Vitamin E Supplementation of Cattle and Shelf-Life of Beef for the Japanese Market[1]

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ABSTRACT: Feeder steers (n = 84) were stratified into four weight groups to provide slaughter groups so that product that had been in vacuum packages at 0 to 2°C for 40, 60, 80, or 100 d postmortem could be simultaneously evaluated. Each of the four groups was randomly divided into three subgroups so that vitamin E could be supplemented in the diet at rates of 0, 1,000, or 2,000 (E0, E1000, and E2000, respectively) IU·steer^{-1}·d^{-1} for 100 d. After slaughtering, chilling, and fabricating, one ribeye-roll and one strip loin from each carcass was transported to the university laboratory for analyses, whereas the paired subprimals were transported to Japan. Based on metmyoglobin formation and lipid oxidation, strip loin steaks deteriorated at a faster rate during retail-display than did ribeye steaks. Steaks from subprimals that were stored for 100 d had inferior ($P < .05$) retail-display characteristics and a shorter ($P < .05$) caselife than steaks from the other storage periods. α-Tocopherol levels in longissimus muscle were lower ($P < .05$) for E0 than for E1000 and E2000 (3.51, 5.54, and 6.10 μg/g of tissue, respectively). Supplementing cattle with vitamin E resulted in steaks that exhibited superior lean color, less surface discoloration, more desirable overall appearance, and less lipid oxidation during retail-display than control steaks; minimal differences were observed between E1000 and E2000 steaks. Steaks from cattle supplemented with vitamin E were preferred over control steaks by 91% of Japanese survey participants (n = 10,941), and 58% of all participants identified muscle color as the most important factor in selecting beef products.

Key Words: Beef, Color, Vitamin E, Exports, Keeping Quality

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Introduction

Consumers have learned through experience that the desirable color of fresh beef is bright red and that any deviation from this creates a degree of unacceptability for the product. This is especially true in Japan; following marbling, Japanese Quality Grading Standards for beef carcasses consider lean color as the second most important criterion. Using simulated retail-display conditions, previous researchers have demonstrated that beef from cattle given supplementary vitamin E in the diet was less susceptible to metmyoglobin formation and lipid oxidation than was conventionally produced beef (Faustman et al., 1989a,b; Arnold et al., 1992a,b, 1993a,b; Sanders et al., 1993; Lavelle et al., 1995; Sherbeck et al., 1995). The latter studies were designed to evaluate effects of dietary vitamin E supplementation on caselife of beef, after vacuum-packaged storage for 0 to 21 d. The objectives of the present study were to determine the importance of muscle color in beef to Japanese consumers and to evaluate the effect of dietary vitamin E supplementation to cattle on fresh beef retail display characteristics following extended periods (40 to 100 d) of vacuum-packaged storage.

Materials and Methods

Angus × Hereford × Saler steers (n = 84) were obtained from a single producer in southwestern Colorado. At the Colorado State University (CSU) research farm in Fort Collins, these feeder steers were processed and stratified by weight into four groups. The four weight groups (n = 21 each) allowed for storage groups so that product that had been in vacuum-packaged storage for 40, 60, 80, or 100 d (hereafter referred to as **40S**, **60S**, **80S**, and **100S**, respectively) could be simultaneously evaluated.

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Feeder cattle in each weight group were randomly assigned to one of three subgroups; vitamin E was provided as a dietary supplement at rates of 0, 1,000, or 2,000 IU·steer^{-1}·d^{-1} (hereafter referred to as **E0**, **E1000**, or **E2000**, respectively) to different subgroups.

Steers representing the four subprimal storage groups were placed on feed at 20-d intervals. One-third of the cattle (a subgroup) in each weight group received only the basal diet (85% whole corn and 25% corn silage, DM basis) for the entire feeding period. Two subgroups in each weight group were supplemented for 100 d with α-tocopheryl acetate (Rovimix™ E-50 SD, Hoffmann-LaRoche, Nutley, NJ). The α-tocopheryl acetate was supplemented so that 0, 1,000 or 2,000 IU of α-tocopheryl acetate was contained in each .23 kg of pulverized corn carrier (applied by top-dressing).

