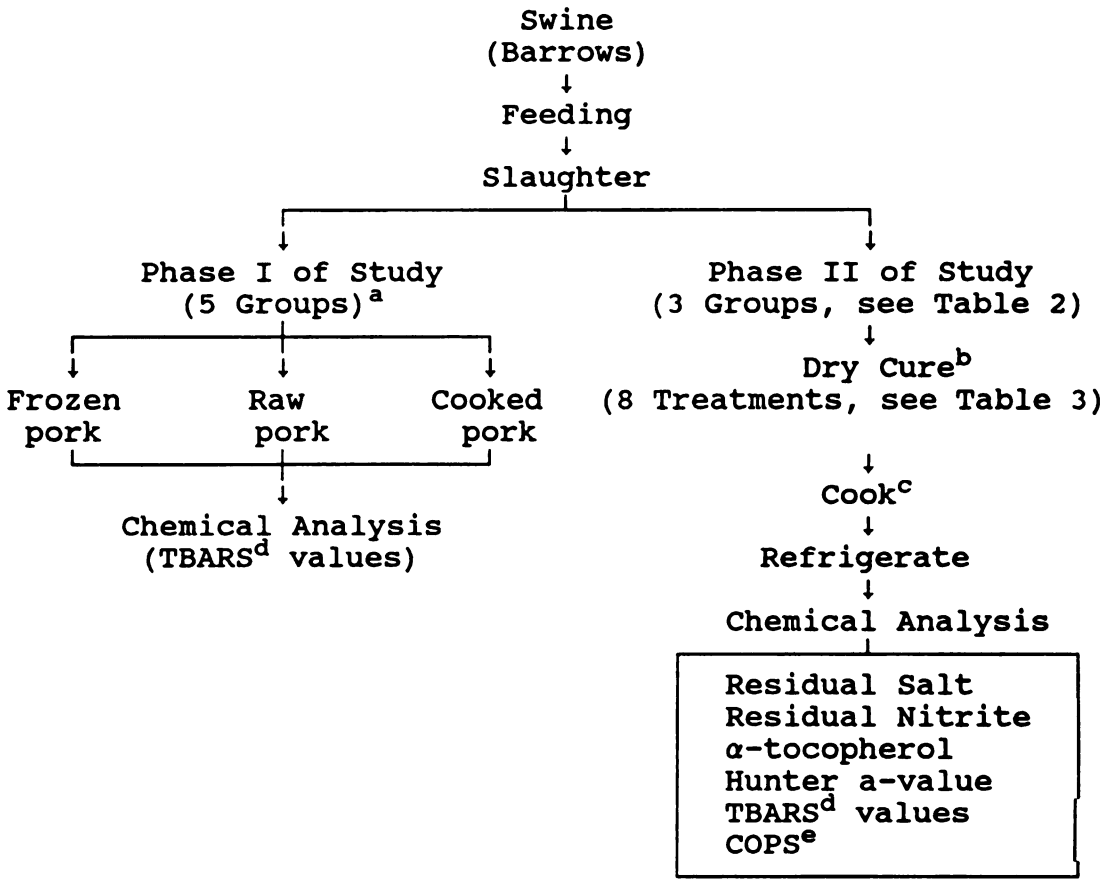
frankfurters vacuum packaged and refrigerated 35 days. Stoick et al. (1991) reported that oleoresin rosemary with sodium tripolyphosphate (STPP) had antioxidant activity in restructured beef steaks. However, Lai et al. (1991) reported that oleoresin rosemary used alone has no antioxidant activity in restructured chicken nuggets. Liu et al. (1992) also reported that oleoresin rosemary used alone has no effect on lipid oxidation in restructured pork steaks. These results may be due to different spices and processed meat products. More work needs to be done to verify the effects of natural antioxidants in meat products.

MATERIALS AND METHODS

All chemical reagents and solvents utilized in this study were analytical grade and/or HPLC grade. Individual standard cholesterol oxides were purchased from Steraloids Inc. (Wilton, NH). Alpha-tocopherol acetate was donated by BASF Corp., (Wyandotte, MI). Oleoresin rosemary and oleoresin sage were donated by Kalsec Inc. (Kalamazoo, MI). Bis (trimethylsilyl) trifluofluoroacetamide (BSTFA) was purchased from Pierce Chemical Co. (Rockford, IL).

Swine Feeding Regimen

The experimental design of this study is summarized in Figure 4. Thirty castrated male pigs (barrows) were randomly divided into 5 groups (each group contained 6 pigs) at Michigan State University Swine Research Farm and fed the diets indicated in Table 1. At 4 months of age, the diets were supplemented with natural antioxidants for 2 months as indicated in Table 2. Group 1, pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate; Group 2, pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate; Group 3, pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary; Group 4, pigs fed a diet supplemented with 750 mg/kg oleoresin rosemary; Group 5, pigs fed a diet supplemented with 750 mg/kg oleoresin sage. Animals were given water and feed *ad libitum*.



[a] Group 1: Pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Group 2: Pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Group 3: Pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.
Group 4: Pigs fed a diet supplemented with 750 mg/kg oleoresin rosemary.
Group 5: Pigs fed a diet supplemented with 750 mg/kg oleoresin sage.

[b] Pork was rubbed with 4 different dry cure mixtures and were assigned to 8 different treatments (Table 3).

[c] Dry-cured pork was placed in a cooking bag and cooked in a smokehouse to an internal temperature of 68°C.

[d] TBARS: 2-thiobarbituric acid reactive substances.

[e] COPS: Cholesterol oxidation products.

Figure 4. Flow chart of experimental design.

Table 1. Percentage composition of the pig diet[ab].

Ingredients	Amounts (%)
Ground shelled corn	70.9
Soybean meal	22.5
Corn oil	0.5
Mono-dicalcium phosphate	1.5
Calcium carbonate	1.1
Sodium chloride	0.3
Vitamin-trace mineral mix	0.5
Selenium 90 premix	0.15
Aureomycin 50	0.05

[a] Calculated concentration of Ca, P and crude protein was 18%, 21% and 14%, respectively.
[b] Calculated amount of α-tocopherol acetate was 10 mg/kg feed.

Table 2. Supplemental diets fed to pigs during the feeding trial[a].

Pigs/Groups	Diets
1 (Control)	10 mg/kg α-tocopherol acetate
2	200 mg/kg α-tocopherol acetate
3	500 mg/kg oleoresin rosemary
4	750 mg/kg oleoresin rosemary
5	750 mg/kg oleoresin sage

[a] The basal diet contained 10 mg/kg α-tocopherol acetate.

Pork Slaughter and Muscle Preparation

The pigs were slaughtered at approximately 105 kg (live weight) using standard industry techniques in the Meat Laboratory at Michigan State University. After chilling for 24 hr at 1°C, cutting and boning were completed. Boneless loins were selected for further study. The study was divided into two distinct phases to evaluate the effects of antioxidants on lipid stability in both raw pork and dry-cured pork.

Phase I: Effects of Dietary Natural Antioxidants on Lipid Oxidative Stability in Raw, Frozen and Cooked Pork

In Phase I of the study, the effects of dietary natural antioxidants on lipid oxidation in raw pork, frozen pork and cooked pork during storage were evaluated. Lipid oxidation in raw pork was evaluated during short-term and long-term storage, while lipid oxidation in cooked pork was monitored during only refrigerated storage. All 5 groups of pigs were included in Phase I of the study. Boneless chops from the right loin from each pig were taken and placed on polystyrene trays, wrapped in an oxygen-permeable PVC stretch overwrap and stored at 4°C under fluorescent light. Lipid oxidation was measured at 0, 3, 6 and 9 days. Samples were also placed on polystyrene trays, wrapped in an oxygen-permeable PVC stretch overwrap and held at -20°C. Lipid oxidation was measured at

0, 2 and 4 months. Additional samples were vacuum-packaged (polyethylene-laminated nylon pouch, oxygen transmission rate: 9 $ml/m^2/24$ hr) and stored at -20°C for evaluation of lipid stability in cooked pork. The frozen chops were thawed, ground, put in self-sealing plastic bags and cooked for 30 min. in a waterbath maintained at 70°C. The cooked pork was then stored at 4°C under fluorescent light. Lipid oxidation was measured at 0, 2 and 4 days.

Phase II: Effects of Antioxidants on Lipid and Cholesterol Stability in Dry-Cured Pork

In Phase II of the study, the effects of antioxidants on lipid and cholesterol stability in dry-cured pork were examined. Only 3 pig groups (groups 1, 2 and 3 in Figure 4) were evaluated. The boneless left loin from each pig was vacuum-packaged (polyethylene-laminated nylon pouch, oxygen transmission rate: 9 $ml/m^2/24$ hr), blast-frozen to -32°C and held for 20 days until the study was initiated.

Preparation of Curing Ingredients

Four different curing mixtures were prepared as described below:

(1) Salt alone

(2) Salt with sodium nitrite

Salt and sodium nitrite were mixed in the ratio 20:0.15

(w/w). The targeted ingoing amounts of salt and sodium nitrite in the rubbed loins were 2% and 150 mg/kg, respectively.

(3) Salt, sodium nitrite and oleoresin rosemary (OR)

Salt, sodium nitrite and OR were mixed in the ratio 20:0.15:0.5 (w/w/w). The targeted ingoing amount of OR in the rubbed loin was 500 mg/kg. It was necessary to dissolve OR in ethanol. The ethanol solution was mixed with a small portion of salt and sodium nitrite from (2) above and evaporated using a rotary evaporator (Buchi, Rotavapor-r, Brinkmann Instrument Inc., Westbury, NY) at 35°C until the ethanol was gone. The mixture was then thoroughly mixed with the remaining salt and sodium nitrite from (2) above.

