Effects of Vitamin E Supplementation on Lipid Peroxidation and Color Retention of Salted Calf Muscle from a Diet Rich in Polyunsaturated Fatty Acids

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The color of fresh meat is one of the most important quality criteria of raw muscle foods. This red color is principally due to the presence of oxymyoglobin. The present study was undertaken to examine the effect of a diet rich in polyunsaturated fatty acids (PUFA), the addition of NaCl, and the influence of dietary supplementation with vitamin E on calf muscle oxymyoglobin oxidation (color) and lipid peroxidation. Vitamin E was added to the feed at a concentration of 4000 mg/day for 90 days before slaughter. This diet increased the α-tocopherol concentration in muscle membrane from 2.6−2.8 to 6.5−7.0 μg/g of fresh weight. It was found that the diet rich in PUFA and, especially, the addition of NaCl increased muscle lipid peroxidation and oxymyoglobin oxidation as indicated by the contents of thiobarbituric acid-reactive substances and substances that impaired color value readings during storage at 4 °C. Both undesirable reactions during storage were controlled very efficiently by the presence of a critically high concentration of α-tocopherol in the muscle tissues. The findings concerning the antioxidant activity of α-tocopherol in this study form additional evidence of its efficient protection against oxidative reactions during storage of muscle tissues and its potential to maintain a high nutritional value in them.

Keywords: *Vitamin E; PUFA; lipid oxidation; oxymyoglobin; NaCl*

INTRODUCTION

The color of fresh meat is perhaps the most important characteristic by which consumers judge the freshness and quality of raw muscle foods. The red color of muscle is principally due to the presence of oxymyoglobin. The redox state of the heme iron, and the presence or nature of the ligand bond to myoglobin, account for the color of muscle foods. Oxidation of heme iron from the ferrous state in oxymyoglobin (Mb-Fe^{2+}-O_2) or deoxymyoglobin (Mb-Fe^{2+}) to the ferric state, metmyoglobin (Mb-Fe^{3+}), results in the undesirable brownish color (see ref *30*).

Dietary fat supplementation to animal foods and the tendency of the species to accumulate fatty acids in the membrane phospholipids affect the lipid composition of the membrane and its susceptibility to peroxidation. Increasing the degree of unsaturation of muscle membrane lipids reduces the oxidative stability of the muscle (*1*−*7*).

Polyunsaturated fatty acids (PUFAs) present in membranes of muscle cells are particularly susceptible to peroxidation during storage at low temperatures (*5*). Several authors have postulated that oxymyoglobin oxidation and lipid peroxidation are interrelated (*8*−*13*).

We have recently used a model system that includes sarcosomes, Mb-Fe^{2+}-O_2, and an iron redox cycle-catalyzed membrane lipid peroxidation to demonstrate that oxymyoglobin oxidation occurs by two possible pathways: (a) autoxidation and oxidation by superoxide ($O_2^{\bullet -}$), H_2O_2, and hydroxyl radicals, ($HO^{\bullet}$); and (b) lipid hydroperoxides and lipid oxyradicals (*14*, *15*). It was found possible to reduce oxidation by almost 50% through addition of catalase and superoxide dismutase (SOD) and to prevent most of the remaining oxidation by addition of catechin, a well-known antioxidant, which acts as a scavenger of free radicals (*5*, *15*). Addition of vitamin E to the feed increased α-tocopherol concentration in sarcosomes and thus significantly increased the stability of the lipids and Mb-Fe^{2+}-O_2 against oxidation (*15*).

Vitamin E has been found to improve the quality of farm animal products. Feeding with vitamin E-supplemented diets reduced lipid peroxidation in turkey muscle (*16*−*19*), in chicken meat (*18*, *20*−*22*), in pork (*23*), in fish (*24*, *25*), and in beef (*26*, *27*). Increased tissue α-tocopherol was found to prevent not only muscle lipid peroxidation but also oxymyoglobin oxidation (*28*−*30*).

Sodium chloride, which is an important additive in the meat industry and is used in kosher meat processing, has been reported to act as a pro-oxidant (*5*). This effect of NaCl seems to be attributed in part to the capability of the ions to displace iron ions from binding macromolecules (*31*) or to increase iron solubility (*32*). It is well-known that Cl^- ions also increase the autoxidation of oxymyoglobin (*33*).