After each storage group's appropriate feeding periods, the cattle were transported to the Monfort/ConAgra beef packing plant (Greeley, CO), where they were humanely slaughtered using conventional procedures. Within 1 h postmortem, a 20-g lean-tissue sample was obtained from the longissimus lumborum muscle near the last lumbar vertebra of each of the carcasses. Refrigerated samples were saponified and analyzed for muscle α-tocopherol according to procedures of Bilich et al. (1994). After a 24-h chill period (1 to 2°C), USDA Yield Grade and Quality Grade factors were obtained (USDA, 1976). After an additional 24-h chilling period (2 to 3°C), five carcasses with the most similar marbling scores were selected from each vitamin E supplementation level. From each of the selected carcasses, a ribeye-roll (IMPS 112, hereafter referred to as **RE**) and a striploin (IMPS 180, hereafter referred to as **SL**) were individually vacuum-packaged, boxed, and stored at 0 to 2°C at the beef processing plant storage facility.

When cattle in the final storage group (40 d) had been slaughtered, fabricated, and packaged, all boxed products (n = 60 SL and RE, each) were transported to the CSU Meat Laboratory. Following arrival at the meat laboratory, one-half of the RE and SL from each slaughter group were placed in boxes designed for export purposes and transported via air carrier to Japan, and the remaining one-half of the beef subprimal product mix remained in storage (0 to 2°C) at CSU for subsequent laboratory analyses.

Visual Evaluations

After appropriate subprimal storage periods were obtained, subprimals were aseptically removed from vacuum bags and cut into nine steaks; each steak was placed on a styrofoam tray and overwrapped with oxygen-permeable (1,000 to 1,050 mL of 0_2/645 cm^2 during a 24-h period) polyvinyl chloride packaging film. The steaks were then placed in a commercial retail-display case (2 to 4°C) under continuous, cool-white fluorescent lighting (2,200 to 2,500 lx). Meat color was evaluated twice daily throughout a 7-d retail display period. Steaks were visually evaluated on d 0 through 7 by a three- to five-member panel consisting of CSU personnel. Each steak was evaluated for lean color (8 = bright cherry-red, 1 = extremely dark brown or green), fat color (8 = creamy white, 1 = dark brown or green), surface discoloration (7 = no discoloration, 4 = 26 to 50% discoloration, 1 = completely discolored), and overall appearance (8 = extremely desirable, 1 = extremely undesirable). In this study, overall appearance was used as a descriptive term that resulted from the combined effects of lean and fat color as well as surface discoloration ratings.

On each day of display, percentage of muscle pigment as metmyoglobin was evaluated for two steaks per treatment combination at two locations on each steak. The percentage of metmyoglobin present among the meat surface myoglobins was calculated using the specific wavelength method as described by Krzywicki (1979) and a Hewlett-Packard 8452A Diode Array Spectrophotometer (Waldbronn, Germany) with a Labsphere RSA-HP-84 Reflectance Spectroscopy accessory (North Sutton, NH).

Thiobarbituric Acid Analyses

On retail-display d 0, 1, 4, and 7, duplicate cores (12 cm^2 × .8 cm thick; 10 g) of muscle were obtained from one steak from each steer for lipid oxidation analysis. This analysis was performed using the thiobarbituric acid (**TBA**) test procedure of Salih et al. (1987), except that 5% (wt/vol) aqueous trichloroacetic acid (Mallincrodt, Paris, KY) was used as the extraction solvent.

Total Plate Count Microbiological Analyses

Duplicate samples from one steak from each steer were analyzed for total plate count on retail-display d 0, 1, 4, and 7. Samples were blended in 200 mL of .1% peptone water and appropriate decimal dilutions were plated onto plate count agar (Difco, Detroit, MI) and were allowed to incubate for 48 h at room temperature. The pH of the blended samples was determined at each sampling time, upon completion of the desired decimal dilutions, using an Orion Model 610 pH meter (Orion Research, Boston, MA).