(4) Salt, sodium nitrite and α-tocopherol (prepared from synthetic phytol)

Salt, sodium nitrite and α-tocopherol were mixed in the ratio 20:0.15:0.5 (w/w/w). The targeted ingoing amount of α-tocopherol in the rubbed loin was 500 mg/kg. The method used to mix the ingredients was similar to (3) above. In the rubbing process, it was estimated that 15% of the ingredients would be lost. Therefore, the amounts were compensated by adding an extra 15% of ingredients. The purpose was to insure that the correct amount of the targeted ingredients went into the muscle tissues.

Dry-Curing Procedure

The frozen loins were thawed at 2°C for 2 days. Approximately 300 g of pork were taken from each of 24 loins (12 loins from group 1, 6 loins from group 2 and 6 loins from group 3), labeled as samples for day 0 analyses. Lipid oxidation and Hunter color values were analyzed within 4 hr. The remaining pork from each loin was rubbed with 4 mixtures of ingredients indicated in Table 3.

Table 3. The ingredients of dry-curing treatments.

Treatment[a]	Ingredients
1	2% salt
2	2% salt+150 mg/kg nitrite
3	2% salt+150 mg/kg nitrite+500 mg/kg OR
4	2% salt+150 mg/kg nitrite+500 mg/kg α-tocopherol
5	2% salt
6	2% salt+150 mg/kg nitrite
7	2% salt
8	2% salt+150 mg/kg nitrite

[a] Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

The loins were assigned to 8 treatment groups. Treatments (1, 2, 3 and 4) were from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate. Treatments (5 and 6) were from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate. Treatments (7 and 8) were from pigs fed a diet supplemented with 500 mg/kg OR. The rubbed loins were placed in a double oxygen-permeable PVC bag, secured with two clips and stored at 4°C in a cooler for 2 weeks. After 1 week of the storage, the rubbed loins were overhauled. After 2 weeks of equilibration, approximately 600 g pork were taken from each treatment, labeled as day 14 samples and subjected to further chemical analyses.

Cooking and Storing of Pork

The remaining pork from the loins from each treatment was placed in cooking bags (temperature range: -23°C to 82°C, moisture vapor transmission rate (MVTR): 4.7 gm/m^2/atm/24 hr, O_2 Barrier: 2868 ml/m^2/atm/24 hr, (Package Concepts & Materials, South Carolina), and cooked in a forced air smoke house. The cooking conditions are presented in Table 4. It took approximately 5.5 hr to reach the internal temperature of 68°C. After cooking, the loins were removed from the smokehouse and placed at 4°C to chill for 24 hr. They were weighed and the percent yield calculated as described below:

$$(\%)\ \text{Yield} = \frac{\text{Weight after cooking}}{\text{Weight before cooking}} \times 100\%$$

After chilling, the loins were sliced to a thickness of 0.5 cm, placed in polystyrene trays, wrapped in an oxygen-permeable PVC stretch overwrap, stored at 4°C under fluorescent light for two weeks. Samples were taken at each storage time (days 15, 22 and 29) for further analyses.

Table 4. Cooking schedule for loins.

	Time	Internal-Temp.	Dry-Bulb	Wet-Bulb
1	30 min	32°C	54°C	35°C
2	110 min	46°C	60°C	46°C
3	180 min	49°C	66°C	52°C
4	360 min	68°C	82°C	71°C
Shower	20 min	38°C		

Methods of Analysis

(1) Assessment of lipid oxidation

The thiobarbituric acid reactive substances (TBARS) values in dry-cured pork loin chops were measured in duplicate at days 0, 14, 15, 22 and 29 using the distillation method of Tarladgis et al. (1964), as modified by Crackel et al. (1988a). Sulfanilamide was added to all samples containing nitrite before distillation to bind the nitrite through diazonium salt formation (Zipser and Watts, 1962). After color development, the absorbance of the solution was determined

using a spectrophotometer (Spectronic 2000, Bausch and Lomb, Rochester, NY) at 532 nm and converted to mg malonaldehyde/kg of meat (TBARS values) by multiplying by a factor of 6.2.

(2) Color analysis

Color changes in the dry-cured pork were measured using Hunter L (luminance), a (redness) and b (yellowness) values for samples at day 0, 14, 15, 22 and 29. The muscle sample was placed in a petri dish (100 mm x 15 mm) and its color read using a HunterLab ColorQuest spectrophotometer (Hunter Associates Laboratory, Inc., Restone, Virginia). These are relative color difference values obtained by comparing to the values of a standard pink plate (L: 66.66, a: 15.66 and b: 8.92).

(3) Residual sodium nitrite

Residual sodium nitrite in dry-cured pork loin chops was measured in samples at day 14 and 15 using the AOAC (1990) procedure. After color development, the absorbance was measured at 540 nm using a spectrophotometer. A standard curve was obtained by preparing 50 ml sodium nitrite solutions containing 1, 2, 5, 10, 20, 30 and 40 mg/kg of $NaNO_2$.

(4) Residual salt

Residual sodium chloride in dry-cured pork loin chops was measured in samples at day 14 and 15 using the AOAC (1990) procedure. The (%) NaCl was calculated as below:

(%) NaCl in g sample = (15 ml x 0.1 N ($AgNO_3$) - titration volume x 0.1 N (NH_4SCN)) x 58.5 x 100 % / 1000 ml / 3 g sample

(5) Lipid extraction

Lipid extraction was accomplished using the method described by Marmer and Maxwell (1981) for total lipid content. This extraction employed a dry column filled with glass wool, 0.1 g MgO and 10 g mixture of Celite : $CaHPO_4$ (9:1). A 5 ± 0.1 g meat sample was weighed, cut into small pieces and thoroughly mixed with 15 g Celite using a mortar and pestle. The mixture was transferred to the dry column. Sixty mL CH_2Cl_2 solvent were eluted through the column, followed by 150 mL of CH_2Cl_2 : methanol (9:1). The solvents were collected in a round-bottom flask and evaporated using a rotary evaporator at 35°C. The lipid was weighed and the total lipid content in the pork was calculated. The extracted lipid was frozen at -9°C and analyzed for COPS within 1 day.

(6) Cholesterol oxidation products

Cholesterol oxidation products in the samples were quantitated at days 14, 15 and 22 using essentially the procedure described by Monahan et al. (1992b). The COPS in the meat extracts were derivatized by adding 100 µl BSTFA, vortexed for 30 sec. and held in a dark place at room temperature for 30 min. The derivatized COPS were dried under nitrogen gas and redissolved in 200 µL

hexane for chromatographic analysis.

A gas chromatograph (GC) (Model 5890A, Hewlett Packard, Avondale, PA) equipped with a flame ionization detector was used to separate COPS. A 4 μL aliquot of the sample was injected into the GC. A 0.25 mm x 15 m capillary column (DB-1, 100% dimethyl-polysiloxane, non-polar, J and W Scientific, Folsom, CA) was used and operated with a helium carrier gas (split flow rate: 2.43 cm/min, column flow rate: 0.98 cm/min and split ratio: 13.825). The GC oven temperature was initially held at 170°C and increased at a rate 10°C/min to 220°C, 0.4°C/min to 236°C and finally 10°C/min to 320°C. Identification of COPS was based on comparison of sample retention times to the mixed COPS standards. Peak areas were integrated using a HP 3392A Integrator.

(7) Alpha-Tocopherol Content of Muscle Tissues

The α-tocopherol concentrations in muscle samples from the pigs fed diets supplemented with α-tocopherol acetate or in loins cured with a mix containing α-tocopherol were determined using the method of Asghar et al. (1991a). A high performance liquid chromatograph (HPLC) system (Waters Associates, Milford, MA) equipped with a ODSpak reverse phase C_{18} column (Ultrasphere, 5μm, 4.6 mm x 150 mm, Beckman Instruments Inc., Fullerton, CA) and a fixed wavelength detector was used (Waters, 440 absorbance detector) set at 280 nm. The eluting solvent was 100%

methanol at a flow rate of 1 mL/min. Peak areas were integrated using a HP 3380A integrator. A standard curve was obtained by preparing dl-α-tocopherol (Sigma Chemical Co., St. Louis, MO) containing 0.85 mg/μL, 1.7 mg/μL, 2.55 mg/μL and 3.4 mg/μL. The residues of α-tocopherol in meat tissue were obtained from the standard curve.

(8) Statistical Methods

In this study, the experiment was designed as a three factor (replication x treatment x time) randomized model with balanced data. The rubbed loins were the experimental units and were replicated three times. Mean, standard errors, mean square errors, one factor ANOVA (analysis of variance), two factor ANOVA, correlation and interaction of main effects were done using the MSTAT software (version C, 1989, Michigan State University, East Lansing, MI). Correlation was calculated using a pooled within-groups method. Mean separations were performed using Tukey's test with the mean square error term at the 5% level of probability.

RESULTS AND DISCUSSION

Phase I: Effects of Dietary Natural Antioxidants on Lipid Oxidative Stability in Raw, Frozen and Cooked Pork

The effects of natural antioxidants (α-tocopherol, OR and oleoresin sage) in the diet of pigs on lipid oxidative stability in pork were evaluated using TBARS values during retail display, during frozen storage and during retail display after cooking.

The mean TBARS values for raw pork stored at 4°C under fluorescent light are presented in Table 5. The TBARS values of all treatments on day 0 are similar and less than 0.2. After 3 days of storage, there were significantly ($p<0.05$) lower TBARS values in samples from pigs fed the diet supplemented with α-tocopherol acetate than the control treatment from pigs fed a basal diet. These differences were consistent to the end of the 9 day storage period. These results indicated that dietary α-tocopherol acetate (200 mg/kg) enhanced lipid stability in pork after 9 days of storage at 4°C. The observation is consistent with previous reports (Astrup, 1973; Buckley and Connolly, 1980; Buckley et al., 1988; Monahan et al., 1990; Monahan et al., 1992a). However, supplementation of the diets with OR and oleoresin sage had no effect on lipid stability in pork. Although some researchers reported that OR (Barbut et al., 1985; Resurrection and Reynolds, 1989) and sage

Table 5. TBARS[1] values (mg malonaldehyde/kg meat) of raw pork stored at 4°C under fluorescent light.

