Introduction

Biological tissues are commonly subjected to free radical attack and subsequent oxidative damage due to imperfections in oxygen metabolism (Halliwell et al., 1995). Skeletal muscle is particularly susceptible to oxidative reactions since it contains high concentrations of lipid membrane systems (e.g. mitochondria and sarcoplasmic reticulum), prooxidants (e.g. transition metals and heme-containing proteins) and in some cases oxygen. To protect against these prooxidative conditions, skeletal muscle contains a multi-component antioxidant system. This endogenous skeletal muscle antioxidant defense system is composed of nonenzymic antioxidants in both the cytosolic and lipid phases as well as antioxidant enzymes. Concentrations of endogenous antioxidants are a function of animal species, muscle type and in some cases diet.

Endogenous antioxidants provide a natural level of oxidative stability to all muscle foods. However, meat processing operations such as size reduction, sodium chloride addition and cooking can accelerate oxidative reactions and inactivate antioxidants to the point where endogenous antioxidants cannot adequately protect the muscle. In these meat products, exogenous antioxidant additives, dietary antioxidants or food packaging techniques must be utilized to control oxidation. Control of these oxidative reactions is crucial in order to produce high-quality, wholesome meat products since oxidation causes deterioration of flavor, color and texture and the formation of potentially toxic compounds (Addis and Park, 1989; Vercellotti et al., 1992; Faustman, 1993; Xiong and Decker, 1995).

Endogenous Antioxidants

The endogenous antioxidants can be classified as lipid-soluble, cytosolic or enzymic. The function of these antioxidants is to control prooxidant activity, scavenge free radicals and inactivate reactive oxygen species.

Lipid-Soluble Nonenzymic Antioxidants

Tocopherols: The major naturally-occurring lipid soluble antioxidant in skeletal muscle is tocopherol. Tocopherols consist of four different isomers, α, β, gamma and δ. Tocopherols contain a side chain which can be either saturated (tocols) or unsaturated (tocotrienols), resulting in eight possible isomers (Shahidi and Wanasundara, 1992). All tocopherols contain a phenolic structure which scavenges lipid and oxygen radicals through the formation of a tocopherol quinone radical whos energy is 2 to 3 times lower than most fatty acid radicals (Buettner, 1993). Formation of the low-energy tocopherol quinone radical minimizes the chance that the free radical can further promote lipid oxidation. Once the tocopherol radical is formed, the molecule is no longer an active antioxidant. However, other compounds, such as ascorbic acid and possibly reduced glutathione, can reduce the tocopherol radical, thus regenerating its antioxidant ac-
activity (Parker, 1989). Tocopherols are not synthesized by animal tissues and must be obtained from vegetable sources. Therefore, tocopherol content in skeletal muscle is largely dependent on diet. This subject has been reviewed in more detail by Faustman (1993), Buckley et al. (1995) and Schaefer et al. (1995).

**Carotenoids:** Carotenoids are another group of lipid-soluble endogenous antioxidants in skeletal muscle which are obtained from the diet. Carotenoids are a diverse group (>600 different compounds) of yellow to red colored polyenes, consisting of 3 to 13 double bonds and in some cases six carbon hydroxylated ring structures at one or both ends of the molecule (Olson, 1993). The major antioxidant mechanism of carotenoids is through their ability to interact with singlet oxygen. Singlet oxygen is produced from atmospheric (triplet) oxygen through the transfer of light energy to oxygen via a photosensitizer (e.g. riboflavin and heme-containing proteins; Bradley and Min, 1992). Since singlet oxygen is capable of forming lipid peroxides, its presence must be tightly controlled in biological systems. Carotenoids react with singlet oxygen, resulting in formation of the triplet state of both oxygen and the carotenoid. The energy of triplet carotenoids can then be diminished through radiationless transfer to the surrounding solvent (Olson, 1993). Carotenoids can also inhibit oxidation reactions by accepting or donating electrons (Bradely and Min, 1992). Since singlet oxygen is capable of forming lipid peroxides, its presence must be tightly controlled in biological systems. Carotenoids react with singlet oxygen, resulting in formation of the triplet state of both oxygen and the carotenoid. The energy of triplet carotenoids can then be diminished through radiationless transfer to the surrounding solvent (Olson, 1993). Carotenoids can also inhibit oxidation reactions by accepting or donating electrons (Bradely and Min, 1992). The resulting carotenoid radical forms a stable carbon center radical in the presence of low oxygen concentrations or an unstable peroxyl radical in the presence of high oxygen concentrations (Burton and Ingold, 1984). Through these reactions, carotenoids can be either antioxidative (low oxygen) or prooxidative (high oxygen).