Japanese Export Procedures

Subprimals that were transported to Japan were sent to the U.S. Meat Export Federation (USMEF) office in Tokyo. Upon arrival, RE and SL subprimals were transported to the University of Nippon, Food Technology building. The RE and three SL subprimals were randomly selected from each vitamin E supplementation level of each of the four vacuum-packaged storage periods and fabricated into steaks. The steaks were placed on styrofoam trays, overwrapped with oxygen-permeable film, and displayed

under simulated retail conditions in a refrigerated cooler (4°C) for 3 d. At the end of d 3 of retail display, steaks were transported to the Nippon Convention Center for the International Food Expo. On arrival at the Expo, retail beef packages were placed into a multiple-deck retail meat case (4°C) under continuous, cool-white fluorescent lighting (2,000 to 2,500 lx). Approximately 111,000 Pacific Rim food manufacturers, meat buyers, meat retailers, and food service personnel were able to observe the steaks in the retail-display case at the Expo. Using a survey instrument developed by the USMEF-Tokyo staff, attendees were asked to list their most important considerations when selecting beef items. Additional questions were designed to determine 1) how important color is to Japanese consumers in their decision making process for purchasing beef, 2) whether participants could distinguish between beef from vitamin E-supplemented and control steers, and 3) whether the participants believed that beef from vitamin E-supplemented steers was more appealing in lean color than beef from control steers. Survey participants did not know whether beef retail cuts originated from cattle supplemented with vitamin E.

Statistical Analyses

Results were analyzed by analysis of variance using the GLM procedure of SAS (1985). For variables that were measured over time, a split-plot analysis was performed to account for repeated measures (Freund et al., 1986). Orthogonal contrasts were used to compare certain means (Steel and Torrie, 1980).

Results and Discussion

Because no detectable differences ($P > .05$) in animal performance or carcass characteristics were observed among the four storage groups in this study, all results were stratified and analyzed by vitamin E supplementation level. Feedlot performance may be improved by vitamin E supplementation during the initial weeks of starting cattle on feedlot diets (Hill et al., 1990). Feedlot performance and carcass characteristics of cattle in the present study were not affected ($P > .05$) by feeding supplemental vitamin E (Arnold et al., 1992a, 1993a; Table 1).

Calculated average consumption of vitamin E was 50, 820, and 1,600 IU·steer^{-1}·d^{-1} for E0, E1000, and E2000 steers, respectively. Storage group did not affect vitamin E intake ($P > .05$).

Muscle α-tocopherol concentrations were increased ($P < .05$) by feeding supplemental vitamin E (Table 2). For each storage group, muscle α-tocopherol concentrations were lowest for carcasses from the E0 supplementation level. For 40S, 60S, and 100S muscle, α-tocopherol levels from cattle supplemented with E1000 and E2000 did not differ ($P > .05$) from each other; however, all were greater ($P < .05$) than muscle α-tocopherol levels from cattle fed E0. For 80S, E2000 cattle had higher ($P < .05$) muscle levels of α-tocopherol than did E1000 cattle.

Vitamin E supplementation was effective in stabilizing the retail-display life of RE and SL steaks. Effects of vitamin E supplementation on lean color of beef steaks are presented in Figure 1. At the time of the initial fabrication of RE and SL subprimals into steaks, no difference ($P > .05$) was observed among steaks from cattle of E1000 and E2000 supplementation levels; however, steaks from steers supplemented with vitamin E were brighter ($P < .05$) than steaks from E0 steers at d 0. Therefore, vitamin E did have

Table 1. Feedlot performance and carcass characteristics by treatment[a]

	Vitamin E level[b]			
Characteristic	E0	E1000	E2000	SE
Final weight, kg	608	600	608	13.06
ADG, kg/d	1.54	1.46	1.55	.15
DMI, kg/d	10.94	11.13	11.05	1.01
DMI/ADG	7.10	7.62	7.13	.60
Hot carcass weight, kg	360.72	357.16	364.92	9.31
Dress, %	59.33	59.33	60.02	.36
Actual PYG[c]	3.05	3.12	3.12	.09
Adjusted PYG	3.13	3.21	3.15	.09
Ribeye area, cm^2	84.88	85.13	86.00	.25
KPH, %[d]	1.85	1.90	1.77	.09
YG	2.6	2.7	2.6	.13
Skeletal maturity[e]	161	158	163	397
Lean maturity[e]	155	154	155	3.05
Marbling score[f]	437	452	459	17.75

[a]Vitamin E level effects were not significant ($P < .05$).
[b]E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}; E2000 = 2,000 IU·steer^{-1}·d^{-1}.
[c]PYG = preliminary yield grade.
[d]KPH = kidney, pelvic, and heart fat.
[e]100 = A, 200 = B, 300 = C.
[f]300 = slight; 400 = small; 500 = modest.