Dietary Treatment[2]	Storage Time (Days)			
	0	3	6	9
Control	0.1[a]	0.3[a]	1.0[a]	2.1[a]
Alpha-tocopherol acetate (200 mg/kg)	0.1[a]	0.1[b]	0.2[b]	0.4[b]
Oleoresin rosemary (500 mg/kg)	0.1[a]	0.3[a]	0.7[a]	1.7[a]
Oleoresin rosemary (750 mg/kg)	0.1[a]	0.3[a]	0.7[a]	1.7[a]
Oleoresin sage (750 mg/kg)	0.1[a]	0.3[a]	0.7[ab]	1.7[a]

[1] Values represent means of 6 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).
[2] Control: from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.

(Korczak et al., 1988) had antioxidant activity in meat products upon addition into meat products, there are no reports in the literature on the antioxidant activity of dietary OR and oleoresin sage in meat foods.

The TBARS values for all treatments after 6 days of fresh retail storage were 1.0 or less. Previous research indicates that above a TBARS threshold value of 1.0 (Tarladgis et al., 1960; Liu et al., 1992), oxidized flavors are detectable by

experienced panelists. Although sensory evaluation would be necessary to determine if differences in TBARS values reflect differences in off-flavor intensity, these threshold values suggest that pork from all treatments except the dietary α-tocopherol acetate had considerable oxidized flavor after 9 days refrigerated storage. However, the oxidized flavor may not have been detectable at days 3 and 6.

The mean TBARS values of pork stored at -20°C in an oxygen permeable film are presented in Table 6. The TBARS values for all treatments were significantly ($p<0.05$) lower than the control treatment after 2 months of frozen storage. The TBARS values for the control treatment (0.4) were also low and indicated that very little lipid oxidation had occurred. These results indicated that diets supplemented with α-tocopherol acetate, OR, and oleoresin sage significantly ($p<0.05$) improved lipid stability in pork after 2 months of frozen storage. The treatments of dietary OR and oleoresin sage supplementation having antioxidant effects in the early frozen storage stage of pork were different from those for raw pork. These responses may be explained by the different time /storage temperature (2 months/-20°C) than that for raw pork (3-6 days/4°C). However, after 4 months of frozen storage, only dietary α-tocopherol acetate had an effect on lipid stability but OR and oleoresin sage did not show antioxidant effects. These results indicated that the antioxidant effects of dietary OR and oleoresin sage were not very strong and less

Table 6. TBARS[1] values (mg malonaldehyde/kg meat) of raw pork stored at -20°C for up to 4 months packaged in an oxygen permeable film.

Dietary Treatment[2]	Storage Time (Months)		
	0	2	4
Control	0.1[a]	0.4[a]	0.4[a]
Alpha-tocopherol acetate (200 mg/kg)	0.1[a]	0.1[b]	0.2[b]
Oleoresin rosemary (500 mg/kg)	0.1[a]	0.1[b]	0.3[ab]
Oleoresin rosemary (750 mg/kg)	0.1[a]	0.1[b]	0.3[ab]
Oleoresin sage (750 mg/kg)	0.1[a]	0.2[b]	0.3[ab]

[1] Values represent means of 6 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).
[2] Control: from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.

than α-tocopherol.

The TBARS values for cooked pork during retail display are presented in Table 7. Immediately after cooking, the TBARS values were greater than those for raw pork at day 0. The results confirm that cooking enhances lipid oxidation because cooking disrupts the muscle membranes, thus exposing lipid substrates to oxidative catalysts (Rhee, 1988). These results

Table 7. TBARS[1] values (mg malonaldehyde/kg meat) of pork cooked at 70°C for 30 min. and stored at 4°C for up to 4 days under fluorescent light.

Dietary Treatment[2]	Storage Time (Days)		
	0	2	4
Control	0.8[a]	5.5[a]	6.9[a]
Alpha-tocopherol acetate (200 mg/kg)	0.2[b]	1.8[b]	3.0[b]
Oleoresin rosemary (500 mg/kg)	0.6[a]	5.4[a]	6.7[a]
Oleoresin rosemary (750 mg/kg)	0.8[a]	7.7[a]	7.1[a]
Oleoresin sage (750 mg/kg)	0.5[a]	4.9[a]	6.5[ab]

[1] Values represent means of 6 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).
[2] Control: from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.

are consistent with results of previous studies (Sato and Hegarty, 1971; Monahan et al., 1992b). The TBARS values of pork from the dietary α-tocopherol acetate treatment were significantly ($p<0.05$) lower than the control treatment on day 0, 2 and 4. Supplementary α-tocopherol acetate had an antioxidant effect on lipid stability on day 0 after cooking and throughout the 4 days of storage at 4°C. This observation is consistent with the data of Monahan et al. (1992b). Dietary OR and oleoresin sage had no effects on lipid

oxidation in cooked pork during 4 days of retail display.

The data collected in Phase I of the study indicated that dietary α-tocopherol improved lipid stability in raw pork, frozen pork and cooked pork during storage. Dietary OR and oleoresin sage had no significant ($p>0.05$) antioxidant effects in those products. These different responses of these natural antioxidants in pork through supplementation in the diets may be due to: (1) different efficiencies of deposition in muscle tissue because of their different structures, and (2) different locations in muscle tissue. Alpha-tocopherol is primarily located in the membrane portion of the cell, thus protecting tissue lipids from free radical attack (McCay and King, 1980). Oleoresin rosemary and oleoresin sage may be deposited in other portions of the cell or little was deposited in cell membrane, thus reducing their antioxidant activity.

Phase II: Effects of Antioxidants on Lipid and Cholesterol Stability in Dry-Cured Pork

Evaluation of Yields and Non-meat Ingredients in Dry-Cured Pork Loins

The objective of evaluating residual concentrations of salt, nitrite and α-tocopherol was to verify the anticipated and reasonable concentrations of non-meat ingredients in dry-cured pork after processing. Unreasonable residual concentrations of non-meat ingredients would indicate

inappropriate processing, and thus, the products could not be used for further studies of color, lipid and cholesterol oxidation in dry-cured pork.

Cooking yields of dry-cured pork

The cooking yields of dry-cured pork are presented in Table 8. The average cooking yield of dry-cured pork was 73%. The variation of cooking yield between all treatments was small. This demonstrates that the non-meat ingredients present in the various treatments had little effect on cooking yield.

Salt concentration in dry-cured pork

The salt concentrations in dry-cured pork before and after cooking are presented in Table 8. Average salt concentrations of dry-cured pork were 1.9% (before cooking) and 1.4% (after cooking). These data (1.9%) verify that the target concentration (2%) was reached. The salt concentration after cooking reflects the fact that about 26% of salt was lost with the moisture during cooking.

Nitrite concentration in dry-cured pork

The nitrite concentrations in dry-cured pork before and after cooking are presented in Table 8. Mean values of nitrite levels in dry-cured pork were 30 mg/kg (before

Table 8. Cooking yields, residual nitrite and salt concentration of dry-cured pork before and after cooking.

Treatment[d]	Day 14 (Uncooked)		Day 15 (Cooked)		
	Nitrite[a] (mg/kg)	Salt[b] (%)	Nitrite (mg/kg)	Salt (%)	Cooking[c] Yield(%)
1. S	-	2.0	-	1.4	72
2. S+N	29	1.9	15	1.4	71
3. S+N+OR	30	1.9	13	1.4	74
4. S+N+V	26	1.9	11	1.5	75
5. S	-	1.9	-	1.4	72
6. S+N	34	1.9	14	1.4	74
7. S	-	2.0	-	1.4	72
8. S+N	32	1.9	10	1.4	70
mean	30[e]±9.7	1.9[f]±0.06	12[e]±3.3	1.4[f]±0.04	73[f]±2.85

[a,b,c] Values represent means of 3 replications.
[d] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.
[e] Value represents the mean ± standard error of 5 means.
[f] Value represents the mean ± standard error of 8 means.

cooking) and 12 mg/kg (after cooking). The nitrite concentration is much smaller than the target level (150 mg/kg) and is partly due to the dissociation of nitrite during storage. The dissociation of nitrite is due to the Van Slyke reaction: $RCHNH_2COOH + HONO \rightarrow RCHOHCOOH + N_2 + H_2O$. This reaction demonstrates that gaseous nitrogen can be liberated

from the α-amino acids with the production of the corresponding α-hydroxy acid. In uncooked cured meat, nitrite reacts with myoglobin in muscle tissues to develop cured meat color and to form nitrosyl heme pigments. Tarladgis (1962) concluded that the pigment of heated cured muscle is dinitrosylhemochrome due to the reaction of nitrosylmyoglobin with an additional molecule of nitrite. Lee and Cassens (1976) studied the distribution of ^{15}N in heated and unheated pigment in a model system, and found that the heated samples contained about twice as much ^{15}N as unheated samples. The result supports the conclusion of Tarladgis (1962). Frouin et al. (1975) provided proof that the reaction of nitric oxide with unsaturated fatty acids could result in the loss of nitrite during storage. Woolford et al. (1976) concluded that the reaction of nitrite with non-heme proteins is a major pathway for loss of nitrite. Morrissey and Tichivangana (1985) found that nitrite can strongly react with free iron on heating. In addition, nitrite would be converted to nitrate during storage. These reactions all contribute to the depletion of nitrite during storage. Cassens et al. (1976) concluded that 1-5% of nitrite is dissociated to nitrogen, 5-15% of nitrite reacts with myoglobin, 1-5% of nitrite reacts with lipid, 20-30% of nitrite reacts with protein, 5-15% of nitrite reacts with sulfhydryl groups and 1-10% of nitrite is converted to nitrate. Because of its reactivity with meat components, only about 50% of the nitrite

added to meat is detectable immediately after processing. The residual nitrite undergoes depletion as the product is stored. In this study, the residual nitrite concentration was 30 mg/kg after 14 days of storage at 4°C and was similar to those of Bacus and Brown (1981), who reported that residual nitrite concentrations of bacon ranged from 20 to 40 mg/kg after 21 days of storage at 4.4°C.