Like tocopherols, animals also obtain carotenoids from dietary sources, such as plants or plankton. Due to the pigmentation of carotenoids, high concentrations can lead to the coloration of fats, skeletal muscle and associated skin. Carotenoids are purposely added to the diets of aquacultured salmon species and poultry to influence the color of the final product (Faustman, 1993). Whether dietary carotenoids are always antioxidative is not well understood, since in some cases they do not reduce lipid oxidation rates in muscle foods (King et al., 1995). In addition to problems associated with the prooxidative and antioxidative nature of carotenoids, their potential as dietary antioxidants is limited by their effects on their visual properties of muscle foods.

*Ubiquinone:* Ubiquinone or coenzyme Q is a benzoquinone with an isoprenoid side chain found in the electron transport chain of mitochondria. Ubiquinone's side chain contains 2 to 10 isoprenoid units with 10 being the most common (Zubay, 1983). Ubiquinones inhibit lipid oxidation by their ability to scavenge free radicals (Cabrini et al., 1986). Very little is known about the antioxidant role of ubiquinones in skeletal muscle, but their presence in mitochondria suggests that they could be especially important in the oxidative stability of red muscle.

**Cytosolic Nonenzymic Antioxidants:**

*Histidine Containing Dipeptides*

Carnosine and anserine are N-ß-alanyl-L-histidine and N-ß-alanyl-3-methyl-L-histidine dipeptides, respectively, endogenous to skeletal muscle (Figure 1). Carnosine and anserine concentrations in beef, pork, chicken and fish range from 10-70 mM (Table 1; Crush, 1970; Plowman and Close, 1988). White muscle fibers generally have higher anserine and carnosine concentrations than red muscle with chicken breast and leg muscle having combined dipeptide concentrations of 1.2% (71 mM) and 0.2% (12.2 mM), respectively, of the wet weight of the muscle (Crush, 1970).

Similarities in the structure of anserine and carnosine suggest similar physiological functions. Since the pK's of

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>MUSCLE</th>
<th>CARNOSINE mg/100 g tissue</th>
<th>ANSERINE (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Leg</td>
<td>50 (2.9)</td>
<td>167 (9.3)</td>
</tr>
<tr>
<td>Gallus gallus</td>
<td>Pectoral</td>
<td>278 (16.4)</td>
<td>983 (54.6)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Oryctolagus cuniculus</td>
<td>70 (4.1)</td>
<td>400 (22.2)</td>
</tr>
<tr>
<td>Atlantic Salmon</td>
<td>Salmo salar</td>
<td>0</td>
<td>400 (22.2)</td>
</tr>
<tr>
<td>Beef</td>
<td>Leg</td>
<td>150 (8.8)</td>
<td>50 (2.8)</td>
</tr>
<tr>
<td>Swine</td>
<td>Sus sp.</td>
<td>276 (9.2)</td>
<td>20 (1.1)</td>
</tr>
<tr>
<td>Human</td>
<td>Quadriceps</td>
<td>362 (21.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

Crush, 1970.
the imidazole ring of carnosine and anserine are 6.83 and 7.04, respectively, these histidine-containing dipeptides exhibit excellent buffering capacity at physiological pH values. Concentrations of anserine and carnosine are such that they can provide up to 40% of pH-buffering capacity of skeletal muscle (Davey, 1960). The buffering capacity of muscle has been correlated with carnosine concentrations in horse, dog and man (Harris et al., 1990). The role of carnosine and anserine as buffers could explain why higher concentrations are found in white muscle fibers where anaerobic metabolism is common.

Boldyrev and co-workers (1987; 1988) were the first to report that carnosine has membrane-protecting properties. In a system containing sarcoplasmic reticulum membranes, they demonstrated that carnosine and anserine could decrease lipid oxidation rates as determined by thiobarbituric acid reactive substance (TBARS; Boldyrev et al., 1988). The antioxidant activity of carnosine and anserine has since been demonstrated in numerous model systems (for review see Chan and Decker, 1994).

