

Beef Color Update: The Role for Vitamin E¹

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ABSTRACT: Dietary supplementation of livestock with vitamin E results in improved quality of meat subsequently obtained from these animals. The effect is especially noteworthy in cattle, in which the primary effects are delayed discoloration and lipid oxidation. A threshold level of α -tocopherol in muscle ensures a detectable effect; dietary strategies for attaining this threshold must consider tocopherol status of cattle arriving at the feedyard and duration and level of supplementation. The α -tocopherol concentration in muscle must be determined before proper interpretation of experimental results can be made. Muscles vary in their color stability, and this relative difference is not changed by vitamin E

supplementation. Several in vitro models have been used to characterize the interaction between α -tocopherol, lipid oxidation, and oxymyoglobin oxidation. α -Tocopherol seems to exert its color-stabilizing effect by indirectly delaying oxymyoglobin oxidation via direct inhibition of lipid oxidation. However, recent results demonstrating a protective effect of α -tocopherol toward oxymyoglobin in low-oxygen atmospheres indicate that additional mechanisms may exist. A better understanding of the fundamental bases for protection of water-soluble myoglobin by lipid-soluble α -tocopherol is needed to optimize this beneficial effect.

Key Words: Vitamin E, Tocopherols, Oxidation, Meat Quality

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Introduction

Vitamin E is a generic term that includes all entities that exhibit the biological activity of α -tocopherol. The most biopotent vitamin E compound is d- α -tocopherol (Pryor, 1996). The acetate ester of chemically synthesized, or all-racemic, α -tocopherol is the common supplemental form of vitamin E in ruminant diets. Naturally occurring d- α -tocopherol, which is found in plant oils, and all-*rac* α -tocopherol, arising from de-esterification by presumably pancreatic esterases, are absorbed from the small intestine and distributed via chylomicrons and lipoproteins

to tissues. α -Tocopherol is located within cell membranes and seems to function as the principal chain-breaking antioxidant in biological systems (Burton et al., 1983).

The protective effect of α -tocopherol against lipid oxidation in biological systems and food has been known for some time. Early investigators reported that dietary supplementation of dairy cattle with vitamin E yielded an antioxidant effect in milk (Krukovsky et al., 1949; DeLuca et al., 1957). Muscle-based food products also benefit from higher levels of α -tocopherol (Webb et al., 1972; Astrup, 1973; Marusich et al., 1975; Tsai et al., 1978).

More recently, the color-stabilizing effect of α -tocopherol within meat has renewed interest in dietary strategies for improving overall quality of beef and other meat products (Faustman, 1993; Buckley et al., 1995; Liu et al., 1995; Schaefer et al., 1995a,b, 1996). The purpose of this review is to summarize recent progress in understanding the effects of dietary vitamin E on beef quality and to highlight issues raised by this research.

Vitamin E and Meat Quality

The concentration of α -tocopherol within membranes is tissue- and organ-dependent. In general,

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Table 1. Effect of dietary supplementation of vitamin E on meat quality in meat-producing livestock

Indication of meat quality and type of meat	Fresh ^a	Cooked ^a	Frozen ^a	Reference ^b
Lipid stability				
Beef	+	+	+	1,2,3,4
Lamb	+	NA	+	5,6,7
Pork	+	+	+	8,9,10,11
Veal	+	+	+	12,13
Poultry	+	+	+	14,15,16
Color stability				
Beef	+	NA	+	1, 17-19
Lamb	+	NA	+	6,7
Pork	+	NA	+	20,21
Veal	NA	NA	NA	—
Poultry	+	NA	NA	22
Moisture retention				
Beef	+	NA	NA	23
Pork	+	+	+	20,24

^a+ indicates a positive/desirable effect; NA = not available.

^b1, Faustman et al. (1989); 2, Liu et al. (1994); 3, Lanari et al. (1994); 4, Liu et al. (1996b); 5, Strohecker et al. (1997); 6, Wulf et al. (1995); 7, Guidera et al. (1995); 8, Buckley and Connolly (1980); 9, Cannon et al. (1996); 10, Monahan et al. (1990); 11, Monahan et al. (1992); 12, Igene et al. (1976); 13, Engesteth et al. (1993); 14, Marusich et al. (1975); 15, Sheehy et al. (1993); 16, Lin et al. (1989); 17, Arnold et al. (1993a); 18, Lanari et al. (1993); 19, Sherbeck et al. (1995); 20, Asghar et al. (1991); 21, Monahan et al. (1994); 22, Sante and Lacourt (1994); 23, Mitsumoto et al. (1995); 24, Cheah et al. (1995).

liver and adrenal glands contain the greatest concentrations of α -tocopherol, and muscle and adipose contain the least (Arnold et al., 1992). It is important to note that the additional α -tocopherol obtained through vitamin E-supplemented diets is distributed among subcellular fractions in the same proportions as in the muscle of unsupplemented animals (Asghar et al., 1991; Arnold et al., 1993a).