Alpha-tocopherol concentrations in dry-cured pork

The α-tocopherol concentrations in dry-cured pork before cooking are presented in Table 9. The average α-tocopherol concentration in muscle tissue of pigs fed a basal diet (treatment 1) was 3.1 μg/g. This value is similar to the value (3.2 μg/g) reported by Monahan et al. (1990; 1992a). The mean α-tocopherol concentration in muscle tissue of pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate was 4.5 μg/g. The value is lower than the value (7.0 and 8.0 μg/g) reported by Monahan et al. (1990; 1992a) and may be due to (1) a shorter feeding period and (2) small sample size (n=3).

Asghar et al. (1991a) reported that supplementation of swine with α-tocopherol acetate increased plasma α-tocopherol concentrations relative to those of control pigs. Asghar et al. (1991b) also demonstrated that the deposition of α-tocopherol in L. dorsi muscle of pigs was dependent upon the concentration of α-tocopherol acetate in the feed. In this

Table 9. The α-tocopherol content (μg/g) of dry-cured pork.

Treatment[a]	α-tocopherol[bc] (μg/g)
1. Salt	3.1 ± 0.6
4. Salt+nitrite+α-tocopherol	303.7 ± 17.8
5. Salt	4.1 ± 0.2
6. Salt+nitrite	5.0 ± 1.3

[a] Treatment 1 are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatment 4 are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate and rubbed with 500 mg/kg α-tocopherol.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
[b] Values represent means of 3 replications.
[c] Mean ± standard deviation of the mean.

study, the concentration of α-tocopherol in muscle tissue through supplementation in the diets was consistent with those reported by Asghar et al. (1991a; 1991b). The average α-tocopherol concentration in muscle tissue of pigs fed a basal diet and dry-cured with 500 mg/kg α-tocopherol (treatment 4) was 304 μg/g meat. There is limited literature information in α-tocopherol concentrations in muscle tissue introduced through the dry-cure mixture. The greater α-tocopherol concentration in dry-cured pork processed by direct addition of α-tocopherol was expected as only a small portion of dietary α-tocopherol is deposited in muscle tissue.

Evaluation of Cured Color Development in Dry-Cured Pork

Changes of surface color in meat are expressed by Hunter L, a and b values. The L value is white when the reading is 100 and is black when the reading is zero. The a value is red when positive, gray when zero and green when negative. The b value is yellow when positive, gray when zero, and blue when negative. The Hunter L, a and b values of dry-cured pork for each storage time were measured and analyses of variance for these data are presented in Appendices A, B and C, respectively. For the purposes of this study, the surface color change is based on Hunter a-values. There was a highly significant ($p<0.001$) main effect of storage time and treatment and a significant interaction ($p<0.001$) between these two main effects. Differences in a-values due to treatment were tested at each storage time.

Hunter a-values in dry-cured pork stored at 4°C on days 0, 14, 15, 22 and 29 are presented in Figure 5. Individual means and statistics are presented in Table 10. Hunter a-values of all treatments ranged from 5.4 to 6.5 and were similar ($p>0.05$) on day 0. These values are typical values of fresh pork color and are similar to those reported by Asghar et al. (1991a). The small variation of a-values confirms the uniform condition of the heme pigments in the muscle at 0 time. As storage time increased, a-values in treatments without nitrite gradually decreased from 6.4 to -0.1 (Table 10). There are many factors contributing to the discoloration

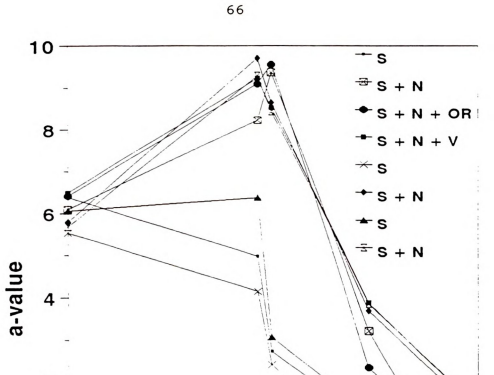


Figure 5. Hunter a-values from dry-cured pork stored at 4°C. Retail display (fluorescent light) conditions began at day 15 (one day after cooking). S: salt; N: nitrite; OR: rosemary; V: α-tocopherol. Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate. Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate. Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

Table 10. Hunter a-values[1] from dry-cured pork stored at 4°C.

	Storage Time (Days)[2]				
	Uncooked[3]		Cooked[3]		
Treatment[4]	0	14	15	22	29
1. S	6.4^{a} ±0.83	5.0^{bc} ±0.87	2.8^{b} ±0.22	1.1^{ab} ±0.72	0.0^{a} ±0.33
2. S+N	6.1^{a} ±0.43	8.2^{ab} ±1.19	9.4^{a} ±1.27	3.2^{ab} ±0.79	-0.6^{a} ±0.18
3. S+N+OR	6.4^{a} ±1.04	9.1^{a} ±1.00	9.6^{a} ±1.13	2.4^{ab} ±0.32	0.2^{a} ±0.86
4. S+N+V	6.5^{a} ±0.95	9.2^{a} ±1.72	8.5^{a} ±2.07	3.9^{a} ±1.74	1.7^{a} ±1.25
5. S	5.5^{a} ±0.17	4.2^{c} ±0.69	2.4^{b} ±1.03	0.2^{b} ±1.64	-0.1^{a} ±1.26
6. S+N	5.8^{a} ±0.89	9.7^{a} ±1.37	8.7^{a} ±0.76	3.7^{a} ±0.87	1.6^{a} ±1.42
7. S	6.1^{a} ±0.68	6.4^{abc} ±1.14	3.1^{b} ±0.16	1.3^{ab} ±1.48	0.5^{a} ±1.23
8. S+N	5.7^{a} ±1.76	9.3^{a} ±1.53	8.4^{a} ±1.48	3.9^{a} ±0.91	1.7^{a} ±0.95

[1] Means ± standard deviation of the mean.
Values represent means of 3 replications.
Mean values in a column with the same superscript are not significantly different from each other ($p>0.05$).

[2] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).

[3] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.

[4] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

of fresh pork. The simplest and most reversible discoloration in muscle tissues is when the myoglobin is oxidized to metmyoglobin in the presence of oxygen, or by the action of peroxides present in meat. The color turns to brown, gray or green as the porphyrin ring is partly destroyed (Erdman and Watts, 1957; Rikert et al., 1957; Fox, 1966). Exposure to light is another factor in meat discoloration. Light has been shown to cause dissociation of oxygen from heme in oxymyoglobin (Gibson, 1954). In addition, metal ions, bacteria and salt also cause meat discoloration (Nicol, et al., 1970; Castro, 1971; Ockerman and Cahill, 1977).

The Hunter a-values of treatments with nitrite increased before day 15 (one day after cooking), and gradually decreased after day 15 to the end of 29 days of storage. After 29 days (14 days after cooking and retail display), a-values were low and not significantly ($p>0.05$) different. These results can be explained by the mechanism of cured color development and fading (Jay and Fox, 1987). First, after adding nitrite, myoglobin reacts with nitrite, which results in the formation of nitrosyl heme pigments. Nitrosyl heme pigments are red which explains the increase in a-values. During the first 14 days, the amounts of nitrite in muscle tissues increases because of equilibration from the surface. Therefore, more nitrosyl heme pigments are formed and cured meat color will predominate the entire muscle. When cured meat is cooked, dinitrosylhemochrome is formed by reacting nitrosylmyoglobin

with another molecule of nitric oxide. The dinitrosyl-hemochrome pigments are pink. The denatured heme pigments will be oxidized as storage time increases. This explains the decreasing Hunter a-values during subsequent storage. The light fading of cured meat is due to the dissociation of the nitric oxide from heme pigments in the presence of light, followed the oxidation of nitric oxide and heme pigments by oxygen.

On day 15, treatments with nitrite and without nitrite were significantly different ($p<0.05$). After 29 days (14 days of retail display), there was no difference ($p>0.05$) between treatments with nitrite and without nitrite. The results are due to the oxidation of most of the nitric oxide heme pigments. The color was grey/green because of the oxidation of heme pigments and the growth of molds.

Effects of Antioxidants on Lipid Oxidative Stability in Dry-Cured Pork

The analysis of variance for TBARS values in dry-cured pork is presented in Appendix D. The results indicate that there were highly significant effects ($p<0.001$) in TBARS values for treatments and storage time, respectively. In addition, there was a strong interaction ($p<0.001$) between storage time and treatment. Differences in antioxidant efficacy due to treatments were tested at each storage time.