Increasing endogenous carnosine concentrations from 9.2 to 31.3 mM increased the oxidative stability of raw, salted (2% NaCl), frozen (Decker and Crum, 1991) and cooked, refrigerated ground pork (Decker and Crum, 1993). In addition to inhibiting lipid oxidation, carnosine also decreased myoglobin oxidation in salted ground pork (Decker and Crum, 1991). Carnosine’s ability to inhibit myoglobin oxidation is not related to its ability to stabilize oxymyoglobin or reduce metmyoglobin, suggesting that the color stabilizing effect of carnosine is related to its antioxidant activity (Decker et al., 1995).

The antioxidant mechanism of carnosine and anserine involves both metal chelation and free radical scavenging. Carnosine is capable of chelating metals, yet its chelating activity is dependent on metal ion type (Brown and Antholine, 1979; Brown, 1988). Studies on the oxidation of biomolecules and the NMR spectra of carnosine indicate that carnosine chelates copper much more strongly than iron (Kohen et al., 1988; Decker et al., 1992).

While carnosine is a weak iron chelator, it is still capable of inhibiting iron-catalyzed lipid oxidation (Decker et al., 1992). Electron paramagnetic resonance (EPR) studies have shown that carnosine could inactivate hydroxyl radicals generated by iron and H$_2$O$_2$ or H$_2$O$_2$ and UV-irradiation (Rutstov et al., 1991; Chan et al., 1994). Carnosine and anserine also quench singlet oxygen (Dahl et al., 1988; Egorov et al., 1992) but do not scavenge superoxide anions (Aruoma et al., 1989; Yoshikawa et al., 1991). These data indicate that carnosine’s antioxidant activity is also related to its ability to quench high energy free radicals such as singlet oxygen and hydroxyl radicals.

The antioxidant mechanism of anserine and carnosine is multifunctional since the dipeptides can inactivate copper by chelation and scavenge free radicals. Anserine and carnosine are active antioxidants at in vivo concentrations and are capable of inhibiting the oxidation of both water-soluble compounds and lipid membranes. Therefore, the antioxidant role of anserine and carnosine in biological systems seems to be to inactivate prooxidant metals and free radicals in the aqueous phase of tissue. The higher concentrations of anserine and carnosine found in fast twitch (white) muscle fibers where anaerobic metabolism and oxidative burst are common suggests that the dipeptides could provide a crucial line of defense against oxidatively induced ischemic reperfusion injury.

**Polyamines, Nucleotides and Related Compounds**

The polyamines, putrescine \([\text{NH}_2(\text{CH}_2)_4\text{NH}_2]\), spermidine \([\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_2\text{NH}_2]\) and spermine \([\text{NH}_2(\text{CH}_2)_2\text{NH}(\text{CH}_2)_3\text{NH}_2]\) are found in nearly all animal tissues. These polyamines have been postulated to play a role in membrane stabilization and antioxidant protection (Lovaas and Carlin, 1991). The polyamines inhibit lipid oxidation by free radical inactivation (Droplet et al., 1986), inhibition of iron catalyzed reactions (Lovaas and Carlin, 1991) and by altering interactions between spermine and phospholipids (Cabrini et al., 1989). Antioxidant activity of the polyamines increases with increasing number of amine groups (e.g. spermine > spermidine > putrescine). The concentration of putrescine (0.035 - 0.42 mM), spermidine (0.17 - 0.95 mM) and spermine (0.25 - 1.9 mM) in fresh skeletal muscle (Lakritz et al., 1975; Yamamoto et al., 1982; Yamanaka et al., 1987) are similar to those found to inhibit lipid oxidation in vitro. However, there is no direct evidence that these compounds significantly contribute to the antioxidant stability of skeletal muscle.