Table 2. Effect of vitamin E supplementation on the concentration of α-tocopherol in lean tissue

	Vitamin E level, μg/g[b]		
Storage group[a]	E0	E1000	E2000
40S[c]	3.70[d]	5.84[e]	5.74[e]
60S[c]	4.06[d]	5.85[e]	6.75[e]
80S[c]	2.90[d]	4.72[e]	6.10[f]
100S[c]	3.37[d]	5.74[e]	5.80[e]

[a]Effects between slaughter groups are not significant ($P > .05$); SE = .37.

[b]E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}; E2000 = 2,000 IU·steer^{-1}·d^{-1}.

[c]40S, 60S, 80S, 100S are storage periods (d) for ribeye and strip loin subprimals.

[d,e,f]Means within the same row are significantly different ($P < .05$) if letters differ; SE = 63.

an effect at the time of retail-cut fabrication on the lean color of beef that was subjected to extended periods (40 to 100 d) of vacuum-package storage. As retail-display period progressed, lean color scores assigned to the steaks from steers of all vitamin E supplementation levels continually decreased, but there were no differences ($P > .05$) in lean color between steaks from E1000- or E2000-supplemented steers. Throughout retail-display, steaks from E0 steers received significantly lower lean color scores than did steaks from vitamin E-supplemented steers (Figure 1). The magnitude of differences in lean color was greatest ($P < .01$) between E0 and E1000 from d 3 through 7 of retail-display.

Percentage of surface discoloration of RE and SL steaks demonstrated that vitamin E supplementation was effective in maintaining a bright red, fresh appearance for a longer portion of the retail-display period compared with control steaks (Figure 2). Steaks from steers supplemented with vitamin E exhibited less ($P < .05$) surface discoloration than control steaks beginning on d 3 and continuing through d 6 of retail-display. Steaks from E1000-supplemented steers had less surface discoloration on d 1 through 3 than steaks from E2000-supplemented steers.

Fat color of RE and SL steaks was not affected ($P > .05$) by vitamin E supplementation (data not shown). Desirability of fat color in RE and SL steaks decreased as the retail-display period progressed ($P < .01$). Visual evaluations of fat color on d 0 demonstrated that as vacuum-packaged storage increased, desirability of fat color decreased. Fat color scores of steaks from 100S were substantially lower than those for 40S, 60S, or 80S steaks on d 0 of retail-display. As

	Retail display, d							
Contrast	0	1	2	3	4	5	6	7
E0 vs E	*	*	*	**	**	**	**	**
E1000 vs E2000	NS	NS	NS	NS	NS	NS	NS	NS
SE	.089	.125	.122	.132	.158	.165	.154	.153

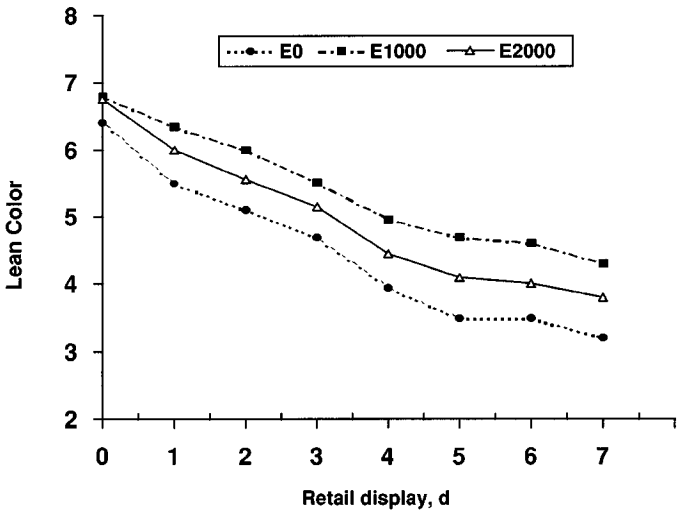

Figure 1. Orthogonal contrasts, least squares means, and standard errors for visual evaluation of lean color by vitamin E supplementation level and day of retail display. E = vitamin E; E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}, E2000 = 2,000 IU·steer^{-1}·d^{-1}. 8 = Bright cherry-red; 1 = dark brown/green. *$P < .05$; **$P < .01$; NS = not statistically significant ($P > .05$).