Meat quality is judged by a variety of variables. To date, studies concerned with vitamin E supplementation to improve meat quality have focused on lipid oxidation, color stability, and moisture retention (Table 1). The lipid profile of meat and rate of accumulation of oxidation products can significantly influence meat flavor. Precooked and minced products are especially susceptible to the effects of lipid oxidation and development of off-flavors. The bright red appearance of beef is due to oxymyoglobin, a ferrous heme pigment that oxidizes to brown metmyoglobin. The rate of oxymyoglobin oxidation is dependent on a variety of factors (Faustman and Cassens, 1990a; Renner, 1990). Moisture retention can impact palatability of the cooked product and may also influence product weight and thus economic return.

The effect of dietary vitamin E supplementation on color stability of three beef muscles is presented in Figure 1. Color and lipid stability of fresh beef longissimus muscle is improved with increased tissue

concentrations of α -tocopherol to a threshold of ca. 3.0 to 3.3 μg α -tocopherol/g tissue (Faustman et al., 1989; Arnold et al., 1993a). Concentrations of α -tocopherol as high as 10 μg α -tocopherol/g of psoas major have been achieved with dietary supplementation (Chan et al., 1996). Because muscles accumulate α -tocopherol to different extents, the threshold concentration varies among muscles (Liu et al., 1996a). Liu et al. (1995) recently reported that supplementation of ca. 500 mg α -tocopheryl acetate per animal daily for 126 d was sufficient to obtain the color-stabilizing effect of vitamin E for beef aged 14 d. When beef is to be aged for 56 d (e.g., export), ca. 2,000 mg of α -tocopheryl acetate per animal per day for 126 d is recommended (Liu et al., 1996a). The loss of α -tocopherol from tissue following removal of supplemental vitamin E is slow, requiring 140 to 180 d for depletion to reach equilibrium with dietary intake (Arnold et al., 1993a).

Dietary delivery of α -tocopherol via supplementation with α -tocopheryl acetate seems to be the most efficacious means for obtaining the color-stabilizing effect; Mitsumoto et al. (1993) provided excellent support for this concept. These investigators compared the color stability of beef obtained from vitamin E-supplemented cattle with that of beef from unsupplemented cattle; sufficient exogenous vitamin E was added to control beef to ensure the same total level of α -tocopherol in meat from both treatments. Beef from supplemented cattle had superior color stability; the authors concluded that proper physiological placement of vitamin E was as critical as its concentration for providing an optimal color-stabilizing effect.



Figure 1. Color of beef from a control and a vitamin E-supplemented (E-2,000) steer. α -Tocopherol concentrations for control and E-2,000 psoas (top row), longissimus (middle row), and gluteus (bottom row) were 1.2 and 9.0 $\mu\text{g}/\text{g}$ tissue; .9 and 8.8 $\mu\text{g}/\text{g}$ tissue; and 1.2 and 9.5 $\mu\text{g}/\text{g}$ tissue, respectively.

In studies concerned with vitamin E supplementation of cattle for the purpose of obtaining beef with improved quality, it is absolutely critical to determine the concentration of α -tocopherol in the muscle. The concentration will be a consequence of the level of α -tocopheryl acetate actually received by the cattle and the duration for which the supplemented diet was fed. The extent to which an effect of vitamin E supplementation may be noted will depend, in part, on the α -tocopherol status of cattle upon initiation of supplementation. Plasma α -tocopherol concentration can be used as an indicator of dietary vitamin E intake, but it is not an accurate indicator of muscle α -tocopherol concentration unless dietary vitamin E intake is known to have been constant for the previous 84 to 126 d (Arnold et al., 1993a). This is the duration required for muscle α -tocopherol concentration to equilibrate with dietary vitamin E intake. Biopsy samples can be used to evaluate muscle α -tocopherol status (Arnold et al., 1993a).