TBARS values of dry-cured pork stored at 4°C for up to 29

days are presented in Figure 6. The mean TBARS values and the appropriate statistical information are presented in Table 11. The TBARS values of all treatments were similar on day 0 and were below 0.5. There were significant ($p<0.05$) TBARS values in treatments with salt only after 14 days of storage (treatments 1 and 7). These were 7 times higher than TBARS values at day 0. These data (treatment 1) confirm previous work indicating that salt is a prooxidant (Lea, 1937; Chang and Watts, 1950; Tappel, 1952; Banks, 1961; Ellis et al., 1968; Powers and Mast, 1980; Kanner and Kinsella, 1983). Salt increased lipid oxidation in raw muscle after 14 days of storage at 4°C. Pork from pigs fed a diet supplemented with 500 mg/kg OR (treatment 7) had TBARS values similar to those for treatment 1 after 14 days of storage. Loins from pigs fed a diet supplemented with α-tocopherol acetate and rubbed with salt (treatment 5), or rubbed with salt and nitrite (treatment 6), improved lipid stability after 14 days of storage. The antioxidant effect of dietary α-tocopherol acetate supplementation is consistent with previous reports on pork (Astrup, 1973; Buckley and Connolly, 1980; Buckley et al., 1988; Monahan et al., 1990; Monahan et al., 1992a).

On the 15th day (day 1 after cooking), all samples which were dry-cured with nitrite and salt, had low TBARS values. The trends were similar to those demonstrated on the 14th day; however, pork from pigs fed a diet supplemented with α-tocopherol acetate and cured with salt alone also had low

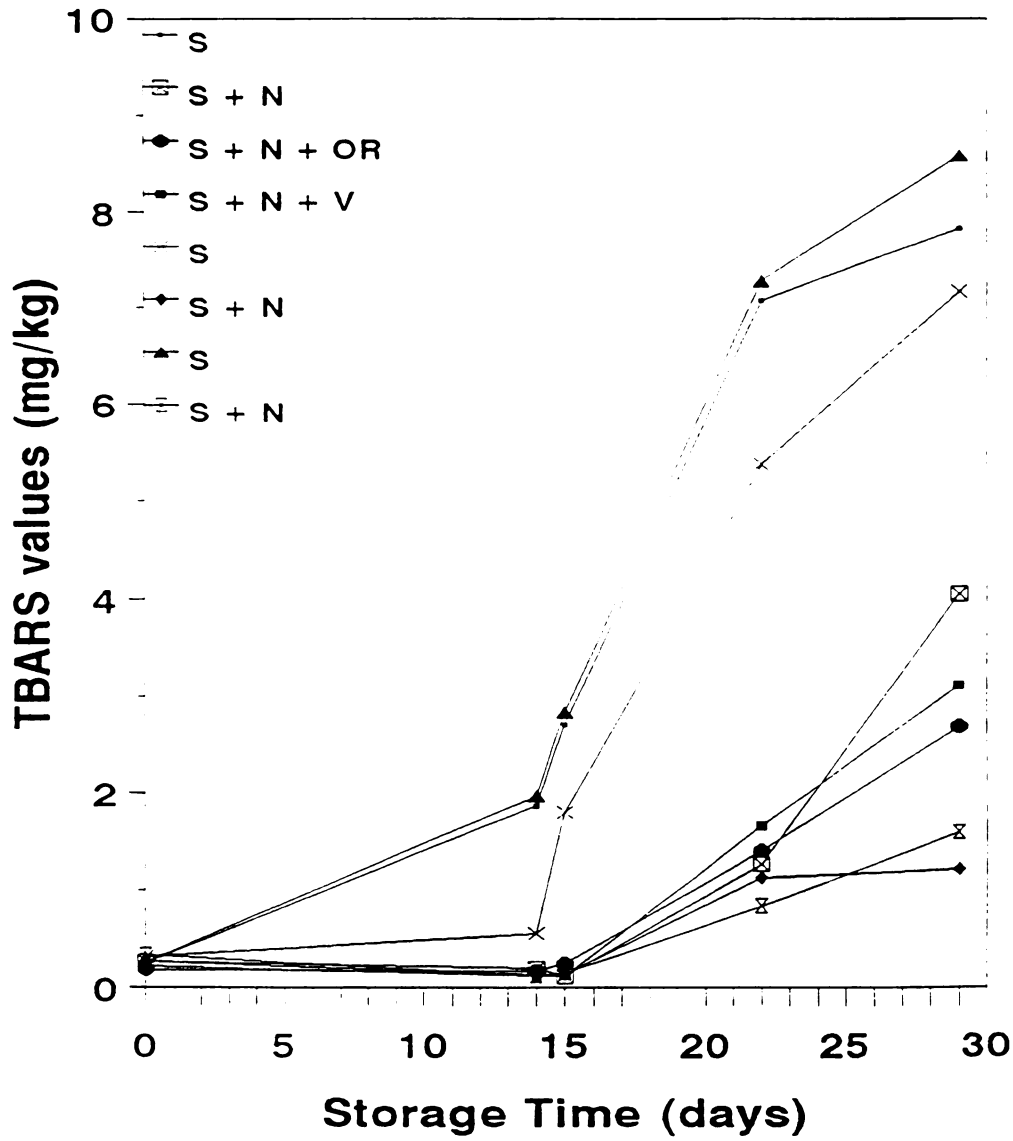


Figure 6. TBARS (mg malonaldehyde/kg pork) values of dry-cured pork stored at 4°C. Retail display (fluorescent light) conditions began at day 15 (one day after cooking). S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol. Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate. Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate. Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

Table 11. TBARS[1] values (mg malonaldehyde/kg meat) of dry-cured pork stored at 4°C.

	Storage Time (Days)[2]				
	Uncooked[3]		Cooked[3]		
Treatment[4]	0	14	15	22	29
1. S	0.3^{a} ±0.01	1.9^{a} ±0.25	2.7^{a} ±0.13	7.1^{a} ±0.55	7.8^{a} ±0.37
2. S+N	0.3^{a} ±0.06	0.2^{b} ±0.01	0.1^{c} ±0.02	1.3^{b} ±0.90	4.1^{b} ±0.53
3. S+N+OR	0.2^{a} ±0.04	0.2^{b} ±0.02	0.2^{bc} ±0.10	1.4^{b} ±0.90	2.7^{bc} ±1.19
4. S+N+V	0.2^{a} ±0.08	0.1^{b} ±0.02	0.1^{bc} ±0.02	1.7^{b} ±0.89	3.1^{bc} ±1.20
5. S	0.3^{a} ±0.06	0.6^{b} ±0.16	1.8^{ab} ±0.78	5.4^{a} ±0.51	7.2^{a} ±1.29
6. S+N	0.3^{a} ±0.09	0.1^{b} ±0.03	0.1^{bc} ±0.02	1.1^{b} ±0.53	1.2^{c} ±1.00
7. S	0.3^{a} ±0.02	2.0^{a} ±0.51	2.8^{a} ±1.41	7.3^{a} ±0.38	8.6^{a} ±1.58
8. S+N	0.3^{a} ±0.06	0.1^{b} ±0.02	0.2^{bc} ±0.01	0.8^{b} ±0.10	1.6^{bc} ±0.85

[1] Mean ± standard deviation of the mean.
Values represent means of 3 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).

[2] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).

[3] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.

[4] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

TBARS values ($p>0.05$) when it was compared to these treatments (1 and 7). The antioxidant ability of nitrite is consistent with results presented in earlier studies (Morrissey and Tichivangana, 1985; Igene et al., 1985). Because of the antioxidant ability of nitrite, researchers (Zipser et al., 1964; Igene and Pearson, 1979) have proposed that nitrite can efficiently prevent the development of warmed-over flavor in cured meat products. This research indicates that nitrite is a strong antioxidant and is better than α-tocopherol and OR in dry-cured pork regardless of method of addition. The difference in TBARS values between day 14 and 15 are due to cooking which enhances the lipid oxidation. The effect of cooking overpowers the antioxidant effect of α-tocopherol supplementation. This observation is consistent through the end of the storage period. Cooking enhances lipid oxidation due to more heme iron released from heme pigments, thermal destabilization of lipids and thermal oxidation of lipids (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979; Monahan et al. 1992b). Treatments from pigs fed a diet supplemented with OR had no effect of lipid stability. After 22 days of storage, TBARS values increased rapidly, indicating that dietary supplementation with α-tocopherol acetate and OR alone had no effect ($p>0.05$) on lipid stability, while other samples treated with nitrite, or in combination with dry cure mixture of α-tocopherol or OR, produced a significant ($p<0.05$) antioxidant effect.

Effects of Antioxidants on Cholesterol Oxidative Stability in Dry-Cured Pork

The analysis of variance for total COPS in dry-cured pork is presented in Appendix E. There was a highly significant ($p<0.001$) main effect of storage times and treatments as well as a highly significant interaction ($p<0.001$) between the two main effects. Means were statistically evaluated at each storage time.

Total COPS concentrations in dry-cured pork stored at 4°C are presented in Figure 7. Individual means and standard deviations are presented in Table 12. Gas chromatograms of mixed standard COPS and isolated COPS from dry-cured pork (treatment 1) after 22 days storage are presented in Figures 8 and 9. The COPS concentrations in dry-cured pork were not measured at day 0. It is assumed that the COPS in all treatments were similar at the initial time. This assumption was based upon non-significant Hunter L, a, b values and TBARS values in all treatments at 0 day and from literature observations (Park and Addis, 1987; Monahan *et al.*, 1992b). Park and Addis (1987) reported low TBARS values and essentially zero COPS contents in raw ground beef and turkey at day 0 of storage. Monahan *et al.* (1992b) reported that Hunter a-values, TBARS values and COPS in raw pork chops were either not significantly different or non-detectable immediately after slaughter.