The aim of the present study was to examine the effects of vitamin E supplementation and high PUFA

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Table 1. Composition of the Basal Calf Diets[a] (Grams per Kilogram Dry Matter)

ingredient	control	linseed
barely grains	123	114
corn grains	504	464
linseeds		81
soybean meal	33	30
vetch hay	62	54
wheat silage	183	174
cotton gin trash	62	54
urea	9	8
minerals and vitamins	24	21
calculated composition (units/kg or kg/DM		
ME, Mcal	2.70	2.83
crude protein, g	137	147

[a] Premix of vitamins, microelements, and monensin (Vitopar 2000, Kofolk Ltd., Tel Aviv, Israel). One kilogram of dietary DM provided the following: vitamin A, 6000 IU; vitamin D3, 1200 IU; vitamin E, 30 IU; monensin, 22 mg; NaCl, 5.5 g; Ca, 8 g; P, 4 g; Mn, 18 mg; Fe, 18 mg; Cu, 7 mg; I, 5 mg; Co, 0.12 mg; Se 0.18 mg.

content in the feed on calf muscle lipid peroxidation and fresh muscle color retention, following NaCl addition and storage at 4 °C.

MATERIALS AND METHODS

Twenty-four head of Fresian calves were raised at the Neve Ya'ar Experimental Station of the ARO (Agricultural Research Organization). For 90 days prior to slaughter, the cattle received four different diets: feed containing linseed (PUFA); basal diet without linseed; and each of these with added vitamin E, at 4 g/day in a Kopeluk barley premix. Table 1 shows the composition of the basal and linseed diets. Blood was drawn monthly from the animals and centrifuged for 20 min at 2000 rpm; the pellet was frozen and freeze-dried for tocopherol analysis.

Following slaughter at the age of 14 months, the forequarters of the carcasses were placed in a well-ventilated 0 °C cold storage unit for 24 h. For the experiments, a ~2-kg section of the longissimus dorsi (LD) muscle was removed from the distal end of the forequarter of an animal from each feeding treatment. All fat was trimmed away, and the muscle was cut into parallelepipeds. A parallelepiped from each feed treatment was then cut into cubes, one cube serving as a control and one being kosher-salted according to ritual practice.

The kosher-salting process consisted of soaking the LD cubes in tap water for 30 min at room temperature (23 °C) and then salting heavily in coarse salt, sold specifically for this purpose and leaving them salted, on a drain board, for 1 h, at room temperature. The salt was then shaken off each cube, and they were rinsed in two changes of cold tap water and allowed to drain for ~5 min. The control cubes were kept in plastic bags in a cold room at 2 °C.

Following draining 1-cm-thick slices were cut from the six surfaces of each cube, under a laminar flow hood and with sterilized utensils. The slices were then placed aseptically, face up, into Petri dishes, which were closed and removed to a cold room at 2 °C. The color of the samples was recorded, and color changes during storage were monitored at regular intervals.

Vitamin E Content. A simple method was developed for the extraction of tocopherols from muscle tissues; it showed higher extraction efficiency than the method of Burton et al. (*34*). A 5-g sample of muscle tissue was cut thinly and frozen at −70 °C. The tissue was freeze-dried and ground in a Waring blender (100-mL cup). α-Tocopherol from the muscle powder (500 mg) was extracted with 4 mL of ethanol containing 1% of BHT, in a Teflon homogenizer and a vortex mixer. The homogenate was centrifuged for 15 min at 5000*g*. The supernatant was filtered through a 0.2-μm membrane. A 20 μM sample was injected into an HPLC (LKB Broma) and separated with a Merck Lichrocart column RP-18 125-4-micromolar, eluted by an isocratic mobile phase of methanol (flow rate = 1 mL/min), and detected with an HPLC spectrofluorometer detector (Jesco FP 210). The eluent was excited at 290 nm and emitted at 329 nm.