It is important to note that if a given nutritional program delivers sufficient vitamin E to obtain the threshold level in muscle, then additional supplementation is unnecessary. Hill and Williams (1993) have reported little or no benefit for fresh beef color stability from vitamin E supplementation. Their experiments have typically been conducted with yearling cattle that had access to grass pasture immediately before the vitamin E experiment. Plasma α -tocopherol concentration was 5 to 7 $\mu\text{g/mL}$ at the time cattle were removed from pasture. Because α -tocopherol depletion from muscle is slow, even the unsupplemented cattle possessed longissimus α -tocopherol concentrations in excess of 3.0 μg α -tocopherol/g tissue (G.M. Hill, unpublished data).

The scenario of grazing before confinement finishing for cattle is common in the industry. Green forage is a good dietary source of α -tocopherol. When pasture quality allows for high levels of α -tocopherol consumption, α -tocopherol concentration in muscle may not be an accurate indicator of oxidative stability of the meat. Larick and Turner (1989) reported that diet influences the polyunsaturated fatty acid composition of phospholipids in pectoralis muscle. Access to rye and ryegrass pasture vs corn/corn silage or wheat/corn silage diets resulted in increased concentrations of C18:2, C18:3, C20:3, C20:4, and C22:5 fatty acids in the phospholipid fraction of this muscle. Apparently, biohydrogenation by the ruminal microflora is not complete for some dietary lipid sources. This information poses the situation in which muscle from cattle that grazed green forage could contain a high α -tocopherol concentration but the significance of this antioxidant could be compromised by a phospholipid fraction that is more susceptible to oxidation. It would be interesting to learn the rates at which the fatty acid profile of muscle lipid fractions change during the finishing phase when the muscle of grazed cattle

comes into equilibrium with the fatty acid profile of a high-grain diet. Comparison of the equilibration rates of the lipid fractions with the equilibration rate for α -tocopherol would address the reliability of muscle α -tocopherol as the sole, or major, indicator of oxidative stability in beef.

The Relative Color Stability of Different Muscles is Not Changed by α -Tocopherol

Meat cuts from a given carcass are characterized by differences in several sensory characteristics. These are a reflection of the compositional and metabolic differences that exist in different muscles. Several investigators have reported muscle-dependent differences in meat color stability (O'Keefe and Hood, 1980–81, 1982; Ledward, 1985; Genot et al., 1991; Anton et al., 1993); the biochemical bases for these differences seem related to pro-oxidative and pro-reductive forces present in postmortem muscle (Faustman and Cassens, 1990a). The effect of α -tocopherol on meat color stability of three muscles known to discolor at markedly different rates was recently investigated by Chan et al. (1996). Their investigation built on the results of O'Keefe and Hood (1982), who demonstrated that color stability of longissimus > gluteus medius > psoas. Chan et al. (1996) reported that this same relationship existed in beef from control or vitamin E-supplemented cattle. Within a given muscle type, meat cuts from cattle on the vitamin E treatment were superior in color stability to meat cuts from controls. Interestingly, the α -tocopherol concentration of the three muscles followed the order psoas > gluteus medius > longissimus lumborum. A similar inverse relationship between color stability and α -tocopherol concentration of different muscles within a carcass was reported by Liu et al. (1996a,b). Thus, factors other than α -tocopherol concentration seem primarily responsible for muscle-dependent color differences.

Chan et al. (1996a) also used a microsome:myoglobin model system to further investigate muscle-dependent differences in color stability. The microsomal fraction of postmortem skeletal muscle contains a relatively high concentration of α -tocopherol (Asghar et al., 1991; Arnold et al., 1993a). The concentration of α -tocopherol in microsomes increases to the same degree as that in other subcellular fractions obtained from muscle of vitamin E-supplemented pigs (Asghar et al., 1991) and cattle (Arnold et al., 1993a). Chan et al. (1996) isolated microsomes from three muscles for use in the microsome:myoglobin model. They found that muscle microsomes from vitamin E-supplemented cattle demonstrated less lipid oxidation than those from controls; oxymyoglobin oxidation was also less in treatment microsome preparations than in controls. There were no differences in fatty acid composition among muscles within a treatment, and there was no effect of vitamin

E supplementation on microsomal fatty acid composition. In addition, there were no differences in oxymyoglobin stability for the three muscle microsome preparations within a treatment. Thus, differences in color stability among muscles do not seem related to oxidative stability of microsomal fractions.