The COPS concentrations in all pork chops increased with

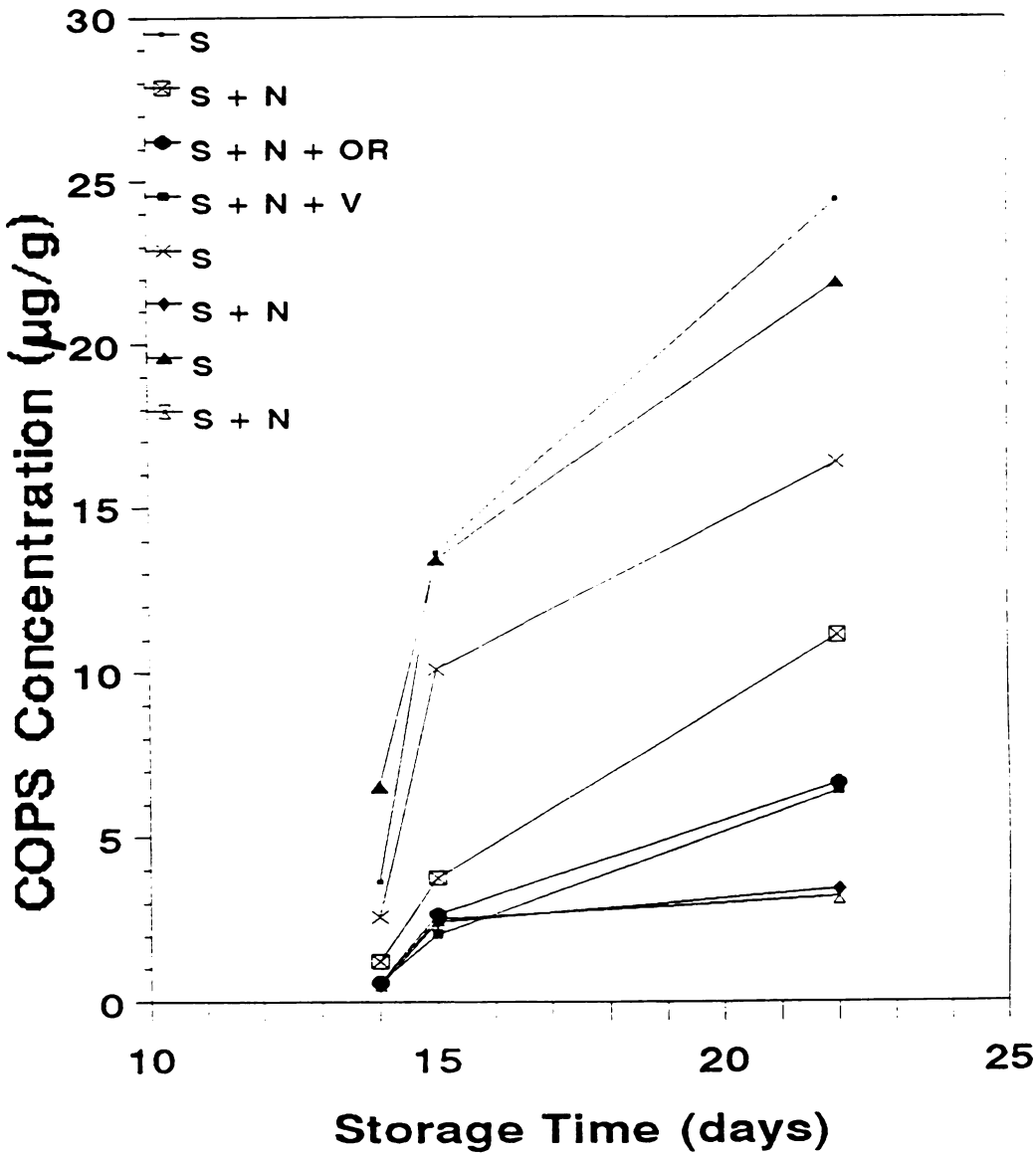


Figure 7. Total COPS concentrations (µg/g) in dry-cured pork stored at 4°C. Retail display (fluorescent light) conditions began at day 15 (one day after cooking). S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol. Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate. Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate. Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

Table 12. Total COPS[1] concentrations (μg/g) in dry-cured pork stored at 4°C.

	Storage Time (Days)[2]		
	Uncooked[3]	Cooked[3]	
Treatment[4]	14	15	22
1. S	3.6[b] ±0.13	13.6[a] ±0.31	24.4[a] ±2.31
2. S+N	1.2[cd] ±0.15	3.8[c] ±0.78	11.1[c] ±2.22
3. S+N+OR	0.6[d] ±0.04	2.7[c] ±0.54	6.7[cd] ±1.53
4. S+N+V	0.6[d] ±0.06	2.1[c] ±0.40	6.4[cd] ±1.82
5. S	2.6[bc] ±0.52	10.1[b] ±1.74	16.4[b] ±1.30
6. S+N	0.5[d] ±0.24	2.4[c] ±0.40	3.4[d] ±1.37
7. S	6.5[a] ±1.37	13.4[a] ±2.21	21.9[a] ±1.42
8. S+N	0.6[d] ±0.09	2.5[c] ±0.25	3.2[d] ±0.64

[1] Mean ± standard deviation of the mean.
Values represent means of 3 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).

[2] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).

[3] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.

[4] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

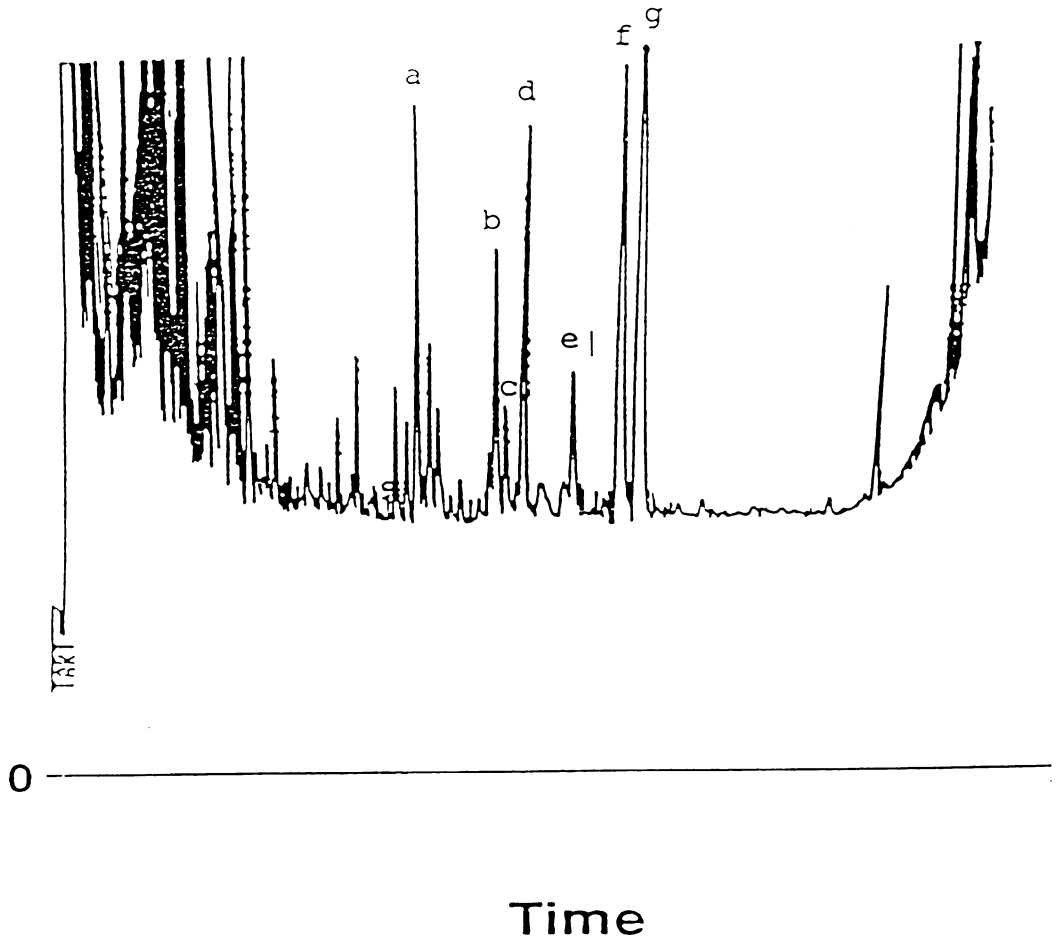


Figure 9. Gas chromatogram of cholesterol oxidation products isolated from dry-cured pork (treatment 1) stored at 4°C after 22 days of storage. a: 7α-hydroxycholesterol; b: β-epoxidecholesterol; c: α-epoxidecholesterol; d: 7β-hydroxycholesterol; e: 6-ketocholesterol; f: 7-ketocholesterol; g: 25-hydroxycholesterol.