	Retail display, d							
Contrast	0	1	2	3	4	5	6	7
E0 vs E	NS	NS	NS	**	**	**	**	NS
E1000 vs E2000	NS	*	*	*	NS	NS	NS	NS
SE	.048	.072	.099	.114	.141	.138	.156	.153

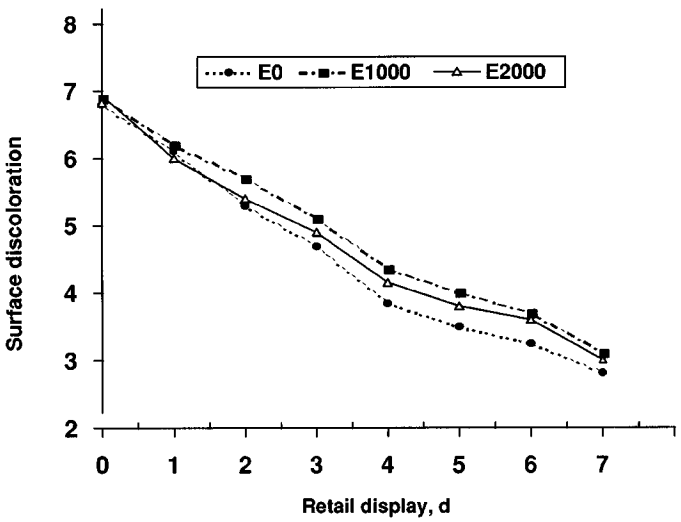

Figure 2. Orthogonal contrasts, least squares means, and standard errors for visual evaluation of surface discoloration by vitamin E supplementation level and day of retail display. E = vitamin E; E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}; E2000 = 2,000 IU·steer^{-1}·d^{-1}. 7 = No discoloration; 1 = complete discoloration. *$P < .05$; **$P < .01$; NS = not statistically significant ($P > .05$).

Contrast	Retail display, d 0	1	2	3	4	5	6	7
E0 vs E	NS	NS	NS	**	**	*	*	NS
E1000 vs E2000	NS	*	NS	*	NS	*	NS	NS
SE	.075	.099	.134	.135	.147	.139	.157	.132

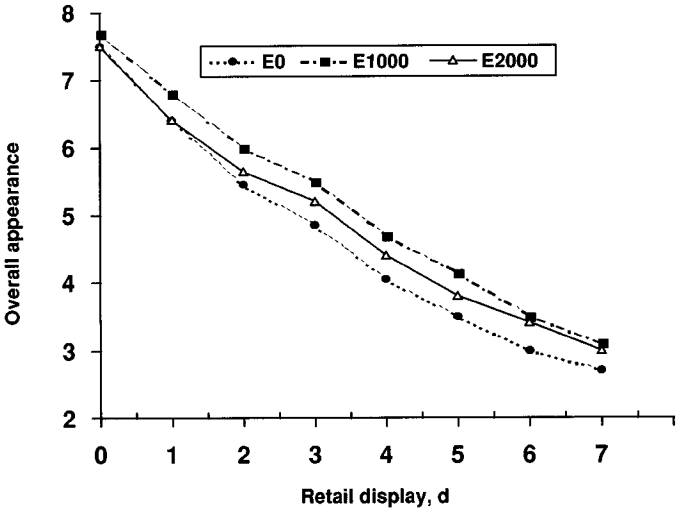

Figure 3. Orthogonal contrasts, least squares means, and standard errors for visual appearance by vitamin supplementation level and day of retail display. E = vitamin E; E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}; E2000 = 2,000 IU·steer^{-1}·d^{-1}. 8 = Extremely desirable; 1 = extremely undesirable. *P < .05; **P < .01; NS = not statistically significant (P > .05).

retail-display progressed through d 4, steaks from 100S received the lowest scores for fat color, but after d 4, fat color scores for steaks from other storage levels were similar to those for steaks from 100S.