Potential Mechanisms by Which Vitamin E Maintains Oxymyoglobin in Meat

The mechanism whereby lipid-soluble α -tocopherol protects oxymyoglobin, a cytosolic protein, is not understood. α -Tocopherol resides in the phospholipid membranes, where it functions as a radical-quenching antioxidant to delay oxidative damage to membrane constituents. The process of lipid oxidation affects flavor in foods but can also result in production of potentially toxic oxidation products (Kubow, 1992). α -Tocopherol has been demonstrated to reduce the incidence of cholesterol oxidation products in cooked pork (Monahan et al., 1992).

The improved stability of oxymyoglobin with elevated concentrations of α -tocopherol seems to demonstrate a link between lipid oxidation and oxymyoglobin oxidation. Greene (1969) was one of the first investigators to show a correlation between these two degradative processes in meat. Meat is a complex food, and investigators have used model approaches to characterize the relationship between lipid oxidation and heme protein oxidation (Chan et al., 1997).

LaBrake and Fung (1992) used liposome models for studying the kinetics of oxyhemoglobin oxidation in the presence of phospholipid. The rate of oxyhemoglobin oxidation was higher in the presence of liposomes. Butylated hydroxytoluene, a lipid-soluble phenolic antioxidant, did not affect the oxidation of oxyhemoglobin; lipid oxidation was not measured in the oxyheme:lipid system. Yin and Faustman (1993) used an oxymyoglobin:liposome model and reported that oxidation of heme protein and lipid was enhanced with increased fatty acid unsaturation. The oxidation of phospholipid and oxymyoglobin were delayed by the addition of α -tocopherol to multilamellar phospholipid vesicles (Yin et al., 1993). Interestingly, α -tocopherol was more effective than ascorbyl palmitate at inhibiting oxymyoglobin oxidation in a liposome system (Havens et al., 1995). Havens et al. have also found the relative antioxidant effectiveness of different tocopherols toward lipid oxidation to be $\alpha = \gamma > \delta$ ($P < .05$) at a total tocopherol concentration of 1.4 μM (unpublished data).

Microsomes have also been used to study heme protein:lipid oxidation interactions (Lin and Hultin, 1977; Anton et al., 1991; Yin and Faustman, 1994; Chan et al., 1996). Microsomes obtained from control and vitamin E-supplemented livestock have provided a means to study oxymyoglobin stability in the presence of tissue membrane lipids. Oxymyoglobin stability was improved with increased concentrations

of α -tocopherol in liver (Yin and Faustman, 1994) and muscle (Chan et al., 1996) microsomes.

Schaefer et al. (1995a) recently proposed a model of oxidation-reduction relationships in beef to explain the stabilization of oxymyoglobin by α -tocopherol. The general hypothesis is that products of lipid oxidation are more water-soluble than their parent compounds and can enter the cytoplasm, where they interact with oxymyoglobin to hasten its oxidation. Several investigators have recently reported the pro-oxidative effect of several specific products of lipid oxidation (Agerbo et al., 1992; Vaughn and Gardner, 1993; Windsor et al., 1993; Blasig et al., 1995). Thus, it is proposed that α -tocopherol maintains oxymyoglobin indirectly via its direct inhibition of lipid oxidation.

Interaction of Vitamin E and Partial Oxygen Pressure on Fresh Meat Color Stability

The partial pressure of oxygen in a packaging atmosphere affects the form of myoglobin that predominates in meat (Faustman and Cassens, 1990a). Low oxygen partial pressures (4 to 10 mm Hg) favor oxidation of oxymyoglobin, and high $p\text{O}_2$ stabilizes oxymyoglobin (George and Stratmann, 1952; Ledward, 1970). High $p\text{O}_2$ atmospheres have been used to extend fresh meat color shelf-life (Daun et al., 1971; Taylor and MacDougall, 1973; Ordonez and Ledward, 1977; Lopez-Lorenzo et al., 1980). The color shelf-life of fresh meat decreases with increased length of anoxic storage of meat before retail display (O'Keefe and Hood, 1980–81). Elevated concentrations of α -tocopherol can help to extend retail shelf-life for meat cuts obtained from primals stored for extended time periods (Liu et al., 1996a). In cases in which an anoxic atmosphere is desired, the pro-oxidative activity of low $p\text{O}_2$ toward oxymyoglobin has necessitated the use of strategies appropriate for ensuring complete oxygen removal. Recently, oxygen-scavenging sachets have been used to accomplish this (Sante et al., 1994; Allen et al., 1996). For meat stored in anoxic conditions, the subsequent color shelf-life of fresh meat products stored aerobically was greater for meat exposed to oxygen-scavengers than for controls.