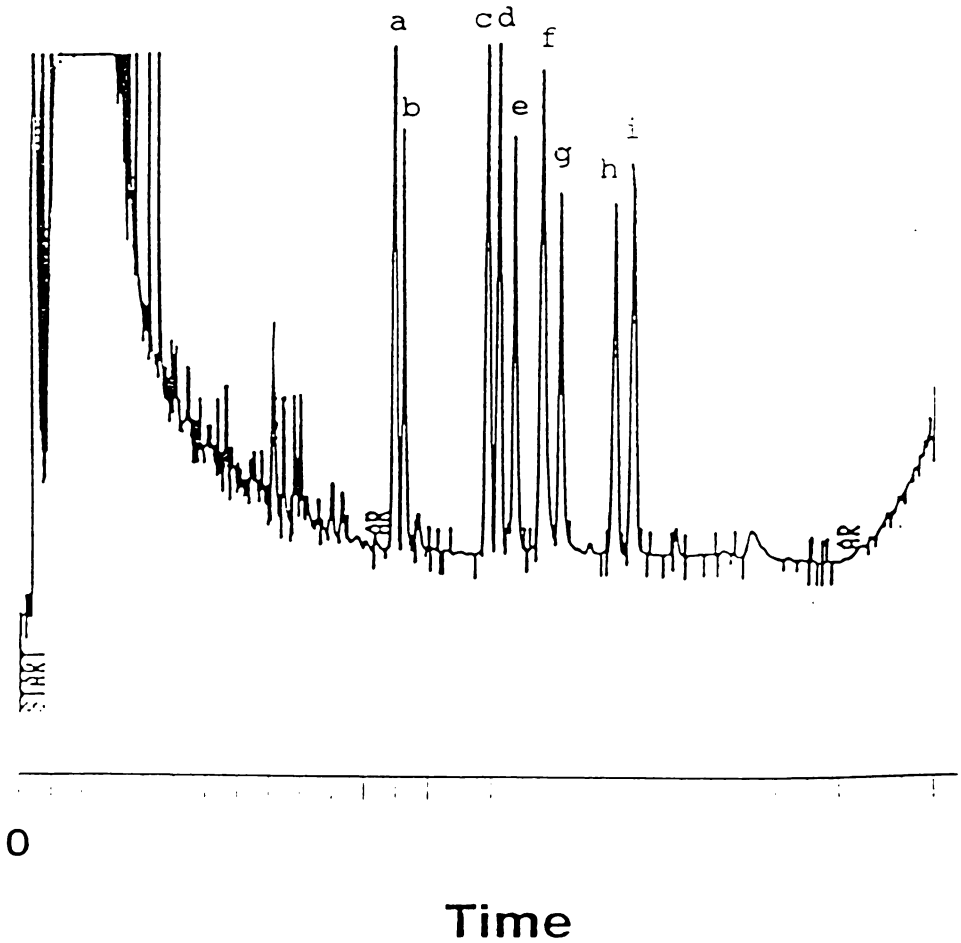


Figure 8. Gas chromatogram of mixed standard cholesterol oxidation products. a: cholesterol; b: 7α-hydroxycholesterol; c: β-epoxidecholesterol; d: α-epoxidecholesterol; e: 7β-hydroxycholesterol; f: 20-hydroxycholesterol; g: 6-ketocholesterol; h:7-ketocholesterol; i: 25-hydroxycholesterol.

storage time. After 14 days of storage, total COPS were significantly ($p<0.05$) higher in those treatments containing only salt (treatments 1, 5 and 7). These data again indicate that salt is a prooxidant and can enhance the development of COPS. Total COPS in all treatments after cooking (day 15) are higher than those before cooking (day 14), especially for treatments (1, 5 and 7) cured with salt alone. These data again indicate that the cooking process can enhance cholesterol oxidation. These observations indicate that cholesterol oxidation may undergo oxidation by a mechanism similar to that for lipids in muscle tissues. In this study, COPS concentration in samples containing only salt before and after cooking were higher than those reported by Pie et al. (1991) in raw and cooked minced pork. These different results were due to (1) salt enhancing cholesterol oxidation in dry-cured pork, (i.e., without nitrite) and (2) the longer storage period.

The COPS concentrations in samples treated with salt plus nitrite were significantly ($p<0.05$) lower than those in samples containing only salt. This observation was true at all storage times. The COPS concentrations in treatments from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate were smaller than those samples treated with salt alone after cooking and after 7 days of cooked storage (day 22). These results are consistent with those of Engeseth et al. (1993) and Monahan et al. (1992b). Engeseth et al. (1993)

demonstrated that feeding veal calves a diet supplemented with α-tocopherol acetate can reduce the total COPS in cooked veal. Monahan et al. (1992b) demonstrated that dietary vitamin E can significantly decrease total COPS in cooked pork. This study demonstrates α-tocopherol effectiveness even after 7 days of retail display, which was different from that measured by TBARS values for lipid oxidation (Table 12). Diets supplemented with OR had no effect on cholesterol stability in dry-cured pork.

Specific cholesterol oxidation products in dry-cured pork

In this study, six specific COPS (7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, β-epoxide-cholesterol, α-epoxidecholesterol and 25-hydroxycholesterol) were identified in dry-cured pork. Concentrations of these individual COPS are presented in Tables 13, 14 and 15. The predominant COPS in samples treated with salt alone were 7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-keto-cholesterol (Table 13). These COPS are the primary oxidation products. Smith (1981) reported that these COPS are formed initially and that 7β-hydroxycholesterol was more thermodynamically stable than the 7α-isomer. Pie et al. (1991) also reported that the concentrations of 7β-hydroxycholesterol were greater than those of 7α-hydroxy-cholesterol in beef, veal and pork. These observations are not consistent with the results of this study. Amounts of 7β-

Table 13. Primary COPS[ab] (μg/g) in dry-cured pork stored at 4°C.

		Uncooked[c]			Cooked[c]					
		Day 14			Day 15			Day 22		
	Treatment[d]	7α[e]	7β[e]	7keto[e]	7α	7β	7keto	7α	7β	7keto
1.	S	0.72	0.48	1.22	3.07	2.97	4.79	4.45	4.72	9.88
2.	S+N	0.16	0.05	0.30	0.78	0.40	1.08	3.31	2.12	2.61
3.	S+N+OR	0.09	ND	0.19	0.61	0.06	0.67	2.07	0.83	1.50
4.	S+N+V	0.09	ND	0.16	0.53	0.05	0.38	1.76	0.88	1.57
5.	S	0.43	0.35	0.71	2.28	2.19	3.31	3.19	2.51	6.95
6.	S+N	0.09	ND	0.02	0.38	0.08	0.74	0.56	0.33	0.88
7.	S	0.92	1.85	1.67	2.97	3.07	4.46	4.01	4.09	9.01
8.	S+N	0.08	ND	0.14	0.42	0.12	0.50	0.57	0.45	0.77

[a] Values represent means of 3 replications.
ND: Non-detectable.
[b] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).
[c] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.
[d] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.
[e] 7α: 7α-hydroxycholesterol; 7β: 7β-hydroxycholesterol; 7keto: 7-ketohydroxycholesterol.

Table 14. Secondary COPS[ab] (μg/g) in dry-cured pork stored at 4°C.

	Uncooked[c]		Cooked[c]			
	Day 14		Day 15		Day 22	
Treatment[d]	α[e]	β[e]	α	β	α	β
1. S	0.15	0.30	0.34	1.30	1.06	2.49
2. S+N	0.13	0.09	ND	0.22	0.44	1.43
3. S+N+OR	0.12	0.01	0.17	0.05	0.24	0.56
4. S+N+V	0.08	0.05	0.03	0.03	0.09	0.59
5. S	0.09	0.18	0.20	0.93	0.70	1.22
6. S+N	0.12	0.03	0.13	0.05	0.22	0.14
7. S	0.20	1.04	0.36	1.32	1.00	2.04
8. S+N	0.10	0.02	0.10	0.08	0.07	0.28

[a] Values represent means of 3 replications.
ND: Non-detectable.
[b] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).
[c] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.
[d] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.
[e] α: α-epoxidecholesterol; β: β-epoxidecholesterol.

Table 15. Tertiary COPS[ab] (μg/g) in dry-cured pork stored at 4°C.

	25-hydroxycholesterol		
	Uncooked[c]	Cooked[c]	
Treatmentd[d]	Day 14	Day 15	Day 22
1. S	0.77	1.18	1.81
2. S+N	0.50	1.30	1.23
3. S+N+OR	0.21	1.11	1.46
4. S+N+V	0.22	1.03	1.55
5. S	0.84	1.18	1.84
6. S+N	0.25	1.04	1.30
7. S	0.63	1.23	1.72
8. S+N	0.22	1.29	1.04

[a] values represent means of 3 replications.
ND: Non-detectable.
[b] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).
[c] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.
[d] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

hydroxycholesterol were not greater than amounts of 7α-hydroxycholesterol in the salt-only treatments. However, Park and Addis (1987) demonstrated that the concentrations of 7β-hydroxycholesterol were lower than those of 7α-hydroxy-cholesterol in 3 year-old freeze-dried pork. These different results may be due to (1) different meat products, (2) different storage conditions, and (3) interconversion of COPS during storage.

Secondary oxidation products (α- and β-epoxide-cholesterol) develop following the epoxidation of the primary products. The relative amounts of secondary products (Table 14) were smaller than the amounts of primary products (Table 13). These results were consistent with previous report of Smith (1981), who summarized that primary products were the major products in cholesterol oxidation. However, in the study of Pie et al. (1991), not all primary products were greater than secondary products in raw and cooked minced meat. These different results may be due to different meat products and different storage and cooking conditions.

One tertiary oxidation product (25-hydroxycholesterol) was isolated from dry-cured pork muscle (Table 15). The amounts of 25-hydroxycholesterol were less than those in primary and secondary products. These results were consistent with those reported by Pie et al. (1991). The effect of nitrite on 25-hydroxy is not as great as it is on 7α, 7β, 7-keto or α-epoxide and β-epoxidecholesterol. However, dietary α-

tocopherol acetate and OR did not show similar responses as nitrite. No previous study has reported inhibition by nitrite of the development of primary and secondary products more than on the development of tertiary products. This response may be because nitrite can protect the C-7 position from free radical attack and subsequent epoxidation of primary products with cholesterol, but cannot protect the side chain carbon from free radical attack.

Correlation between TBARS values, COPS and Hunter a-values

Both lipid and cholesterol undergo oxidation by a free radical mechanism. In addition, nitrosyl heme pigments developed in cured meat also undergo oxidation. Therefore, the correlations of Hunter a-value with TBARS values and COPS were calculated.

Correlation coefficients (r) of TBARS values, COPS and Hunter a-values are 0.15 (TBARS values with COPS), 0.03 (Hunter a-value vs TBARS values) and -0.04 (Hunter a-value vs COPS), respectively. The correlation coefficient of 0.15 (TBARS values and COPS) indicates that changes in TBARS values account for only 2.3% of variation (r^2) in COPS. This correlation coefficient is not consistent with that reported by Monahan et al. (1992b) who reported a correlation coefficient between TBARS values and COPS as high as 0.88 in pork which was supplemented with α-tocopherol acetate in the diet. The higher correlation coefficient may be due to

different statistical methods of calculation (pooled within groups, across groups and mean values only). The correlation coefficient in this study, calculated across groups for TBARS values and COPS, is as high as 0.93 and is similar to that reported by Monahan et al. (1992b). This value does not account for the significant time-treatment interactions, the number of replications or the sample population and may be an overestimate.