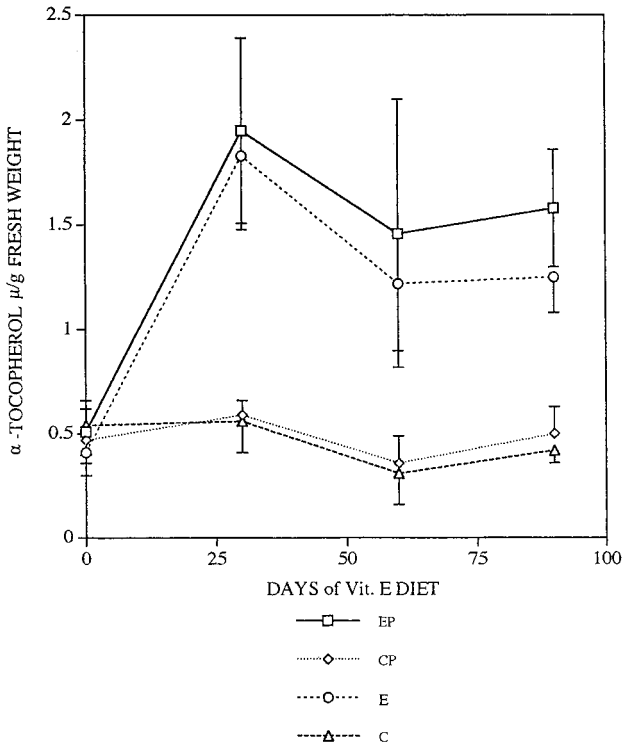

Figure 1. Accumulation of α-tocopherol in red blood cells of calves receiving vitamin E at 4000 mg/day in their feed over a period of 90 days: control (C); vitamin E added to feed (E); PUFA in feed (P).

The results are the average of values for three determinations for each sample. α-Tocopherol (Sigma) was used for calibration of a standard curve.

Lipid Peroxidation. Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Bidlack et al. (*35*). The results are reported as absorption at 532 nm and are the averages of three determinations for each sample.

Meat Color. The color of the meat samples was read directly on the sample with a tristimulus reflectance colorimeter (Minolta CR-200), which was standardized against a white standardizing plate ($L = 97.2$, $a = 0.45$, $b = 2.12$). The changes of the meat color during storage at 4 °C were calculated from the differences between the existing *a* values and those at the start of the storage period for each sample. The results are the averages of three determinations of each sample; in the figures, each bar (I) denotes the standard deviation.

RESULTS

Accumulation of α-Tocopherol in Red Blood and Muscle Cells. The data presented in Figure 1 show that the control diet provided α-tocopherol in an amount which caused the membranes of the red blood cells to accumulate the antioxidant to a concentration of 0.2−0.3 μg/g of fresh weight. Supplementation of vitamin E at a high concentration in the diet increased the accumulation of α-tocopherol in the red blood cells of the treated group to 1.5−1.7 μg/g of fresh weight, almost 5−7 times the level in the control. No significant difference in accumulation of α-tocopherol was found between the group on the regular diet and those groups with high PUFA content in the feed.

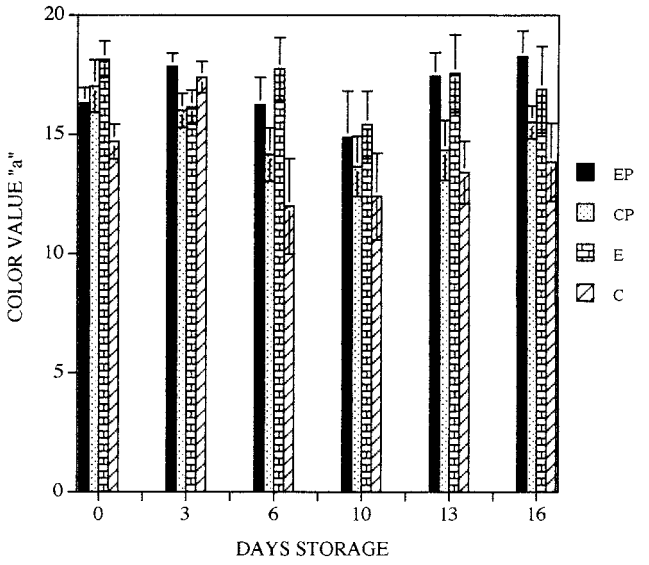

Figure 2. Color values during storage of longissimus dorsi (LD) muscle of calves fed diets supplemented with vitamin E and PUFA: control (C); vitamin E (E); PUFA in feed (P).

