

Preserving Beef Quality with Natural Antioxidants

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BACKGROUND

Consumers spend less time and more money on home-prepared foods opting for convenience products such as pre-cooked entrees (Dumagan and Hackett, 1995). However, they demand acceptable color and flavor, whether the product is ready-to-cook or ready-to-eat. Color and appearance of fresh meat are major factors in consumer purchase decisions because they are presumed to be indicators of meat quality and freshness (Brewer et al., 2002). These characteristics have a significant impact on consumer expectations of satisfaction (Robbins et al., 2003). Lipid oxidation and discoloration (myoglobin oxidation) reveal that color deterioration is an indicator of flavor deterioration (Djenane et al., 2003).

The continued demand for convenient foods has led to an expansion in the variety of fully-cooked, ready-to-eat meat products available. However, cooked meats are susceptible to quality deterioration, particularly oxidative rancidity. The fatty acids in muscle tissue may be saturated or unsaturated (Figure 1). Those in depot fat (subcutaneous, intermuscular, intramuscular) are primarily saturated 16 to 18 carbon straight-chains, while phospholipids in membranes contain more (15 times) unsaturated fatty acids that are much more susceptible to oxidation (Elmore et al., 1999). Oxidation of unsaturated fatty acids can produce numerous compounds, many of which contribute off-odors (Table 1) and are perceptible at very low concentrations. Warmed-over flavor (WOF) has been described as the disappearance of fresh meat flavor and the development of characteristic off-flavors in pre-cooked meats (Kanner, 1994). Usually associated with reheated meats, WOF is often described as “stale”, “cardboard-like”, “painty” or “rancid” (Table 1; Vega and Brewer, 1994, 2007; Love, 1988). In cooked, refrigerated meats, oxidized flavors are easily detected after 48 hours. Prolonged freezer storage can also result in rancidity development in raw meat (Pearson and Young, 1989). Frozen storage prevents microbiological deterioration allowing sufficient time for substantial oxidation to occur and resulting in significant quality deterioration.

Meat color is due to the pigment myoglobin, which is designed to bind and store oxygen in the muscle until it is needed for metabolic processes. Myoglobin is composed of a protein (globin)

and an iron-containing group (heme; Lawrie, 2002). The iron can exist in the reduced (Fe^{2+} ; deficient in two electrons) or oxidized (Fe^{3+} ; deficient in three electrons) states. In food, iron can be a prooxidant. It can catalyze the breakdown of lipid oxidation products by promoting free radical formation from hydrogen peroxide (H_2O_2). In H_2O_2 , each atom (H, O, O, H) shares one electron (•) with another atom so the molecule has an electron structure of H:O:O:H. The H_2O_2 (H:O:O:H) can release H^\bullet , O^\bullet , HO^\bullet , or HOO^\bullet , which can start a chain reaction of oxidation. Free radicals can then attack unsaturated fatty acids.

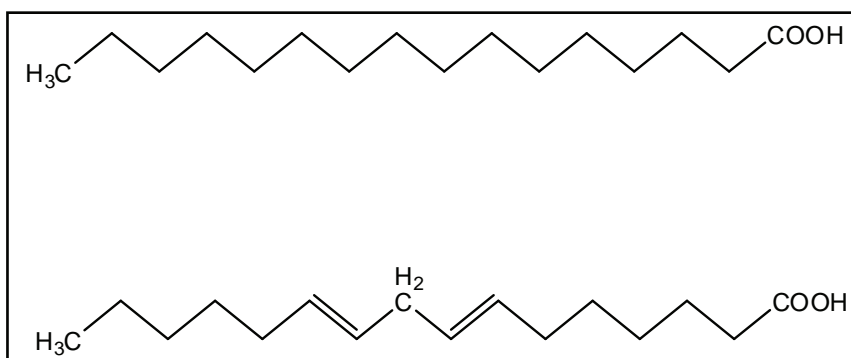


Figure 1. Saturated (top) and unsaturated (bottom) fatty acids

Table 1. Odors in beef and compounds which contribute to them

Beefy	12-methyltridecanal; bis-methyl-thiomethane; 2-methyl-3-(methylthio) thiophene
Cooked	Propanal
Fatty, tallowy	Heptene; nonene; hexanal; octanal; decanal; 3-nonenal; nona-2(E)-enal; E-2-hexenal; E-2-octenal; deca-2, 4-dienal; E,E-nona-2, 4-dienal; 12-methyltridecanal
Green, grassy	Hexanal; E-2-hexenal; E-2-octenal; E-2-decenal; E,E-deca-2, 4-dienal; E,E-octa-2, 4-dienal; E,E-nona-2, 4-dienal; 3-hydroxy-2-butanone; 2-methyl-thiophene
Meaty	Decanal; 2-methyl-3-furanthiol; 2-furyfurythiol; 4-hydroxy-5-methyl-3(2H)-furanone (HMF); 4-hydroxy-2,5-dimethyl-3(2H)-furanone
Pungent	Propanoic acid; pentanal; undecanal; E-2-decenal; nonenone; 1-nonen-3-one
Rancid	Butanoic acid; propanoic acid; hexanal; decanal; 2-hexenal; Trans-4,5-E-2-decenal; E-2-hexenal; 3-hydroxy-2-butanone
Roasted	Bis (2-methyl-3-furyl) disulfide; 4,6-dimethyl-2,3,4,7-tetrathiaoctane; 4,6-dimethyl-2,3,4,7-tetrathiaoctane; methylpyrazine; 2,5- and 2,6-dimethylpyrazine; 3-hydroxy-4,5-dimethyl-2(5H)-furanone
Sweaty	Hexanoic acid; octanoic acid
Warmed-over	Trans-4,5-E-2-decenal; 2,3-octanedione

Source: Brewer 2007, 2009

Oxygen is critical to living tissues and has major effects on the stability and color of the myoglobin which transports it. In molecular oxygen (O₂), each atom shares two electrons with the other atom in a relatively stable, low energy double bond (O::O). However, it can become highly energized or form reactive free radicals, which can react with many other compounds with double bonds, including unsaturated fatty acids (Min and Boff, 2002).

OXIDATION

During the initiation step of lipid oxidation, a hydrogen atom (H•) is abstracted from an unsaturated fatty acid (R) to form an alkyl radical (R•; Fennema, 2008):



Autoxidation then proceeds via traditional pathways (Figure 2). The reaction propagates from one fatty acid to another ultimately forming lipid hydroperoxides (ROOH). Lipid hydroperoxides are tasteless and odorless; however, they decompose to products that are responsible for off-odors and off-flavors (Fennema, 2008). Heat, metal ion catalysis or light can cause hydroperoxide decomposition. This chain reaction terminates when two radicals combine.