Steaks from SL lost their consumer appeal at a faster rate than did RE steaks; at d 6 and 7 of retail-display, SL steaks were scored moderately to very undesirable by panelists, whereas RE steaks were scored as slightly undesirable. Effects of vitamin E supplementation on the overall appearance of beef steaks are presented in Figure 3. On d 3 through 6 of retail display, control steaks were less desirable in overall appearance than were steaks from steers supplemented with vitamin E. Steaks from steers of the E1000 supplementation level received higher (P < .05) scores than steaks from steers of the E2000 supplementation level on d 1, 3, and 5 of retail-display. On d 0 and 7 of retail-display, vitamin E supplementation level had no effect (P > .05) on overall appearance of beef steaks.

Lipid oxidation occurred at a faster (P < .01) rate during retail display in SL steaks than in RE steaks (Figure 4). Thiobarbituric acid values for RE and SL steaks were similar on d 0 of retail-display, but by retail-display d 7, TBA values for SL steaks had increased approximately one and one-half times as much as TBA values for RE steaks. Lipid oxidation

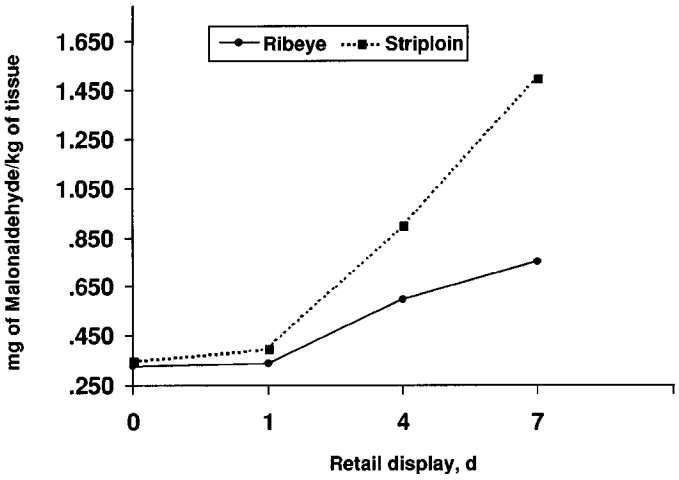

Figure 4. Comparison of ribeye steaks and strip loin steaks for tissue malonaldehyde.

was markedly inhibited (P < .01) for steaks from cattle fed either E1000 or E2000 compared with steaks from cattle fed E0. On d 0 of retail-display, control steaks had higher (P < .02) TBA values than E1000 and E2000 steaks, and the magnitude of these differences increased as retail-display progressed through d 7 (Figure 5).

Vitamin E supplementation did not affect (P > .05) the rate of metmyoglobin formation in either RE or SL

Contrast	Retail display, d 0	1	4	7
E0 vs E	**	**	**	**
E1000 vs E2000	NS	NS	NS	NS
SE	.010	.024	.061	.103

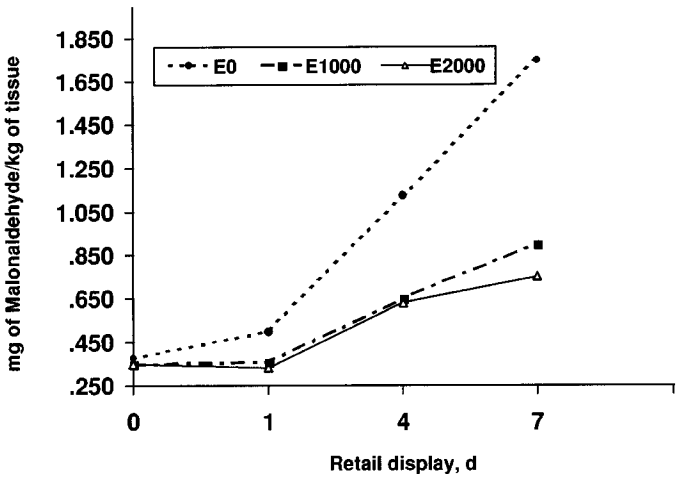

Figure 5. Orthogonal contrasts, least squares means, and standard errors for tissue malonaldehyde by vitamin E supplementation level and day of retail display. E = vitamin E; E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}; E2000 = 2,000 IU·steer^{-1}·d^{-1}. **P < .01; NS = not statistically significant (P > .05).