**Nucleotides including adenosine, xanthine, hypoxanthine and uric acid** are capable of inhibiting lipid oxidation (Matsushita et al., 1963). These compounds are produced in muscle from the anaerobic post-mortem decomposition of ATP (ATP $\rightarrow$ hypoxanthine; Hultin, 1985) and the conversion of hypoxanthine to uric acid byxanthine oxidase upon reoxygenation of anaerobic muscle (Tzeng and Billiar, 1994). Uric acid is the most widely recognized and best described of these antioxidative compounds. Uric acid can inhibit oxidative reactions by both iron chelation and free radical scavenging (Hochstein et al., 1984). Its concentration in blood ranges from 320 to 470 $\mu$M (Hochstein et al., 1984; Wayner et al., 1987; Frei et al., 1988). Uracil acid concentrations in skeletal muscle are not well understood, but since muscle foods contain residual blood and since xanthine oxidase, which converts hypoxanthine to uric acid, has been described in skeletal muscle (Tzeng and Billiar, 1994), this strongly suggests that uric acid is present in muscle. Like the polyamines, more information is needed to determine the importance of uric acid and other nucleotides as endogenous antioxidants in muscle foods.

**Iron Binding Proteins**

Iron catalyzes in the oxidation of lipids and proteins by its ability to accelerate free radical formation. Since skeletal muscle contains high concentrations of iron, it is crucial for
the tissue to control iron reactivity to prevent oxidative damage to cellular components (Decker and Hultin, 1992). Iron is transported and stored in animal systems by transferrin and ferritin, respectively. Transferrin contains two iron-binding sites on a single polypeptide chain with a molecular weight of 80,000. Ferritin is a mult-subunit protein (molecular weight of 450,000) with 4,500 iron-binding sites. Both transferrin and ferritin can inhibit lipid oxidation by binding iron in its inactive ferric state. However, reducing agents (ascorbate, cysteine and superoxide anion) cause the release of iron from ferritin and transferrin, resulting in an acceleration of lipid oxidation reactions. Transferrin is found mainly in blood while ferritin has been reported in muscle at concentrations ranging from 1.1 to 2.8 μg/g beef muscle (Decker and Welch, 1990) and 25-75 μg/g turkey muscle (Kanner and Doll, 1991).

Ascorbic Acid

Turkey dark (Kanner et al., 1991), beef diaphragm (Seman et al., 1991) and mackerel light muscle (Decker and Hultin, 1990a) contain 128, 10 and 51 μM ascorbic acid, respectively. Ascorbic acid either promotes or inhibits lipid oxidation reactions in a concentration-dependent manner. At low concentrations, ascorbate accelerates lipid oxidation through its ability to reduce iron into the active ferrous state. At high concentrations, ascorbic acid inhibits lipid oxidation by inactivating free radicals and lipid oxidation catalysts such as ferryl myoglobin. The antioxidant/prooxidant activity of ascorbate is influenced by iron concentrations with ascorbate showing more pronounced antioxidant activity at lower iron concentrations. Ascorbate is capable of inhibiting the oxidation of phosphatidylcholine liposomes at concentrations of 4 to 6 mM in the presence of 50 ppb iron; but in the presence of 500 ppb iron, ascorbate concentrations must be > 8 mM to exhibit antioxidant activity (Decker and Hultin, 1992). Skeletal muscle contains free or catalytic iron concentrations ranging from 200 to 2500 ppb (for review see Decker and Hultin, 1992) suggesting that endogenous ascorbate concentrations are prooxidative. In addition, enzymic removal of ascorbate from muscle extracts results in a decrease in metal catalyzed lipid oxidation (Decker and Hultin, 1990b; Kanner et al., 1991) again suggesting that endogenous ascorbate activates prooxidant metals in muscle foods. However, endogenous ascorbate concentrations are capable of inhibiting the formation and activity of the prooxidant, ferryl myoglobin (Decker and Hultin, 1990b; Kanner et al., 1991) suggesting that ascorbate could have a dual role in skeletal muscle as both a prooxidant and an antioxidant.

Glutathione

Glutathione is a tripeptide (gamma-Glu-Cys-Gly) found in both reduced (GSH) and oxidized glutathione (GSSG) states. Reduced glutathione inhibits lipid oxidation directly (nondenzymically) by inactivating free radicals or by providing a source of electrons which allows glutathione peroxidase to enzymically decompose hydrogen and lipid peroxides. Total glutathione concentrations in mackerel, bluefish and turkey thigh muscle is 230, 183, 300 nmol/g, respectively (Jia et al., 1996; Lee et al., 1996). Both total and reduced glutathione concentrations did not decrease during the cooking of turkey thigh muscle (Lee et al., 1996) but did decrease during the storage of mackerel and bluefish (Jia et al., 1996).