Solving the problem of WOF is critical to producing high-quality, oven-ready or table-ready, reheatable entree items. Adding antioxidants is nearly always the best strategy to reduce lipid oxidation. Synthetic phenolic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) effectively inhibit WOF; however, consumer concerns regarding their safety have motivated food manufacturers to investigate natural substances as replacements (Decker and Mei, 1996; Formanek et al., 2001). A food product may be labeled as “natural” when

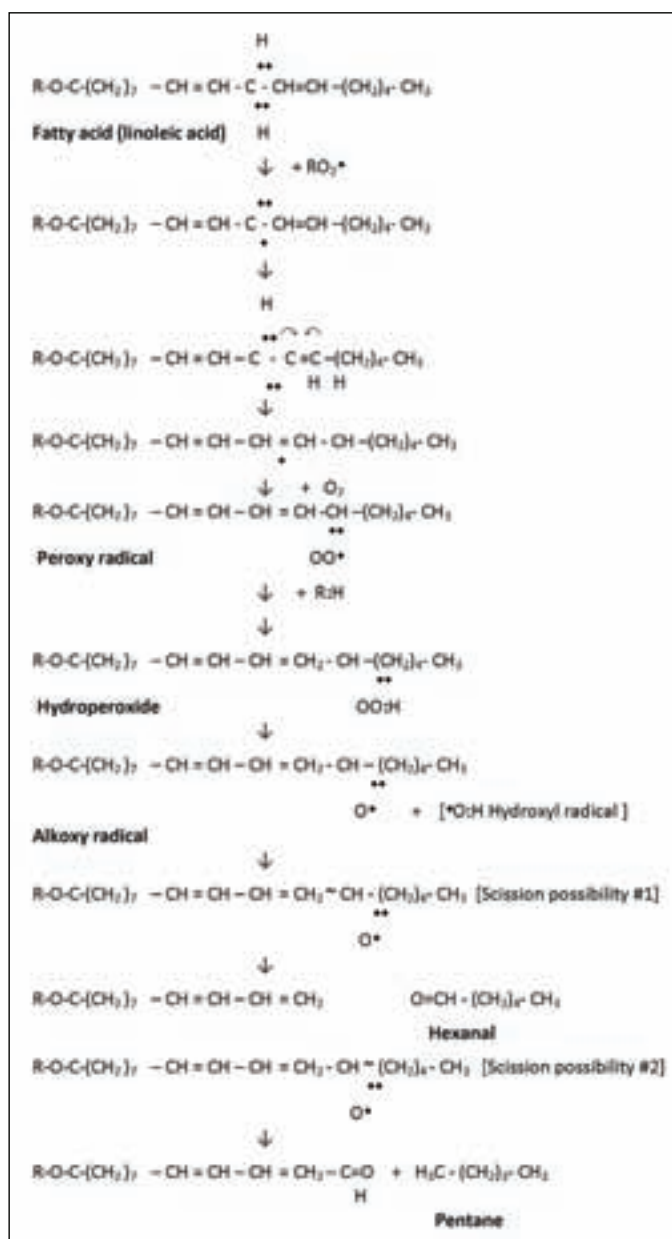


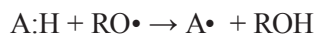
Figure 2. Autooxidation of linoleic acid

the product contains no artificial ingredients and is no more than minimally processed (frozen, ground, smoked or roasted; USDA FSIS, 2005). This is a distinct benefit for meat products which often contain few ingredients.

ANTIOXIDANTS

An antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals which can perpetuate the reaction (Fennema, 1996). Antioxidants can bind metals, scavenge species that initiate or perpetuate oxidation, quench high-energy oxygen species-preventing formation of peroxides, or decompose lipid peroxides. They can improve both color and flavor stability in meats (Xiong et al., 1993). Some vitamins (ascorbic acid, vitamin E) exhibit antioxidative activity. Many herbs and spices (rosemary, oregano, grapeseed, sage, thyme) contain antioxidant components (Ahn et al., 2007; Rojas and Brewer, 2007, 2008; Sasse et al., 2009; Table 2, page 4).

Chelating agents bind metal ions (iron, copper) preventing them from participating in oxidation reactions (Fennema, 1996). However, the most effective antioxidants interrupt the free radical chain reaction and usually contain aromatic rings capable of donating H• to the free radical formed during lipid oxidation (Figure 3). They “sacrifice themselves” by giving up a hydrogen atom, then rearrange to a stable conformation.



Phenolic antioxidants may be either synthetic (BHA, BHT) or naturally occurring (vitamin E, grapeseed extract, pycnogenol, rosemary). Chain-breaking antioxidants differ in their effectiveness because of their chemical characteristics and physical location within a food.

Flavonoids, many of which are naturally occurring pigments, are a major group of plant phenols which have antioxidant properties. In the plant, flavonoids likely function to protect it from damage by high-energy oxygen (Agati et al., 2007). Plant extracts containing high levels of phenolic compounds (clove, cinnamon, marjoram, oregano, cumin, rosemary) with strong H•-donating activity effectively scavenge H₂O₂ and reactive oxygen species (Lugasi et al., 1995). The free-radical-scavenging potential of natural polyphenolic compounds depends on the number and arrangement of free -OH groups on the flavonoid skeleton (Lupea et al., 2008; Shahidi and Wanasundara, 1992; Kondo et al., 2001). Flavonoids can lose a hydrogen-reducing metal rendering them less pro-oxidative (Fernandez et al., 2002). Plant-derived flavonoids include carnosol, rosmanol, rosmariquinone and rosmaridiphenol.

This paper will summarize and describe the uses and mechanisms of action of the more common antioxidants, both natural and synthetic, used in meat products.

Synthetic Antioxidants

Phosphates

Phosphates chelate metal ions preventing them from initiating the oxidation reaction. Pyrophosphates, tripolyphosphates and hexametaphosphates are generally added to increase water holding capacity and finished-product yield. All three compounds protect lipids from oxidation; however, orthophosphates do not. The monovalent cationic phosphates are better antioxidants than the larger cations with higher valences (Weilmeier and Regenstein, 2004; Trout and Dale, 1990).