Ledward (1972) used the relationship between low $p\text{O}_2$ and myoglobin oxidation to demonstrate the ability of meat cuts to reduce metmyoglobin aerobically. The approach requires that fresh meat cuts be packaged in 1% oxygen atmospheres for a time period sufficient to cause metmyoglobin formation (24 to 48 h). Following this pro-oxidative treatment, meat cuts are removed from the packaging environments, the metmyoglobin percentage is determined, and then the meat is stored aerobically for 24 h (Ledward, 1972). At the completion of aerobic storage, the metmyoglobin percentage is again determined. The extent of metmyoglobin reduction is then calculated as a function of the observed decrease in metmyoglobin

concentration relative to the initial metmyoglobin concentration (i.e., after storage in 1% O₂). The method has been used by other investigators (O'Keeffe and Hood, 1982; Faustman and Cassens, 1990b) although the relationship of aerobic reducing ability to enzymatic reduction (metmyoglobin reductase activity) is not clear.

Schaefer et al. (unpublished results) have studied the effect of low pO₂ (i.e., 1% O₂) atmospheres on the stability of oxymyoglobin in fresh beef from control and vitamin E-supplemented cattle (Figure 2). This experimentation was motivated by the desire to develop a rapid method for estimating the α -tocopherol concentration of a meat sample, preferably useful at a very early postmortem date. In the first experiment, longissimus muscle was obtained from carcasses of cattle fed 0, 250, 500, or 2,000 IU α -tocopheryl acetate/animal daily for 126 d (n = 3/dose, heaviest weight block; Liu et al., 1996b) and stored for 10 d postmortem. Vitamin E consumption for the 0, 250, 500, and 2,000 IU dosages was 64, 295, 550, and 2,173 IU/d, respectively. Following storage, the muscle was minced through 9.5- and 3.2-mm plates, formed into triplicate 25-g patties, and subjected to 1% O₂ or air atmospheres for up to 2 h. Metmyoglobin was determined by the method of Stewart et al. (1965). Low pO₂ accelerated metmyoglobin formation compared to air ($P < .001$, data not shown), and this acceleration was delayed by supplemental vitamin E (see Liu et al., 1996b, for longissimus α -tocopherol concentrations). Meat from vitamin E-supplemented cattle at 10 d postmortem demonstrated less metmyoglobin formation after only 1 and 2 h of exposure to 1% O₂ than did control meat ($P < .01$); however, vitamin E treatments were not different ($P > .02$) in their effects (Figure 2b). Similar effects of 1% O₂ and vitamin E supplementation were noted for beef stored only 2 d postmortem (Figure 2a). The diminished sensitivity of this technique to vitamin E supplementation for 2-d (28.7% for E-0 vs 20.8% metmyoglobin for E-500) compared to 10-d beef (31% for E-0 vs 13% metmyoglobin for E-500) and the technique's inability to discriminate among levels of vitamin E supplementation discouraged further use of this approach as a method for estimating α -tocopherol concentration.

In subsequent work, Chan et al. (unpublished data) examined the pO₂ effect in beef slices from control and vitamin E-supplemented cattle. Beef from vitamin E-supplemented cattle demonstrated a significantly reduced susceptibility to low pO₂-induced oxymyoglobin oxidation relative to beef from controls. It is not clear how vitamin E could protect oxymyoglobin against oxidation under low pO₂ conditions that would not be expected to be pro-oxidative toward lipid (i.e., relative to atmospheric pO₂). However, lipid oxidation was not measured in these slices. Thus, in follow-up work we examined metmyoglobin formation and lipid oxidation in longissimus slices during 30

h of storage in 1% O₂. The results from two trials are presented in Figure 3. In brief, Holstein steers (n = 3 per treatment per trial) were nominally supplemented with a target dose of 0, 500, or 2,000 mg α -tocopheryl acetate/steer⁻¹·d⁻¹ for 125 d. This resulted in muscle α -tocopherol concentrations of 1.03, 3.76, and 7.39 μ g α -tocopherol/g tissue, respectively. Strip loins were removed at 24 h postmortem, vacuum-packed shipped to the University of Connecticut Meat Laboratory, and stored at 4°C. At 14 d postmortem, meat cores were prepared from longissimus slices (1 cm) and wrapped