Table 2. α-Tocopherol Accumulation in Membrane Muscle Tissue

	α-tocopherol (μg/g of fresh wt)	
treatment	not koshered	koshered
control	2.61 ± 0.04	2.51 ± 0.02
vitamin E supplemented	6.51 ± 0.15	6.76 ± 0.17
control + PUFA	2.83 ± 0.03	2.55 ± 0.03
control + PUFA + vitamin E supplemented	7.07 ± 0.17	7.30 ± 0.18

Table 2 presents the findings on the accumulation of α-tocopherol in muscle membranes. The amount present in the controls was 2.61−2.83 μg/g of fresh weight. In the treated groups, the α-tocopherol in the muscles ranged between 6.51 and 7.07 μg/g of fresh weight on the day of slaughter, almost 3 times the level in the controls.

Color Deterioration. During the 2 week storage period, red color *a* values of meat samples not treated with vitamin E tended to be lower than those from cattle that received 4 g of vitamin E in the feed (Figure 2). No significant color differences were found between meat samples from animals, with or without PUFA in the feed. Kosher-salting greatly increased the deterioration of the meat color during storage. These changes were high for the samples from animals with high PUFA in the feed and low in those from cattle receiving vitamin E supplementation in the diet (Figure 3).

Color deterioration after kosher-salting was also high in the control meat samples from calves on a regular diet, without high PUFA content in the feed and without vitamin E supplementation. Supplementation of the diet with high levels of vitamin E was very effective in preventing color deterioration in the meat samples from calves with or without high PUFA in the feed and following kosher-salting (Figure 3).

Lipid Peroxidation. The kosher-salting process dramatically affected lipid peroxidation, especially in the samples without vitamin E supplementation. Moderate lipid peroxidation was measured in control samples without vitamin E supplementation and without salting. Supplementation with high concentrations of vitamin E in the ration prevented lipid peroxidation in both kosher-salted or non-kosher salted samples (Figure 4).

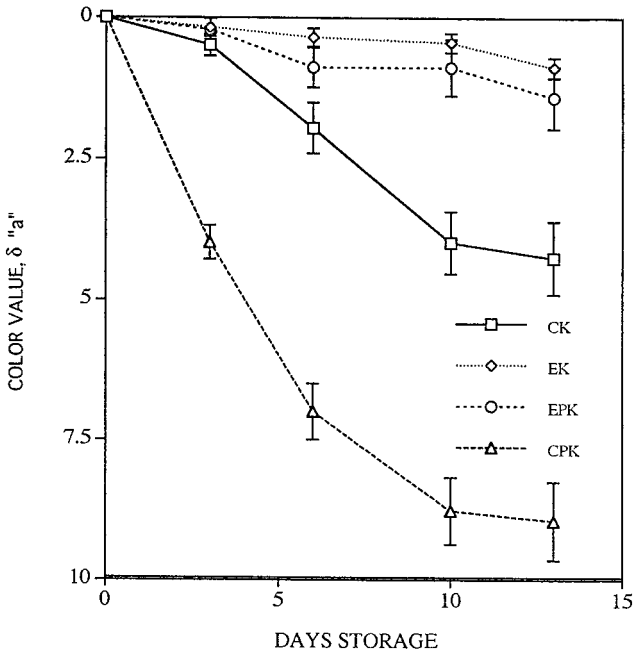

Figure 3. Degradation of color value of LD muscle tissue during storge at 4 °C: control (C); feed supplemented with vitamin E (E); PUFA in feed (P); kosher-salted (K).