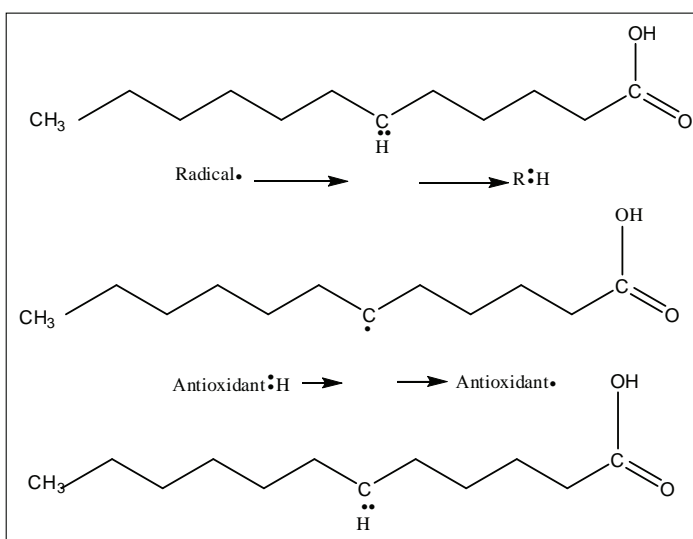


Figure 3. Antioxidant interaction with fatty acid free radical

Iron-binding phosphate compounds appear to be more effective than BHT at preventing rancidity (maintaining low chemical measures of rancidity, preventing rancid odor) in cooked ground beef during refrigerated storage (Vasavada et al., 2006). In precooked roast beef, Murphy et al. (1998) found that sodium tripolyphosphate was more effective than BHA/BHT, oregano and sodium citrate during both refrigerated and frozen storage. Sodium tripolyphosphate (STP) in combination with other antioxidants is even more effective. Alone or in combination with sodium ascorbate and/or tocopherol, STP can decrease thiobarbituric reactive substances (TBARS; a chemical measure of lipid oxidation) and metmyoglobin content, and increase visual color and odor scores in refrigerated ground buffalo meat (Sahoo et al., 2000). Alpha-tocopherol (vitamin E; 0.03%) enhances the antioxidant activity of sodium tripolyphosphate (0.3%; Vara-Ubol and Bowers, 2001). In red meat and poultry products, sodium polyphosphates are limited to 0.5% of the

Table 2. Antioxidative compounds in selected herbs and spices

		Carnosic acid	Carn-osal	Ros-marinic acid	Ros-manol	Ros-madial	Phenolic acids	Flavo-noids	Thymol	Carv-acrol	Epi-catechin	Epicat-gallo-catechin gallate	Querc-itin	Gallic acid	Resver-atrol
Rosemary	<i>Rosmarinus officinalis</i> (Labiatae)	X	X	X	X		X		X		X	X			
Oregano	<i>Origanum vulgare</i> (Labiatae)			X			X	X							
Sage	<i>Salvia officinalis</i> (Labiatae)	X	X	X	X	X	X	X							
Thyme	<i>Thymus vulgaris</i> (Labiatae)			X			X	X	X	X			X		
Marjoram	<i>Origanum majorana</i> (Labiatae)			X			X	X							
Summer savory	<i>Satureja hortensis</i> (Labiatae)		X	X			X	X	X	X					
Green tea	<i>Camellia sinensis</i>							X			X	X		X	
Grapeseed extract								X			X	X	X	X	X

Sources: Yanishlieva et al., 2006; Nakatani and Inatani, 1981; Arouma et al., 1992; Deans and Simpson, 2000; Cuvelier et al., 1994; Carrillo and Tena, 2006; Hernandez-Hernandez et al., 2009; Dorman et al., 2003; Caldwell, 2001; Cabrera et al., 2003; Sato et al., 2007; Lugasi et al., 1995; Shan et al., 2005; Nakatani, 2003; Wang et al., 2008; Miura et al., 2002; Deans et al., 2000; Lee et al., 2005; Jayaprakasha et al., 2001; St. Angelo et al., 1990; Hur et al., 2004.

finished product (5% of pickle at 10% pump level; FSIS, 2000).

BHA / BHT / TBHQ / EDTA

BHA, BHT and tertiary butyl hydroxyquinone (TBHQ) are synthetic, chain breaking antioxidants (Figure 4). They are aromatic rings that can donate H• to an oxidizing lipid (free radical; Figures 3 and 4). This stops the oxidative process by forming a more stable compound. Jayathilakan et al. (2007) reported that TBHQ suppressed oxidation in cooked, refrigerated beef, pork and lamb by >90%; it reduced hexanal production, non-heme iron release and WOF. Chastain et al. (1982) and Love (1987) reported similar results. BHA (0.1%)-impregnated film can maintain beef redness during refrigerated storage as well (Moore et al., 2003). Coating salt, a known pro-oxidant, with BHA and BHT completely inhibits oxidation during cooking and storage (Chen et al., 1984).

Ethylenediamine tetra acetic acid (EDTA) is a metal chelator. By binding iron, it can prevent Fe²⁺-catalyzed oxidation in both raw and cooked meat systems (Igene et al., 1979). The compounds BHA, BHT and TBHQ are limited to 0.01% of the fat when used individually or to a total of 0.02% of the fat when combined with another antioxidant in red meat and poultry (FSIS, 2000).

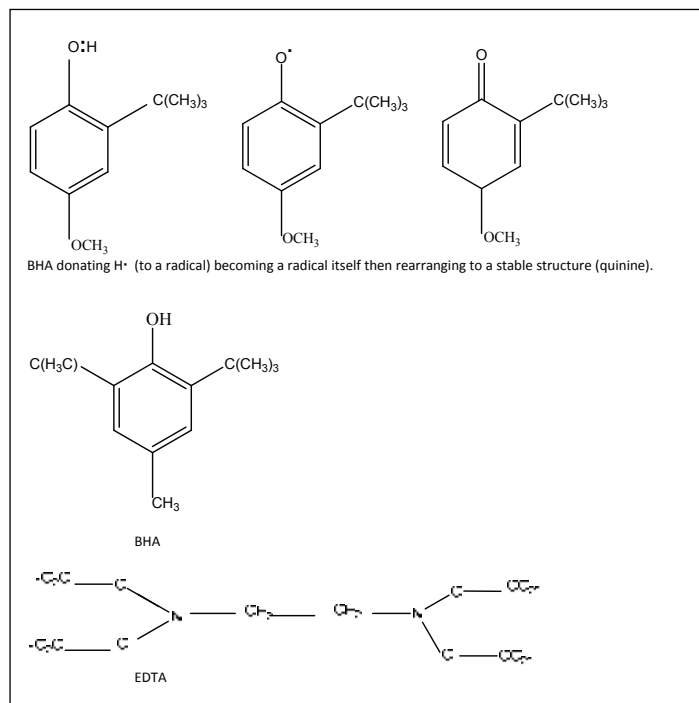


Figure 4. BHA (top), BHA (center), EDTA (bottom)

Natural antioxidants

Endogenous antioxidant systems

Because they are under constant oxidative stress from free radicals, reactive oxygen species and prooxidants, biological tissues from which meat is derived have developed antioxidant systems to protect them from oxidative damage. Carnosine in skeletal muscle inhibits iron-dependent lipid oxidation (Decker et al., 1992). The endogenous antioxidant enzymes catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) break down H_2O_2 delaying the onset of rancidity (Pradhan et al., 2000).

Gorelik and Kanner (2001) found that the inclusion of SOD in a meat-model system decreased oxymyoglobin oxidation by 10% without affecting lipid peroxidation. Adding catalase (which breaks down H_2O_2) decreased oxymyoglobin oxidation by about 40% but not lipid peroxidation. The authors conclude that oxymyoglobin stability was affected by two pathways: activated oxygen species (O_2^- , H_2O_2 , $HO\cdot$) which oxidize myoglobin Fe^{2+} ions, and lipid radicals ($ROO\cdot$, $RO\cdot$) and hydroperoxides generated during lipid peroxidation.