Cytosolic Antioxidant Enzymes

Superoxide Dismutase

Atmospheric or triplet oxygen can be converted to superoxide anion (O2−) by the addition of an electron. Superoxide anion promotes lipid oxidation by acting as a reducing agent, which causes the redox cycling of prooxidant metals or by forming its conjugated acid, the perhydroxyl radical (HO2), at low pH (pKα = 4.8). The perhydroxyl radical directly catalyzes hydrogen abstraction from unsaturated fatty acids (Kanner et al., 1987). The reactivity of superoxide anion and perhydroxyl radicals is controlled by superoxide dismutase (SOD). Two forms of SOD are found in eukaryotic cells, one in the cytosol and the other in the mitochondria (Fridovich, 1974). Cytosolic SOD contains copper and zinc in the active site while mitochondrial SOD contains manganese. Both forms of SOD catalyze the conversion of superoxide anion to hydrogen peroxide by the following reaction: 2 O2− + 2 H+ → O2 + H2O2.

Catalase

Hydrogen peroxide can be formed in biological tissues by a variety of mechanisms including the dismutation of superoxide by SOD (Halliwell and Gutteridge, 1990). Hydrogen peroxide is rapidly decomposed by transition metals such as ferrous and cuprous ions to the hydroxyl radical. The hydroxyl radical is an extremely reactive free radical which can oxidize most biological molecules at diffusion limited reaction rates. Therefore, removal of hydrogen peroxide from biological materials is critical to prevent oxidative damage. Catalase (CAT) is an heme containing enzyme which catalyzes the following reaction (Claiborne, 1985): 2 H2O2 → 2 H2O + O2.

Glutathione Peroxidase

In addition to catalase, most biological systems also contain glutathione peroxidase (GSH-Px) to help control peroxides. GSH-Px differs from CAT in that it is capable of reacting with both lipid and hydrogen peroxides. GSH-Px is a selenium-containing enzyme which catalyzes hydrogen or lipid (LOOH) peroxide reduction using reduced glutathione (GSH):

\[ \text{H}_2\text{O}_2 + 2 \text{GSH} → 2 \text{H}_2\text{O} + \text{GSSG} \]

or

\[ \text{LOOH} + 2 \text{GSH} → \text{LOH} + \text{H}_2\text{O} + \text{GSSG} \]

where GSSG is oxidized glutathione and LOH is a fatty acid alcohol. Two types of GSH-Px exist in biological tissues of
which one shows high specificity for phospholipid hydroperoxides (Maiorino et al., 1990; Günzler and Floke, 1995).

Ceruloplasmin

Ceruloplasmin is a copper-containing enzyme which catalyzes the oxidation of ferrous ions:

\[ 4 \text{Fe}^{2+} + 4 \text{H}^+ + \text{O}_2 \rightarrow 4 \text{Fe}^{3+} + 2 \text{H}_2\text{O} \]

This ferroxidase activity inhibits lipid oxidation by maintaining iron in its oxidized inactive state (Gutteridge, 1985). Ceruloplasmin is found in blood but has not been described in skeletal muscle. It would not be unexpected to find low levels of ceruloplasmin activity in muscle foods due to small amounts of residual blood.

Antioxidant Enzymes in Muscle Foods

Several Authors (Mei et al., 1994; Nakano et al., 1992; Aksnes and Njaa, 1981; Lin and Hultin, 1978) have reported SOD, CAT and GSH-Px activities in skeletal muscle. Several generalizations can be made from the enzyme activities reported in the literature including: Red muscle of chicken, turkey and the salt water fish, saithe, have higher SOD and CAT activity than white muscle; Turkey, chicken, carp and mackerel have higher GSH-Px in red than white muscle while salmon muscle shows the opposite trend; GSH-Px and SOD activity in beef > turkey > pork; CAT activity in pork > beef > turkey.