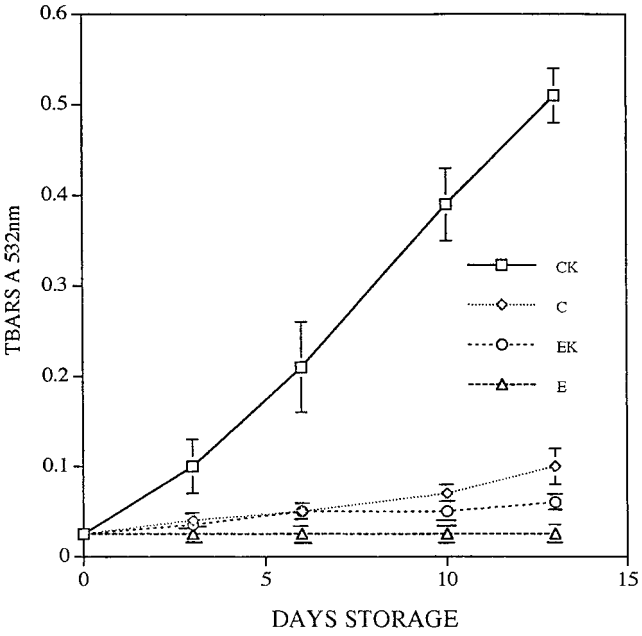

Figure 4. Lipid peroxidation of calf LD muscle tissue stored at 4 °C: control (C); feed supplemented with vitamin E (E); kosher-salted (K).

The rate of lipid peroxidation was higher in meat samples from calves with a high PUFA content in the feed (Figure 5). The accumulation of TBARS in PUFA samples, without supplementation of vitamin E during 6 days of cold storage, after kosher-salting, was $A = 0.44$. This was almost 10-fold that in the control samples without kosher-salting. Supplementation of the calf diets, including those with high PUFA content with vitamin E, prevented lipid peroxidation very efficiently, whether the meat was kosher-salted or not.

Figure 6 shows the losses in α-tocopherol in meat samples during storage at 4 °C. The kosher-salting process increased the loss of α-tocopherol in all of the

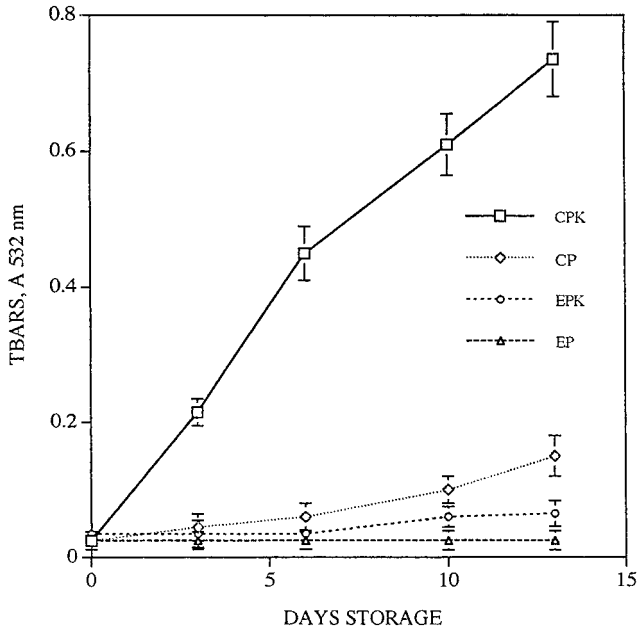

Figure 5. Lipid peroxidation during storage of LD muscle of calves fed diets supplemented with vitamin E and PUFA: control (C); vitamin E (E); PUFA in feed (P); kosher-salted (K).

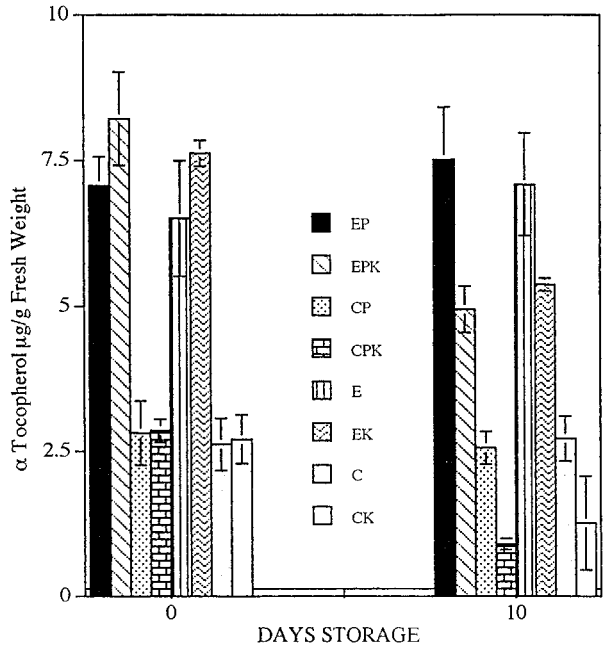

Figure 6. Changes of α-tocopherol levels in LD muscle during 10 days storage: feed supplemented with vitamin E (E); PUFA in feed (P); kosher-salted (K); control (C).

samples compared with those in nonsalted samples. The α-tocopherol losses in all kosher-salted samples during cold storage for 10 days reached ~40% of the amount present at the beginning of the storage period.