Processing can initiate oxidation by damaging tissue integrity and functionality. Cooking denatures the protein structure of these antioxidant enzyme systems (Decker and Mei, 1996; Chen et al., 1999). Salt reduces catalase, GSH-Px and SOD activity (Lee et al., 1997), which may be one of the reasons why TBARS values and lipid peroxides increase as salt content increases.

Propyl gallate

Propyl gallate (PG) is an aromatic antioxidant with three $-OH$ groups on the phenol ring capable of donating H^\bullet (Figure 5). It can reduce lipid oxidation, rancidity and loss of beefy flavor in restructured steaks during extended frozen storage (Stika et al., 2007; Reverte et al., 2003). However, in cow beef, Stika et al. (2008) found that PG provided no additional protection against lipid oxidation beyond that provided by vacuum packaging. Nicolalde et al. (2006) demonstrated that exposing rib bones to PG decreased visual discoloration during storage in high oxygen modified atmosphere packaging (MAP). In red meat and poultry, PG is limited to 0.01% of the fat content when used alone or to 0.02% of the fat when combined with BHA or BHT (FSIS, 2000).

Ascorbic acid

Ascorbic acid (AA) is a chelating agent that binds metal ions; it also scavenges free radicals and acts as a reducing agent (Figure 5). At high levels (> 1000 mg/kg), AA inhibits oxidation; however, at low levels (< 100 mg/kg) it can catalyze oxidation and WOF development (Ahn and Nam, 2004). High AA levels shift the balance between ferrous

(Fe^{2+}) and ferric (Fe^{3+}) iron or act as an oxygen scavenger. In the presence of AA, iron stimulates oxidation in muscle membranes, presumably through the involvement of hydroxyl radicals ($\cdot OH$; Kanner et al., 1994).

Reducing agents can maintain myoglobin in the reduced state. Adding them to ground beef may be one method of preventing premature browning, which is conversion of myoglobin to metmyoglobin at a lower temperature than expected. Sepe et al. (2005) found that sodium ascorbate and sodium erythorbate more effectively maintained red color and maintained myoglobin in the reduced state in cooked ground beef patties than ascorbic acid and ascorbyl palmitate. The solubility of ascorbate affects its ability to prevent discoloration. Mancini et al. (2007) showed that beef lumbar vertebrae treated with AA (aqueous) were redder and less discolored than those treated with ascorbate-6-palmitate (lipid-based). The lack of effectiveness of the hydrophobic antioxidant may be a result of localization of components responsible for bone discoloration within the aqueous phase.

Djenane et al. (2004) found that adding AA (500 ppm) to fresh beef prior to refrigerated storage in modified atmosphere packaging delayed oxidation for 28 days. Wheeler et al. (1996) reported that injecting beef with a sodium ascorbate solution (5% by weight of a 0.25-4% solution) resulted in redder steaks with less surface

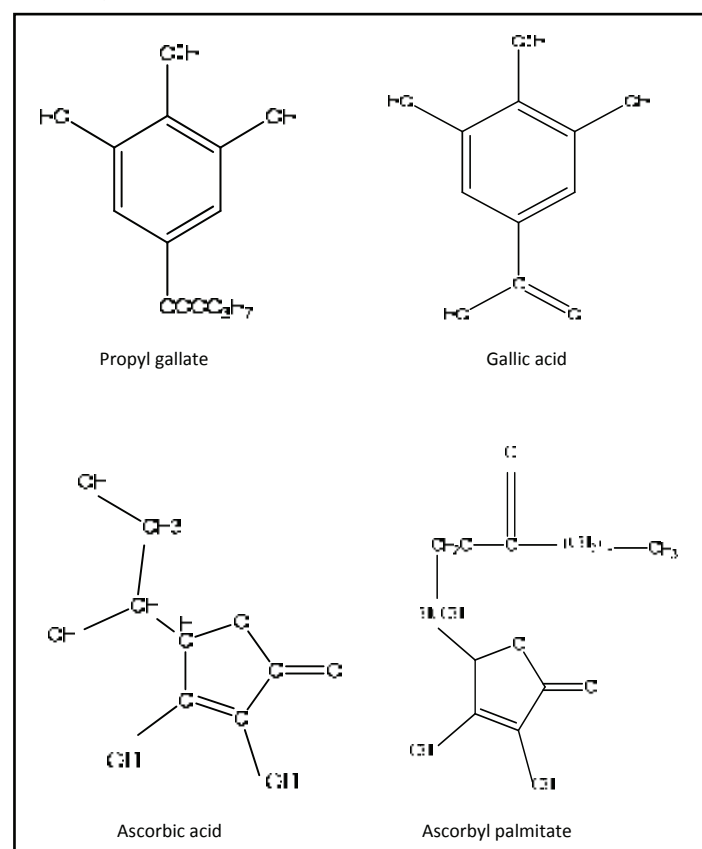


Figure 5. Propyl gallate, gallic acid, ascorbic acid and ascorbyl palmitate

discoloration than control steaks after seven days of display.

AA and phosphates appear to work synergistically to inhibit lipid oxidation (Tims and Watts, 1958; Sato and Hegarty, 1971). AA and tocopherol reduction of lipid oxidation in beef can be enhanced by adding sesamol, especially as storage time increases (Ismail et al., 2008). Adding AA and tocopherol prior to irradiation also maintains redness of irradiated ground beef during storage. Combining AA with tocopherol can more effectively reduce oxidation-reduction potential than adding sesamol. AA, tocopherol and sesamol all reduce aldehydes during storage. Ismail et al. (2009) found that, compared to tocopherol and sesamol alone, ascorbic acid was the most effective in maintaining redness of ground beef which was subsequently irradiated followed by ascorbic acid + tocopherol + sesamol. Mixing the antioxidant into the beef was superior to spraying it on with respect to preventing lipid oxidation and maintaining color. It has been theorized that AA functions to maintain a portion of the iron in the reduced state (Sato and Hegarty, 1970).

The amount of AA permitted in meat products varies depending on the route of introduction: brine, incorporation or surface spray. A 10% AA solution can be applied as a spray to cured beef and pork surfaces. It can be applied directly (500 ppm or 1.8 mg/sq. in.) to fresh beef, pork and lamb surfaces (FSIS, 2000). Sodium ascorbate or erythorbate are also used as cure accelerators.