GSH-Px is the only antioxidant enzyme in muscle foods which has been reported to be influenced by animal diet. DeVore et al. (1983) found that supplementation of chicken diets with 0.25 ppm selenium increased GSH-Px activity in both breast and leg muscle. Selenium supplementation also decreased TBARS formation during the storage of minced muscle at 4°C for 4 days, suggesting that GSH-Px activity could increase oxidative stability.

Oxidation of muscle foods is rapidly accelerated by cooking, leading to the development of what is known as warmed-over flavor. A possible mechanism for the heat-induced acceleration of lipid oxidation is the inactivation of endogenous antioxidants. Research in our laboratory has found that cooking ground pork and beef to an internal temperature of 70°C results in total inactivation of CAT and partial inactivation of GSH-Px (Mei et al., 1994). Inactivation of CAT (r = 0.83-0.86) and GSH-Px (r = 0.83-0.88) correlated well with the development of lipid oxidation. Cooking method also influenced antioxidant enzyme inactivation in ground turkey breast or thigh muscle cooked to 60°C with CAT and GSH-Px being inactivated less by baking than by broiling, frying or microwaving (Figure 2). Among these cooking methods, baked thigh muscle was more oxidatively stable than other cooking methods. Cooking methods did not result in major alterations in the oxidative stability of breast muscle (Figure 3). To better understand how inactivation of antioxidant enzymes were involved in the rapid development of lipid oxidation, ground turkey thigh muscle was cooked to an internal temperature of 80°C to provide minimal activity of both CAT and GSH-Px. Endogenous concentrations of CAT and/or GSH-Px were then immediately added back to the ground muscle and lipid oxidation was monitored during storage. CAT and GSH-Px, both alone and in combination, inhibited less than 15% of lipid oxidation, suggesting that antioxidant enzyme inactivation is not the only factor involved in the rapid development of lipid oxidation in cooked muscle (Lee et al., 1996).

Maillard Reaction Products

While cooking decreases the oxidative stability of muscle foods, it also can cause the formation of antioxidants. Subjecting meats to severe heat treatments such as

![FIGURE 2.](image-url)

Changes in catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity in ground turkey breast (A) and thigh (B) muscle cooked to 60°C by different cooking methods.

![FIGURE 3.](image-url)

Formation of thiobarbituric acid reactive substances (TBARS) in ground turkey breast and thigh muscle cooked to 60°C. Muscle had been stored a 4°C for 4 days prior to analysis.
retorting has been shown to increase oxidative stability compared to less severely cooked meats (Sato et al., 1973; Einerson and Reineccius, 1978; Huang and Greene, 1978). Sato et al. (1973) and Einerson and Reineccius (1978) found that these cooked meats had unidentified, low molecular-weight water-soluble antioxidants. These antioxidants were suggested to be Maillard reaction products (MRP), which are formed from amines and carbonyls at elevated temperatures. MRP's have been proposed to inhibit oxidative reactions by acting as reducing agents and free radical scavengers (Yen and Hsieh, 1995). Unfortunately, little is known about the origin of the amines and carbonyls in skeletal muscle which are responsible for the antioxidative MRP's.

Antioxidant Additives

Skeletal muscle contains a multicomponent antioxidant system capable of controlling oxidative reactions in the live animal and in many muscle foods. However, meat processing operations can decrease oxidative stability, leading to quality deterioration. In these cases, exogenous sources of antioxidants must be used to prolong shelf-life and insure product quality (for review of antioxidant additives in cooked muscle, see Mielche and Bertelsen, 1994).

Phenolic Antioxidants

Lipid oxidation reactions in foods can be controlled by synthetic phenolics. Synthetic phenolics approved for use in the United States include butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and tertiary butylhydroquinone. These lipid-soluble phenolics inhibit lipid oxidation by free radical scavenging. Allowable concentration is generally 0.01% individually or 0.02% in combination (based on fat content) in fresh meats and sausages, 0.003% of total weight in dry sausages and 0.01% of total weight in dried meats (Schmidt, 1986). While synthetic phenolic antioxidants are effective inhibitors of lipid oxidation, consumer demand for “all natural” products has prompted meat processors to utilize natural sources of phenolic antioxidants. Alternative sources of phenolic antioxidants used in the food industry include tocopherol isomers and phenolic-containing plant extracts from sources such as rosemary, sage and green tea.