SUMMARY AND CONCLUSIONS

In Phase I of the study, it was demonstrated that dietary α-tocopherol acetate (200 mg/kg) stabilized the lipids in pork chops during refrigerated storage, during frozen storage, and during retail display after cooking. Dietary OR (500 mg/kg; 750 mg/kg) and oleoresin sage (750 mg/kg) had no effect on lipid stability in pork.

In Phase II of the study, the mean residual salt concentration was 1.9%, which confirmed that the target concentration of 2% was attained. Nitrite concentration was 30 mg/kg, lower than the target level (150 mg/kg), and can be explained by the reactivity with meat components and the depletion as pork is stored. Alpha-tocopherol concentrations in dry-cured pork of pigs fed basal and supplemented diets were 3.1 μg/g and 4.5 μg/g, while α-tocopherol addition to the curing ingredients resulted in 304 μg/g of α-tocopherol in the cured pork.

Significantly ($p<0.05$) higher Hunter a-values were measured in nitrite-treated samples than nitrite-free samples after 15 days storage, but they were similar after 29 days storage. Dietary α-tocopherol acetate and OR had no effect on cured color development. Nitrite acted as a strong antioxidant with regard to lipid and cholesterol oxidative stability. Dietary α-tocopherol acetate resulted in cured pork with less COPS than the control treatment after 22 days

storage (7 days after cooking). Dietary OR had no effect on lipid and cholesterol stability of dry-cured pork.

Six specific COPS were identified in raw and cooked dry-cured pork during storage. The primary COPS products were predominant in cured pork and their concentrations were greater than secondary products.

In conclusion, results from this study demonstrated that dietary α-tocopherol was more effective than dietary OR and oleoresin sage in stabilizing lipids in uncured pork. Nitrite was more effective than α-tocopherol and OR in controlling lipid and cholesterol oxidation in dry-cured pork. Correlation coefficient demonstrated a low relationship between cured color development, lipid oxidation and cholesterol oxidation in dry-cured pork.

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Appendix A

The analysis of variance for Hunter Color L-value in dry-cured pork during refrigerated storage.

Sources	Degrees of Freedom	Sum of Square	Mean Square	F Value	Significance level
A(Rep.)	2	95.345	47.672	8.826	NS
B(Time)	4	16346.274	4086.568	756.568	p<0.001
AB	8	54.453	6.807	1.260	NS
C(Trt.)	7	146.017	20.860	3.862	p<0.05
AC	14	221.754	15.840	2.933	p<0.05
BC	28	193.234	6.901	1.278	NS
Error	56	302.482	5.401		
Total	119	17359.557			

Appendix B

The analysis of variance for Hunter Color b-value in dry-cured pork during refrigerated storage.

Sources	Degrees of Freedom	Sum of Square	Mean Square	F Value	Significance level
A(Rep.)	2	2.944	1.472	3.430	NS
B(Time)	4	108.759	27.190	63.350	$p<0.001$
AB	8	2.299	0.287	0.670	NS
C(Trt.)	7	21.405	3.058	7.124	$p<0.01$
AC	14	9.840	0.703	1.638	NS
BC	28	27.020	0.965	2.248	$p<0.05$
Error	56	24.036	0.429		
Total	119	196.303			

Appendix C

The analysis of variance for Hunter Color a-value in dry-cured pork during refrigerated storage.

Sources	Degrees of Freedom	Sum of Square	Mean Square	F Value	Significance level
A(Rep.)	2	4.514	2.257	2.735	NS
B(Time)	4	859.301	214.825	260.334	p<0.001
AB	8	6.128	0.766	0.928	NS
C(Trt.)	7	219.277	31.325	37.961	p<0.001
AC	14	42.463	3.033	3.676	p<0.005
BC	28	160.936	5.748	6.965	p<0.001
Error	56	46.211	0.825		
Total	119	1338.830			

Appendix D

The analysis of variance for TBARS values of dry-cured pork during refrigerated storage.

Sources	Degrees of Freedom	Sum of Square	Mean Square	F Value	Significance level
A(Rep.)	2	1.379	0.690	2.021	NS
B(Time)	4	336.004	82.751	242.574	p<0.001
AB	8	4.270	0.534	1.565	NS
C(Trt.)	7	244.234	34.891	102.277	p<0.001
AC	14	6.090	0.435	1.275	NS
BC	28	146.024	5.215	15.288	p<0.001
Error	56	19.104	0.341		
Total	119	752.105			

Appendix E

The analysis of variance for total COPS of dry-cured pork during refrigerated storage.

Sources	Degrees of Freedom	Sum of Square	Mean Square	F Value	Significance level
A(Rep.)	2	0.541	0.271	0.177	NS
B(Time)	2	1124.425	562.213	366.851	p<0.001
AB	4	1.349	0.337	0.220	NS
C(Trt.)	7	1639.858	234.265	152.861	p<0.001
AC	14	22.106	1.579	1.030	NS
BC	14	458.222	32.735	21.357	p<0.001
Error	28	42.911	1.533		
Total	71	3289.413			

Appendix F

Hunter L-values[1] from dry-cured pork stored at 4°C.

	Storage Time (Days)[2]				
	Uncooked[3]		Cooked[3]		
Treatment[4]	0	14	15	22	29
1. S	42[a] ±1.1	37[a] ±3.0	63[a] ±3.5	66[a] ±2.0	67[a] ±2.1
2. S+N	43[a] ±1.3	35[a] ±2.7	60[a] ±2.6	62[a] ±1.9	65[ab] ±1.5
3. S+N+OR	45[a] ±0.8	36[a] ±2.5	60[a] ±4.5	59[a] ±3.7	59[b] ±3.3
4. S+N+V	44[a] ±3.5	35[a] ±2.4	62[a] ±4.9	64[a] ±6.0	63[ab] ±3.8
5. S	45[a] ±1.9	36[a] ±0.9	62[a] ±2.4	64[a] ±1.6	64[ab] ±1.4
6. S+N	46[a] ±1.7	35[a] ±2.7	61[a] ±3.2	62[a] ±3.8	65[ab] ±2.8
7. S	43[a] ±2.0	35[a] ±1.7	62[a] ±0.7	64[a] ±1.4	65[ab] ±2.1
8. S+N	47[a] ±3.8	37[a] ±2.1	63[a] ±4.2	64[a] ±4.2	64[ab] ±2.6

[1] Mean ± standard deviation of the mean.
Values represent means of 3 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).

[2] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).

[3] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.

[4] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

Appendix G

Hunter b-values[1] from dry-cured pork stored at 4°C.

	Storage Time (Days)[2]				
	Uncooked[3]		Cooked[3]		
Treatment[4]	0	14	15	22	29
1. S	7.2[a] ±1.33	6.8[a] ±0.90	8.6[a] ±0.57	9.1[a] ±0.75	9.3[a] ±0.73
2. S+N	7.0[a] ±0.23	5.8[a] ±0.66	6.2[b] ±0.49	8.4[a] ±0.37	8.9[a] ±0.20
3. S+N+OR	7.1[a] ±0.06	6.4[a] ±0.46	6.6[b] ±0.43	7.4[a] ±2.16	8.8[a] ±1.04
4. S+N+V	7.7[a] ±1.05	6.3[a] ±0.99	6.4[b] ±0.27	8.5[a] ±0.17	8.5[a] ±0.08
5. S	7.0[a] ±0.68	6.9[a] ±0.80	9.0[a] ±1.14	8.9[a] ±0.56	9.5[a] ±0.65
6. S+N	7.8[a] ±0.57	6.1[a] ±0.72	6.5[b] ±0.25	8.6[a] ±0.45	8.1[a] ±0.61
7. S	7.5[a] ±0.77	6.2[a] ±0.83	8.6[a] ±0.10	9.4[a] ±0.23	9.6[a] ±0.26
8. S+N	7.9[a] ±0.31	6.4[a] ±0.18	6.9[b] ±0.34	8.8[a] ±0.07	7.6[a] ±0.30

[1] Mean ± standard deviation of the mean.
Values represent means of 3 replications.
Means values in a column with the same superscript are not significantly different from each other ($p>0.05$).

[2] Retail display (fluorescent light) conditions began at day 15 (one day after cooking).

[3] Uncooked: samples are stored in the dark at 4°C.
Cooked: samples are stored under fluorescent light at 4°C.

[4] S: salt; N: nitrite; OR: oleoresin rosemary; V: α-tocopherol.
Treatments (1, 2, 3 and 4) are from pigs fed a basal diet containing 10 mg/kg α-tocopherol acetate.
Treatments (5 and 6) are from pigs fed a diet supplemented with 200 mg/kg α-tocopherol acetate.
Treatments (7 and 8) are from pigs fed a diet supplemented with 500 mg/kg oleoresin rosemary.

Appendix H

Calculation of correlation coefficients (pooled, within treatments and storage times) between TBARS values and COPS in dry-cured pork.

X = TBARS values
Y = COPS

i = 1, 2,....8 (treatments)
j = 1, 2, 3, 4, 5 (days)
k = 1, 2, 3 (replications)

Variances (S_x^2) of TBARS values (X) within three replications

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

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

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

$$r_{XY} = S_{XY}/(S^2_X \cdot S^2_Y)^{0.5}$$

$$= \sum_{ij}^{94}\sum S_{XijYij} / (\sum_{ij}^{94}\sum S^2_{Xij} \cdot \sum_{ij}^{94}\sum S^2_{Yij})^{0.5}$$