Alpha tocopherol

Alpha-tocopherol (vitamin E) is a fat-soluble carotenoid (Figure 6). Trolox is a water-soluble derivative of it. Alpha-tocopherol locates in the cell membrane in proximity to phospholipids where it can prevent development of free radicals (Onibi et al., 2000). In meat homogenates, vitamin E has been shown to inhibit oxidation in a concentration-dependent lipid manner. Cort (1974) found that, in beef fat, at the maximum concentration, alpha-tocopherol was equal to BHT; gamma-tocopherol was more antioxidative

than alpha-tocopherol. In general, vitamin E, applied to muscle foods using an oil carrier, targets the neutral lipid fraction (triacylglycerols) rather than the polar lipid fraction (phospholipids) and is not an effective antioxidant. When delta-tocopherol was applied to ground beef using a polar carrier such that it would be incorporated into the phospholipid fraction, it reduced TBARS development after cooking and during storage (Wills et al., 2007).

The contribution of alpha-tocopherol to the oxidative stability of skeletal muscle is largely influenced by diet (Chan and Decker, 1994). The higher the initial concentrations of alpha-tocopherol and beta-carotene in muscle tissue from pasture-finished cattle, the better the color stability of steaks during refrigerated display (Insani et al., 2008). Dietary supplementation increases muscle alpha-tocopherol levels and delays metmyoglobin formation and lipid oxidation in a dose-dependent manner (Garber et al., 1996). Dietary vitamin E can protect against both lipid and pigment oxidation during storage in an oxygen-containing environment (Stubbs et al., 1999; Ying and Ahn, 1998). Tocopherols alone can be added at a level of 0.03% of the fat in red meat; they can be added at a level of 0.02% of the fat in combination with other antioxidants (except TBHQ) in poultry (FSIS, 2000).

Spices and herbs

The antioxidant effect of herbs and spices is due primarily to phenolic -OH groups (Cai et al., 2005). Plants with high levels of phenolic compounds, such as gallic acid (Figure 4), generally have strong H-donating activity and are effective scavengers of H_2O_2 and superoxide radicals (Lugasi et al., 1995). Shan et al. (2005) found that phenolic volatile oils were the principal active ingredients in most spices. The major phenolic constituents are phenolic acids (gallic acid, protocatechuic acid, caffeic acid, rosmarinic acid; Figure 6), phenolic diterpenes (carnosic acid, carnosol; Figure 7), flavonoids (catechin, quercetin, apigenin, kaempferol, naringenin, hesperetin) and volatile oils (eugenol, carvacrol, thymol, menthol). Rosmarinic acid (caffeic acid ester; Figure 8) is the dominant phenol in the six spices in the Labiatae family. Dorman et al. (2003) evaluated herbs belonging to the Lamiaceae family: oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.; Table 2) for their antioxidant properties in terms of $\bullet OH$ radical-scavenging activities and capacity of the extracts to inhibit metal ion-(copper) induced oxidation. The antioxidant characteristics were not completely explained by the total phenolic content of the extracts, but appeared to be strongly dependent on rosmarinic acid. Phenolic acids are effective iron chelators (Andjelkovic et al., 2006). These antioxidative spices, or their components, can be fed to livestock, incorporated into finished products or infused into packaging materials (Formanek et al., 2001; Goldade et al., 1995).

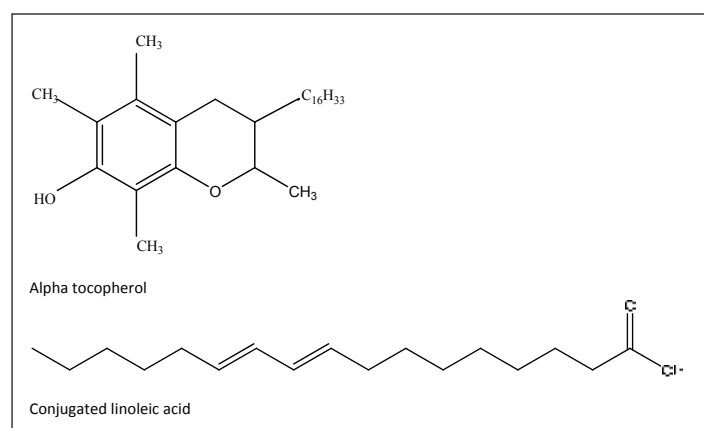


Figure 6. Alpha tocopherol (vitamin E; top) and conjugated linoleic acid (bottom)

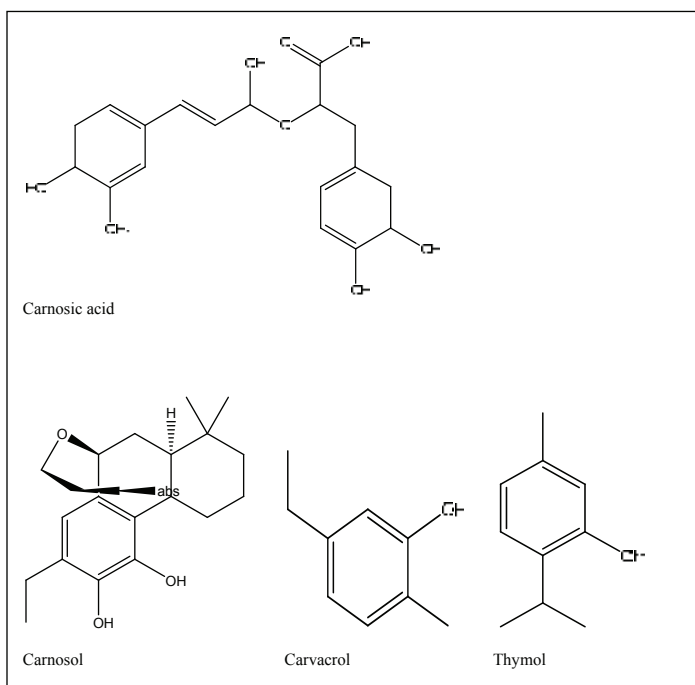


Figure 7. Carnosic acid, carnosol, carvacrol, and thymol

Rosemary

Rosemary (*Rosmarinus officinalis* L.) can inhibit lipid oxidation, chelate metals and scavenge superoxide radicals. The phenolic content of rosemary is ~150 mg/g (Peschel et al., 2007). Nakatani (2003) reported that phenolic diterpenes from rosemary are particularly antioxidative (Figures 7 and 8). The antioxidant activity of carnosic acid is more than twice that of any other phenolic diterpene. It has several times the antioxidative capacity of BHT and BHA but less than TBHQ (Richheimer et al., 1996). In addition, carnosic acid and carnosol chelate iron and scavenge peroxy radicals, especially in lipid-based systems (Arouma et al., 1992).