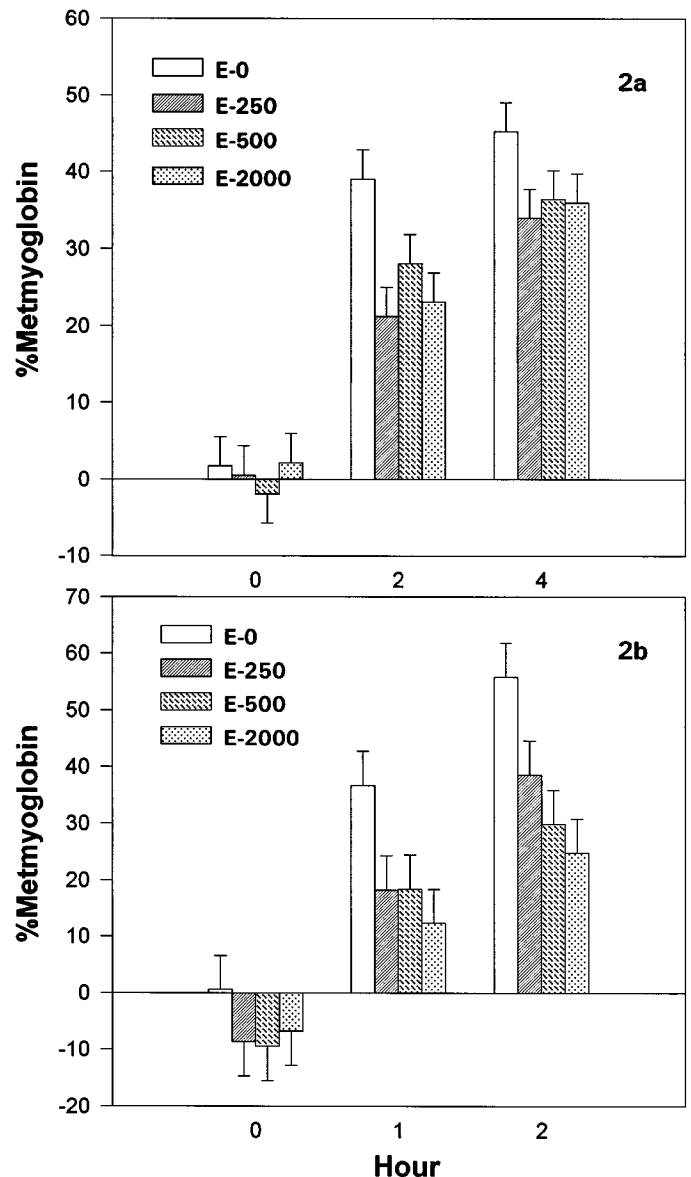


Figure 2. Metmyoglobin formation in minced beef longissimus obtained at 2 d (1a) and 10 d (1b) postmortem and subsequently stored in 1% O₂ for a 2- or 4-hr period. Beef was obtained from cattle supplemented with 0 (E-0), 250 (E-250), 500 (E-500), or 2,000 (E-2,000) IU α -tocopheryl acetate/steer⁻¹·d⁻¹ for 126 d. Standard error bars are indicated.