Tocopherol exists naturally as four different isomers whose antioxidant activity in bulk oils is δ > gamma > β > α (Nawar, 1985). α-Tocopherol, which has the highest vitamin E activity, has been found to be an effective antioxidant when incorporated into muscle via the diet (Faustman, 1993). However, when used as an additive in muscle foods, its effectiveness is less predictable with some investigators (St Angelo et al., 1988; Ramanathan and Das, 1992; Decker and Crum, 1991 & 1993) finding it ineffective or prooxidative and others (Chen et al., 1984; Mitumoto et al., 1991) finding it moderately effective. Tocopherols can also be obtained in the form of mixed tocopherol isomers which are by-product of the vegetable oil industry. Mixed tocopherol isomers appear to be more effective in muscle foods (Calvert and Decker, 1992; Decker et al., 1993; Bruun-Jensen et al, 1994), presumably due to the presence of tocopherol isomers which have superior antioxidant activity compared to α-tocopherol.

Antioxidant phenolics can also be obtained from a variety of plant extracts (for review, see Shahidi and Wanasundara, 1992). The most common commercial source of plant antioxidant phenolics is rosemary (Rosmarinus officinalis L.). Rosemary extracts, at concentrations ranging from 0.02 to 0.05% of total weight, have been reported to inhibit lipid oxidation in beef (Wu et al., 1994; Stoick et al., 1991), pork (Decker et al., 1993), turkey (Barbut et al., 1985), chicken (Lai et al., 1991) and frankfurters (Resurreccion and Reynolds, 1990). The phenolics responsible for the antioxidant activity of rosemary extracts include carnosol, rosmanol, rosmaridiphenol, carnosolic acid and rosmarinic acid (Shahidi and Wanasundara, 1992).

Chelators

Lipid oxidation in muscle foods can also be inhibited by controlling the activity of prooxidant metals. The most common chelators used in muscle foods are citric acid and phosphates. An advantage of citric acid is that it is soluble in lipids, thereby making it effective in all lipid systems. Citric acid can be used in combination with lipid soluble phenolic antioxidants, often resulting in synergistic inhibition of lipid oxidation (Lindsay, 1985).

Phosphates also inhibit lipid oxidation by chelating prooxidant metals with the polyphosphates being more effective chelators than monophosphate (for review, see Sotos, 1986). Since metals are active lipid oxidation catalysts in muscle foods, it is not surprising that the polyphosphates are more effective antioxidants (Tim and Watts, 1958; Shahidi et al., 1986; Trout and Dale, 1990; Mikkelsen et al., 1991). The antioxidant activity of the phosphates is not only dependent on phosphate type but also on the cooked state of the muscle. The polyphosphates are one of the most effective antioxidants in cooked meats, inhibiting lipid oxidation over 85% (Shahidi et al., 1986; Trout and Dale, 1990; Decker and Crum, 1993). However, when used in uncooked meats, polyphosphates are less effective inhibiting 8% to 60% of lipid oxidation in raw beef (Mikkelsen et al., 1991) and even acting as a prooxidant in frozen, salted, ground pork (Decker and Crum, 1991). Decreased antioxidant activity of polyphosphates in raw meats is likely due to their hydrolysis by endogenous skeletal muscle phosphatases during storage. Li et al (1993) found that sodium tripolyphosphate (STP) was completely hydrolysed in raw ground turkey after 1 day of refrigeration storage. STP hydrolysis also occurred at rapid rates during the heating of ground turkey but hydrolysis was not observed when STP was added to muscle which had been precooked to inactivate the phosphatases. While the increased antioxidant activity of polyphosphates in cooked meats seems to be at least partially due to inactivation of phosphatases, it may also be due to the increased importance of iron as a lipid oxidation catalyst. In either case, the...
cooked state of muscle foods should be taken into consideration when using polyphosphates as antioxidants.