Based on TBARS values and sensory characteristics, Pizzocaro et al. (1994) demonstrated that ground fresh rosemary leaves (0.3%) and rosemary plus sage (0.3% + 0.3%) could preserve the oxidative quality of beef hamburger in frozen storage for ten months. Ahn et al. (2004) compared natural antioxidant effectiveness in preserving red color of fresh beef. Beef patties containing antioxidants had higher instrumentally measured lightness (L^*) values after nine days of refrigerated storage. Oleoresin rosemary and grapeseed extract resulted in higher, instrumentally-measured redness (a^*) and yellowness (b^*) values than controls and those containing pinebark extract. Balentine et al. (2006) assessed the influence of the time during the processing of ground beef when rosemary was added on the color and oxidation of the product after six days of refrigerated storage. When rosemary was added prior to grinding of trim and cubed beef, ground beef had the highest a^* values and oxymyoglobin content and lowest TBARS. Rosemary

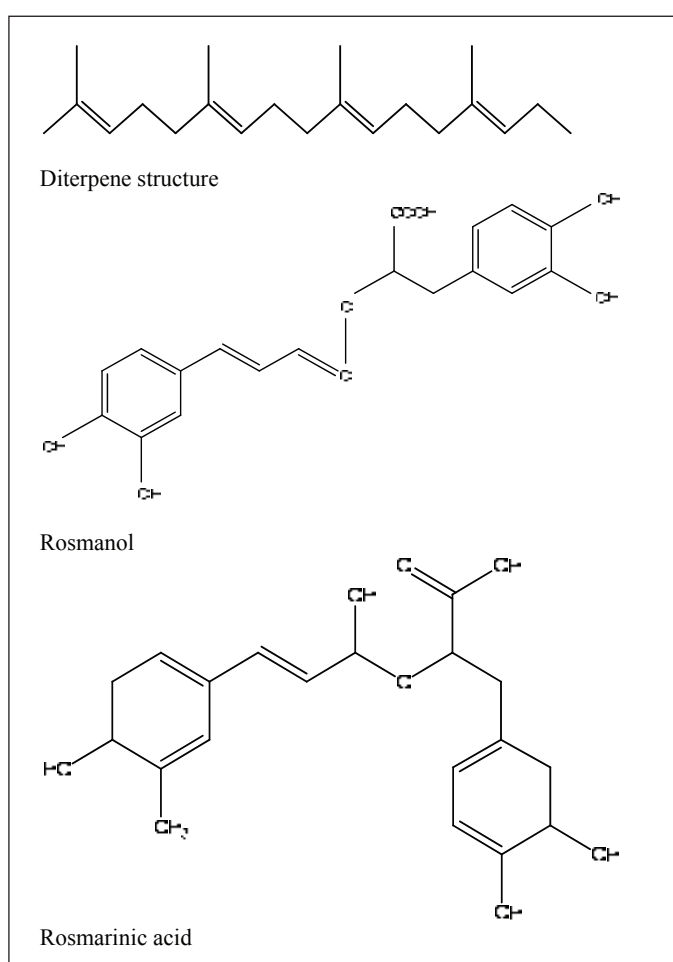


Figure 8. Diterpene structure, rosmanol and rosmarinic acid

(1000 ppm), either alone or with AA (500 ppm), can effectively inhibit both metmyoglobin formation and lipid oxidation in beef patties for 20 days (Sanchez Escalante et al., 2001). Djenane et al. (2003) found that spraying beef steaks with a mixture of rosemary and AA reduced the rates of metmyoglobin formation and lipid oxidation (in the absence of UV radiation) extending display-life from about ten to about 20 days. Thongtan et al. (2005) found that addition of refined rosemary extract to beef patties that were precooked then frozen decreased peroxide values and TBARS values over a 12-week storage period.

In refrigerated beef patties stored in high-oxygen environments, rosemary extracts (0.2%) have antioxidative activities similar to BHA/BHT (0.01%/0.1%; McCarthy et al., 2001). Lund et al. (2007) demonstrated that in high oxygen atmospheres both rosemary extract and ascorbate/citrate can protect fresh beef color; however, ascorbate/citrate is more effective. These antioxidants had no effect on meat stored in a 100% nitrogen environment.

Rosemary essential oil, containing 1,8-cineole, alpha-pinene, camphor, camphene and beta-pinene, has greater antioxidative activity than its individual components (Wang et al., 2008). Rosemary extracts have also been shown to have higher antioxidant activity than the

individual phenolic compounds (carnosic acid, carnosol) separately (Hernandez-Hernandez et al., 2009; Table 2). Under simulated retail-storage conditions, McBride et al. (2007) demonstrated that rosemary extract was more effective than BHA/BHT and vitamin E (through dietary supplementation) in both aerobic and modified atmosphere environments in preserving oxidative and color stability of beef. An optimum concentration of rosemary extract in raw and cooked beef appears to be 0.1% (McBride et al., 2007; McCarthy et al., 2001). Hot-water extracts (10% w/w) from rosemary can reduce the rate of chemically measured lipid oxidation (peroxide oxygen content [POV] and thiobarbituric acid reactive substances [TBARS]), and protect the color (a^* value) and sensory quality of frozen beef patties (Akarpat et al., 2008). Adding rosemary to vitamin E-supplemented beef and pork results in lower TBARS than either compound alone, suggesting a synergistic effect (McBride et al., 2007). However, some of the compounds in rosemary (verbenone, borneol, camphor) can impart an undesirable rosemary odor to foods, even at low concentrations (Carrillo et al., 2006).

Oregano

Evaluating a group of herbs and spices (bay leaves, rosemary, sage, marjoram, oregano, cinnamon, parsley, sweet basil, mint), Muchuweti et al. (2007) reported that oregano (*Origanum vulgare* L.) had the highest antioxidant activity. Oregano extracts contain high concentrations of phenols, primarily rosmarinic acid, which can prevent color deterioration (Hernandez-Hernandez et al., 2009; Table 2). Phenolic carboxylic acids and glycosides are also particularly antioxidative and effectively scavenge superoxide anion radicals (Nakatani, 2003).

Oregano combined with AA can reduce TBARS values and delay surface discoloration (metmyoglobin formation) in ground beef (Sánchez-Escalante et al., 2003). Fasseas et al. (2008) reported that both oregano essential oil (3%) and sage essential oil (3%) significantly reduced oxidation. The effect was much greater in cooked than in raw meat. Camo et al. (2008) compared the effects of direct addition of oregano and rosemary to the use of active films containing oregano and rosemary on the display-life of lamb. Active films containing oregano were significantly more efficient than those with rosemary, extending fresh odor and color from eight to 13 days. Grobbel et al. (2006) found that 0.15% oregano + 0.3% AA was effective at preventing bone discoloration through day two of refrigerated display in polyvinyl chloride (PVC) film in a high-oxygen MAP environment.

Marjoram

Marjoram (*Origanum majorana* L.) essential oil inhibits formation of initial compounds during the oxidation of unsaturated fatty acids (conjugated dienes) by 50% and

the generation of secondary oxidation products (of linoleic acid) by 80% in a model system (Schmidt et al., 2008). Mansour et al. (2006) reported that adding marjoram (as well as rosemary) to a minced-beef system reduced TBARS values during refrigerated storage. Wild marjoram has also been shown effective in refrigerated and chilled pork patties. However, if salt was added and samples were frozen, the effect was lost (El-Alim et al., 1999).