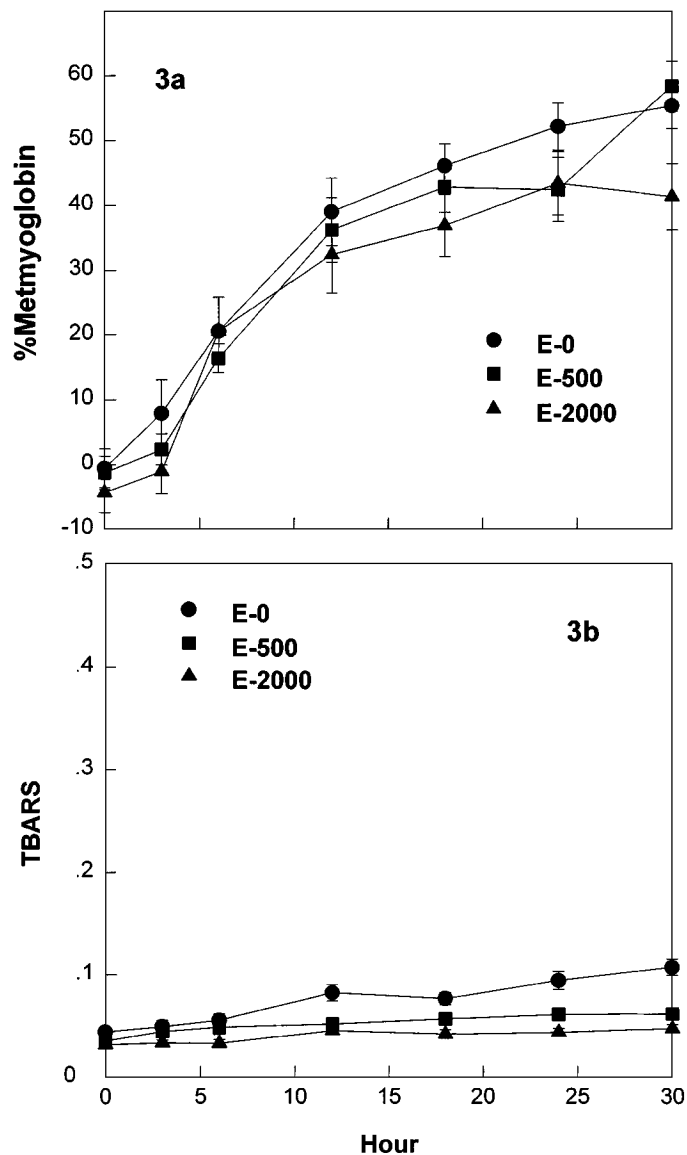


Figure 3. Metmyoglobin (2a) and thiobarbituric acid reactive substances (TBARS) formation (A_{532}) (2b) in beef longissimus (14 d postmortem) slices stored in 1% O_2 for 0 to 30 h. Beef was obtained from cattle supplemented with 0 (E-0), 500 (E-500), or 2,000 (E-2,000) IU α -tocopheryl acetate-animal⁻¹·d⁻¹ for 125 d. Standard error bars are indicated.

as previously described (Chan et al., 1995). Cores were then placed in plastic weigh boats, inserted into oxygen-impermeable pouches, flushed with a gas mixture of 1% O_2 : 99% N_2 , and sealed (Faustman and Cassens, 1990b). The modified atmosphere packages were stored in darkness at 4°C. At 0, 3, 6, 12, 18, 24, and 30 h, duplicate beef cores from each treatment were removed from the packages and analyzed immediately for surface metmyoglobin (Stewart et al., 1965) using a Shimadzu UV-Vis 2100 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD) equipped with an integrating sphere for diffuse reflectance. Following metmyoglobin determination,

beef cores were analyzed for lipid oxidation using a thiobarbituric acid reactive substances (TBARS) assay (Schmedes and Holmer, 1989).

Metmyoglobin formation increased with storage in 1% O_2 during a 30-hr incubation (Figure 3a). Overall, metmyoglobin formation was lower in muscle obtained from E-2,000 cattle than in that from controls ($P < .05$); there was no difference between E-0 and E-500 treatments, or E-500 and E-2,000 treatments, for metmyoglobin formation in 1% O_2 ($P > .05$). Lipid oxidation products expressed as TBARS were detectable for meat slices stored in 1% O_2 and followed the order E-0 > E-500 > E-2,000 ($P < .05$). These results are comparable to those of several other studies in which metmyoglobin formation and lipid oxidation increased in meat slices during storage and an antioxidant effect of vitamin E toward both oxidative processes occurred. However, it is important to note that metmyoglobin formation was greatly accelerated in the 1% O_2 atmosphere relative to normal aerobic storage, whereas lipid oxidation in the slices was lower than that expected for slices displayed under normal aerobic storage (Arnold et al., 1992, 1993b). Even though the rate of TBARS formation needed to increase the rate of metmyoglobin formation by a fixed amount is not known, the link between lipid oxidation and oxymyoglobin oxidation may not be as strong as previously thought.

Implications

Vitamin E supplementation of beef cattle diets is an effective procedure for enhancing the lipid and color stability of meat products subsequently obtained from these animals. This effect has been reported by several investigators and seems consistent with the antioxidant function of α -tocopherol. The exact mechanism by which lipid-soluble α -tocopherol maintains myoglobin in the oxygenated form in fresh beef is unknown. Future research should be directed toward understanding the bases for this protection against oxidative damage so that additional strategies may be developed for enhancing fresh meat shelf-life.

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