DISCUSSION

Lipid peroxidation is one of the primary causes of deterioration of color, flavor, texture, and nutritional value of fresh or processed muscle foods (*5*).

Several researchers have reported that vitamin E not only delays lipid peroxidation in beef but also prevents oxymyoglobin oxidation (*28*−*30*). However, the mode of action of vitamin E in the inhibition of oxymyoglobin oxidation is not clearly understood. Most recently, we used a model system to study membrane lipid peroxidation and autoxidation of oxymyoglobin catalyzed by an iron redox cycle, by simulating in situ muscle oxidative reactions. It was found that active oxygen species such as superoxide and H_2O_2, or hydroperoxides and oxy lipid radicals, are involved in the oxidation of oxymyoglobin to metmyoglobin (*14*, *15*).

In the present study it was found that even though the potential for peroxidation was enhanced by a diet rich in PUFAs or by the addition of NaCl, the enrichment of muscle tissues with α-tocopherol almost completely inhibited muscle myoglobin oxidation, as measured by color degradation of the meat. These results were surprising because it had been anticipated that the addition of α-tocopherol would only partially prevent oxymyoglobin oxidation. One of the differences between our model system and the in situ muscle condition is that the muscle contains enzymes such as SOD, catalase, and glutathione peroxidase (*12*, *36*−*38*). These enzymes readily react with superoxide, hydrogen peroxide, and hydroperoxides and have been found to reduce oxymyoglobin oxidation very significantly (*15*).

Oxymyoglobin oxidation in the muscle tissue is dependent on lipid peroxidation, which is very much reduced by the addition of dietary vitamin E.

Several factors could affect this process in muscle tissue, such as reducing capacity, NaCl, phosphate, oxygen pressure, and temperature (*5*), all of which could also affect oxymyoglobin stability.

Supplementation of vitamin E in the feed increased the concentration of α-tocopherol in the muscle tissues almost 3-fold. This concentration can be achieved by supplementing the diet of the calves with vitamin E at 4000 mg/day for 40−50 days prior to slaughter. These results are in agreement with those reported by Arnold et al. (*28*).

The high concentration of α-tocopherol in the muscle tissues reduced lipid oxidation in muscle tissues by >90%, with or without kosher-salting of the meat. Addition of NaCl to meat samples was previously found by us (*31*) and others (*32*, *34*, *39*−*41*) to greatly enhance lipid peroxidation. Our recent data have shown that addition of NaCl also strongly affects oxymyoglobin oxidation. Although NaCl could enhance autoxidation of oxyhemoproteins (*33*) to superoxide and hydrogen peroxide, these species seem to be controlled very efficiently in in situ muscle tissues by SOD and catalase.

It was found, not surprisingly, that meat tissue from calves fed a ration rich in PUFA was significantly more susceptible to lipid peroxidation and oxymyoglobin oxidation following kosher-salting. However, vitamin E supplementation in the diet prevented these changes, and the meat retained its high sensory properties (color) and nutritive qualities.

It appears from the present study that in fresh muscle foods, oxymyoglobin oxidation levels can be used not only as a criterion of color changes and meat quality but also as a sensitive marker for oxidation in general, as was suggested by the Tappel group (*42*).

In conclusion, fresh beef muscle tissue contains active enzymatic activity (*43*), which controls the level of active oxygen species such as superoxide, hydrogen peroxide, and lipid hydroperoxides. Oxymyoglobin oxidation in fresh muscle tissues in situ seems to be affected by and mostly dependent on lipid peroxidation. Lipid peroxi-

dation in muscle tissue is very well controlled by a critical amount of α-tocopherol in the tissues, even in the presence of increased levels of PUFAs in the membranes, or addition of NaCl, two factors that enhance oxidative processes.

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