A purified component isolated from marjoram, T3b, which is likely a phenolic substance, is a better superoxide anion radical scavenger than BHT, BHA, alpha-tocopherol, AA and a variety of polyphenolic flavonoids (epigallocatechin gallate, quercetin, epicatechin; Jun et al., 2001). The inhibitory mechanism of T3b appears to depend on the action of an endogenous enzyme (superoxide dismutase) which destroys the superoxide anion by converting it to H_2O_2 (note: the author only refers to it by this name and proposes that it is a phenolic substance).

Sage

Sage (*Salvia officinalis* L.) contains a variety of antioxidative substances including carnosol, rosmanol, roseadiol, epirosmanol, isorosmanol, galdosol and carnosic acid (Nakatani, 2003; Miura et al., 2002; Deans et al., 2000; Cuvelier et al., 1994; Table 2). The antioxidative activity of sage oil correlates with the oxygenated diterpene and sesquiterpene concentration (Papageorgiou et al., 2008). The essential oils of sage can reduce oxidation in beef. However, they appear to play a much more important role in cooked meat than in raw meat (Fasseas, 2008). The ethanolic extract of sage can reduce both peroxide oxygen (POV; chemical measure of oxidation) and TBARS values. Sage added to a cooked beef homogenate inhibited lipid oxidation by 47% during refrigerated storage (Wong et al., 1995). Sage is also a strong inhibitor of oxidation in heat-treated meat products during frozen storage (El-Alim et al., 1999).

In model systems, the polar extracts of the *Salvia* species exhibit excellent antioxidant activities compared to BHT and compared to the non-polar sub-fractions of the extract (Tepe et al., 2006). The activity of substances (miltirone, atuntzensin A, luteolin, 7-O-methyl luteolin, eupafolin) which have antioxidant activity comparable to that of BHT appears to be due mainly to the presence of adjacent -OH groups (Miura et al., 2002).

Thyme

A number of species of thyme (*Thymus vulgaris*, *Thymus caespitius*, *T. camphorates*, *T. mastichina*) have antioxidative activity; all contain 1,8-cineole. The antioxidative activity of *T. caespitius* (250 and 500 mg/l) has been reported to be comparable to that of vitamin E and BHT (Migel et al., 2004). The main aroma compounds

in thyme include 1,8-cineole, alpha-terpineol, linalool, carvacrol and thymol (Lee et al., 2005; Table 2). The aroma detection thresholds of carvacrol, thymol and p-cymene (in oil) are 30, 124 and 794 ppm, respectively (Bitar et al., 2008). The compound p-cymene at 335 mg/kg imparts no flavor taints and reduces oxidation rate ~47%. Of the compounds isolated from thyme, Youdim et al. (2002) reported that the order of antioxidative activity was: thyme oil > thymol > carvacrol > gamma-terpinene > myrcene > linalool > p-cymene > limonene > 1,8-cineole > alpha-pinene.

Thyme essential oil exhibits very strong free-radical-scavenging ability. It inhibits both Fe²⁺/ascorbate- and Fe²⁺/H₂O₂ - induced lipid oxidation (Bozin et al., 2006). In a model system, carvacrol and thymol (5 ppm) inhibited oxidation almost completely for 30 days, with antioxidative activity similar to BHT and alpha-tocopherol (Lee et al., 2005a). Thyme-permeated film effectively reduces cooked beef oxidation during refrigerated storage (Golalde et al., 1995).

Green tea

Green tea has substantial antioxidative activity, much of which appears to be due to natural flavonoids (which contain numerous phenolic groups; Figure 9), tannins and some vitamins (Abdullin et al., 2001; Saito et al., 2007). Antioxidant activity of green tea infusions (*Camellia sinensis*) appears to be linearly related to phenol content (Apak et al., 2006). Catechins, polyphenolic flavonoids (Figure 8; Table 2) in green tea, are particularly

effective free radical scavengers (Kondo et al., 2001; Lien et al., 2008). The primary catechin polyphenol constituent and major peroxy-radical-scavenging compound is (-)-epigallocatechin-3-gallate (Caldwell, 2001; Cabrera et al., 2003; Sato et al., 2007). Guo et al. (1996) and Salah et al. (1995) compared the protective effects of four components of "green tea polyphenols" [epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC)] on prevention of iron-induced lipid peroxidation. The inhibitory effects of these compounds on TBARS values was EGCG > ECG > EGC > EC; the free radical scavenging ability of these compounds was ECG > EGCG > EC > EGC. The ability of these compounds to protect membranes from Fe²⁺/Fe³⁺-initiated lipid oxidation appeared to depend not only on their iron-chelating and free-radical scavenging activities, but also on the stability of their free radical forms.

Tea catechins (0.020%) can delay metmyoglobin formation during display of ground beef under both aerobic and MAP conditions (Tang et al., 2006). However, catechin levels over 0.04% do not. Mitsumoto et al. (2005) reported that adding tea catechins (200 or 400 mg/kg) to minced meat inhibited lipid oxidation in both raw and cooked beef to a greater extent than did AA; however, it resulted in discoloration in cooked beef. Adding 300 mg/kg to minced muscle significantly inhibited the pro-oxidative effect of salt (1%) and controlled lipid oxidation of cooked patties during refrigerated storage (Tang et al., 2001). This concentration is similar to that reported to inhibit oxidation in raw and cooked pork patties (McCarthy et al., 2001). Based on TBARS, tea catechins were more effective than rosemary (0.01%), sage (0.05%) and BHT/BHT (0.01%).

Feeding green tea to livestock has had mixed results. Zembayashi et al. (1999) reported that feeding cattle green tea (0.5 kg/d) reduced the iron content, *a** value and intramuscular lipid content of the muscle tissue. While feeding tea catechins to cattle has not been shown to improve lipid or color stability, direct addition (1000 ppm) can improve color of *Longissimus dorsi* slices held in high O₂ (80%) MAP under refrigerated storage (O'Grady et al., 2006).

Grapeseed extract

Grapeseed extracts are rich in phenolic compounds (catechins, epicatechins, epicatechin-3-O-gallate, phenolic acids, caffeic acid, quercetin, myricetin, proanthocyanidins, resveratrol; Jayaprakasha et al., 2001; St. Angelo et al., 1990; Figure 9; Table 2). The total phenolic content of grapeseed extract has been reported to be ~5 g/100 g; the anthocyanin content ranges from 0.14 to 0.68 g/100 g (Rababah et al., 2008).