**Ascorbate**

Use of ascorbate is limited in many meat products due to its ability to maintain the reduced state of myoglobin, thus potentially misleading consumer perception of meat freshness. Despite legal restriction on the use of ascorbate in muscle foods, several researchers have investigated its antioxidant activity. Ascorbate is capable of inhibiting lipid oxidation by inactivating free radicals and by regenerating α-tocopherol. However, ascorbate can also act as a prooxidant by reducing iron to its catalytic ferrous form. Since ascorbate can act as both a prooxidant and antioxidant, it is not surprising that its effect on the oxidative stability of muscle foods is variable. Low concentrations (0.02% to 0.03%) of ascorbate have been reported to be either ineffective or prooxidative in beef (Roozen, 1987; St. Angelo et al., 1988) and fish (Deng et al., 1978; Ramanathan and Das, 1992) while high concentrations (0.5%) can exhibit antioxidant activity in beef (St. Angelo et al., 1988; Shantha et al., 1995) and fish (Deng et al., 1973). Since the prooxidant activity of ascorbate is due to its ability to reduce metals, its antioxidant activity may be improved by using it in combination with chelators which control the activity of iron. Ascorbyl palmitate and ester of ascorbic and palmitic acid, can be used as a lipid soluble source of ascorbate. Ascorbyl palmitate inhibits lipid oxidation in turkey (Bruun-Jensen, 1994; Calvert and Decker, 1992) and beef (Roozen, 1987).

**Nitrite**

Cured meat products are often exposed to conditions which favor lipid oxidation, including reduction of particle size, addition of salt, cooking and prolonged storage times. However, flavor deterioration in these products is not usually a problem due to the presence of nitrite. The antioxidant activity of nitrite has been described by numerous investigators (for review see Shahidi, 1992). The antioxidant mechanism of nitrite is still not completely understood but it is thought to include its ability to stabilize heme-containing proteins (Igene et al., 1985), chelate free iron (Kanner et al., 1984; Morrissey and Tichivangana, 1985), stabilize lipid membranes (Goutefongea et al., 1977; Freybler et al., 1993) and form nitrosated heme compounds which possess antioxidant activity (Kanner and Juen, 1980; Morrissey and Tichivangana, 1985).

**Emerging Natural Antioxidants**

Several compounds from natural sources have been found to have excellent potential as antioxidants in muscle foods. In addition to phenolics from rosemary and related herbs, numerous polyphenols and flavonoids have been found to effectively inhibit lipid oxidation in both raw and cooked fish (Ramanathan and Das, 1992). Phytic acid, a strong metal chelator, inhibits lipid oxidation in its pure form in chicken (Empson et al., 1991) and beef (Lee and Hendricks, 1995) and as part of a wild rice extract in beef (Wu et al., 1994). While phytic acid may be an excellent antioxidant in meat, questions must be answered about how it will effective bioavailability of minerals, such as iron, before it is used in meat products. Carnosine has also been found to inhibit lipid oxidation in meats with maximal effectiveness being observed in raw salted (2% NaCl) pork (Decker and Crum, 1991). Carnosine can not be used economically as a food antioxidant at this time, however, it may be possible to produce carnosine-containing antioxidant extracts from underutilized skeletal muscle (Chan et al., 1993). Several protein sources have also been found to inhibit lipid oxidation, including blood plasma (Faraji et al., 1991), whey protein concentrate (Shantha and Decker, 1995), soybean (Ziprin et al., 1981; Wu and Brewer, 1994), cotton seed (Rhee and Smith, 1983) and peanut (Ziprin et al., 1981). These proteins may have dual benefits as meat additives by both inhibiting flavor deterioration and acting as binding agents.

**Conclusions**

All muscle foods have an inherent resistance to oxidative deterioration due to an endogenous multicomponent antioxidant system which consists of lipid, cytosolic and enzymic antioxidants. The activity of the endogenous antioxidant systems is dependent on animal species, muscle type, dietary factors and heat processing operations which causes antioxidant formation (e.g. Maillard reaction products) and destruction (e.g. antioxidant enzymes). Antioxidant additives which scavenge free radicals and chelate prooxidant metals can be used to increase the oxidative stability of muscle foods. The effectiveness of these additives is dependent not only on their antioxidant activity but also on where they partition into muscle (e.g. membrane vs. adipose lipids) and their stability during processing and storage.

**Acknowledgments**

Portions of the work covered in the review were partially funded by grants from the National Live Stock and Meat Board and the National Pork Producers Council.

**References**