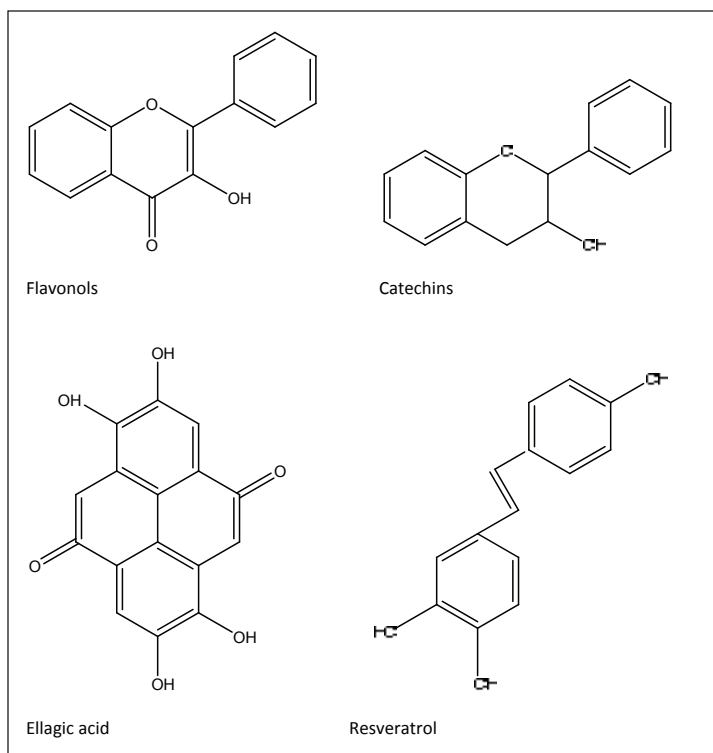


Figure 9. Flavonols, catechins, ellagic acid and resveratrol

The strongest antioxidant and H₂O₂-scavenging activity in all respects is due to phenols with three -OH groups bonded to the aromatic ring adjacent to each other (Sroka et al., 2003). Gallic acid (Figure 5), which has three adjacent -OH substitutions on the aromatic ring, and epigallocatechin, which has three adjacent -OH substitutions on the B ring, have the highest antioxidant activities in the phenolic acid and flavonoid groups, respectively.

In an emulsion system, Hu et al. (2004) found that grapeseed extract (620 µM) inhibited both lipid hydroperoxide and propanal formation. The authors hypothesize that the oligomeric procyanidins are better antioxidants than their monomeric counterparts. In addition, the ability of these antioxidants to concentrate where the oxidative reaction is likely to occur increases their effectiveness.

Sold commercially as a dietary supplement, grapeseed extract can reduce lipid oxidation in raw and cooked meat, and WOF in cooked meat (Ahn et al., 2007; Rojas and Brewer, 2007, 2008). Brannan and Mah (2007) found that in a ground-meat system, grapeseed extract was more effective than gallic acid. Grapeseed extract (0.1% and 1.0%) inhibited formation of hydroperoxides and TBARS. Ahn et al. (2002) reported that grapeseed extract reduced hexanal content and WOF of refrigerated, cooked-minced beef by 97%. It appears to be much more effective in cooked-meat systems than in raw-meat systems (Rojas and Brewer, 2008). The antioxidant activity appears to be concentration-dependent between 0.02 and 0.1% (Ahn et al., 2002; Rojas and Brewer, 2007, 2008; Sasse et al., 2009).

Catechin and epicatechin are generally found in grape seeds, and quercetin, rutin and resveratrol in grape skin extracts (Iacopini et al., 2008). All of these phenols have strong antiradical activity (Pinelo et al., 2004). Resveratrol, largely produced in the grape vine, is a strong antioxidant and a free-radical scavenger (Figure 8). It can retard oxidative processes and maintain color in raw beef patties. Behkit et al. (2003a,b) demonstrated that it was superior to quercetin, rutin and carnosine. In order of antioxidative effectiveness, Murcia and Martinez-Tome (2001) reported that BHA > resveratrol > PG > tripolyphosphate > vanillin > phenol > BHT > alpha-tocopherol. The first three inhibited peroxidation in a concentration-dependent manner. None of the compounds reacted with H₂O₂, and therefore, they would be inefficient in catalyzing subsequent oxidation.

Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a collective term for a group of linoleic acid (18 carbons) isomers with two conjugated double bonds (Figure 5). Hur et al. (2004)

found that adding CLA (0.5%) to ground beef resulted in lower TBARS values and higher oxymyoglobin contents and *a** values during storage. Adding CLA to beef during the grinding process increases CLA content in both raw and cooked ground beef and decreases TBARS values (Cheah et al., 2004). During aerobic storage, it has a greater effect on cooked patties than on raw patties. CLA (1%) added during grinding and prior to irradiation can reduce TBARS values and painty odor of cooked ground beef patties (Cheah et al., 2009).

Realini et al. (2004) reported that total CLA content was higher in beef from pasture-fed than concentrate-fed cattle; however, the meat was not more stable to oxidation. Finishing cattle on pasture enhanced the unsaturated fatty acid profile, including CLA and omega-3 fatty acids, of intramuscular fat. Yang et al. (2000) demonstrated that more than 80% degraded within 110 hours in air at 50°C. Gillis et al. (2007) found that feeding rumen-protected CLA increased the amount of trans fat per serving by 110% and 88% in ribeye steak and ground beef, respectively. It increased CLA cis-9 trans-11 by 58% in ground beef. CLA had minimal effects on lipid oxidation. Feeding CLA to poultry and swine has had some positive results with respect to the fatty acid composition of the raw meat (oleic, linoleic, arachidonic, palmitic, stearic acids) and prevention of oxidation in the cooked meat (TBARS values, hexanal and pentanal concentrations; Du et al., 2002; Lee et al., 2003). However, CLA itself is oxidatively unstable.

SUMMARY

Meat contains a number of unsaturated fatty acids, primarily in the phospholipid fraction in cell membranes, which, by virtue of their double bonds, are prone to oxidize. Oxidation ultimately results in breakdown products which produce off-odors and off-flavors (rancid, warmed-over, cardboard, grassy). This is a particular problem in pre-cooked, frozen, re-heated meat products because heat, added salt and processing can initiate the oxidation process. To produce high-quality, ready-to-heat or ready-to-eat products, the most effective solution is the use of either synthetic or natural antioxidants. Antioxidants such as EDTA and some phosphates can function as chelators to bind metals (iron) that may be present or released during processing (comminution, heating), which initiates oxidation. Antioxidants can also serve as oxidation “chain breakers” by intercepting the free radicals generated from the oxidizing fat at various stages in the process. Antioxidants in the latter category are generally the most effective. Whether synthetic or natural, their common feature is that they have one or more aromatic rings (often phenolic) with one or more -OH groups capable of donating an H• to stop the oxidation process. The synthetic antioxidants BHA, BHT, TBHQ,

and PG have one aromatic ring. The natural antioxidants are derived from plants and include ascorbic acid (vitamin C) and alpha tocopherol (vitamin E; which each have one aromatic ring), catechins, ellagic acid and resveretrol (which each have two aromatic rings), and flavonols (which have three aromatic rings). In general, the effectiveness of these natural antioxidants is proportional to the number

of –OH groups present on the aromatic rings. If their solubility and sensory characteristics are compatible with a particular meat system, the fact that they are natural and have antioxidative activity that is as good as or better than the synthetics makes them particularly attractive for commercial meat processors.

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