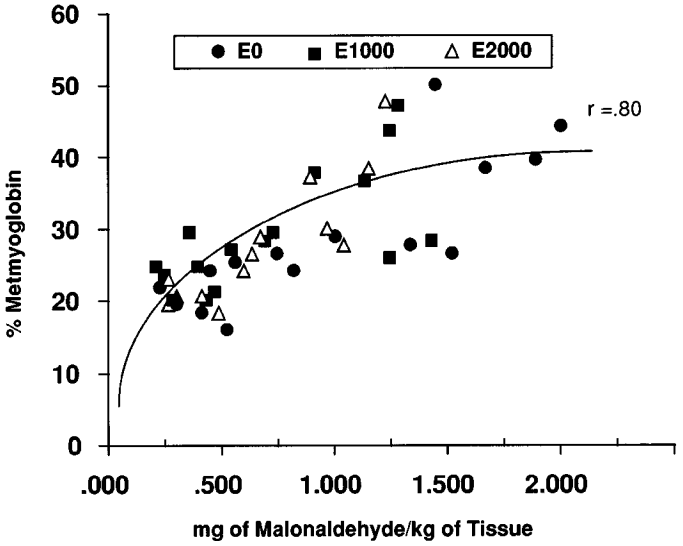

Figure 6. Relationship between metmyoglobin formation and lipid oxidation in strip loin steaks from control and vitamin E-supplemented steers. E0 = control; E1000 = 1,000 IU·steer^{-1}·d^{-1}; E2000 = 2,000 IU·steer^{-1}·d^{-1}.

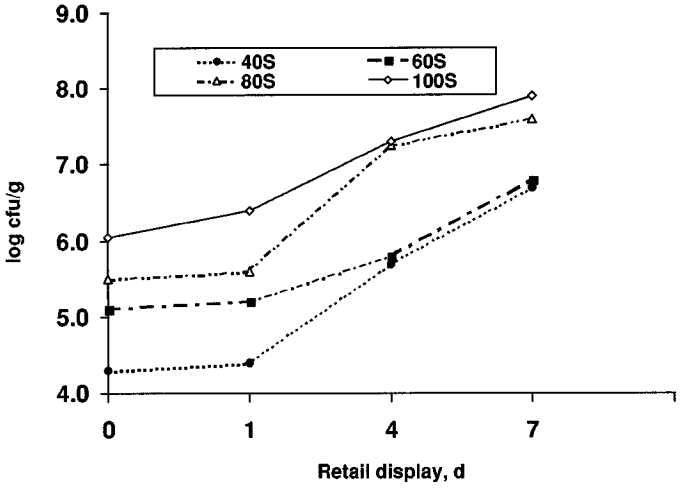

Figure 7. Effect of vacuum-packaged storage on total plate count during retail display. 40S, 60S, 80S, and 100S = 40, 60, 80, and 100 d in vacuum-package storage, respectively.

steaks (data not in tabular form); however, SL steaks formed metmyoglobin faster ($P < .01$) than RE steaks as retail-display progressed. For RE steaks, 20 and 35% metmyoglobin was detectable at d 0 and 7, respectively, of retail display, and for SL steaks, 20 and 43% metmyoglobin was detectable at d 0 and 7, respectively, of retail display. Steaks from 100S exhibited the fastest rate and greatest amount of metmyoglobin formation.

Although vitamin E supplementation did not seem to have an effect on the formation of metmyoglobin, a positive relationship of TBA number and percentage of metmyoglobin (r = .80; $P < .05$) was observed in strip loin steaks (Figure 6).

Microbial counts were not affected ($P > .05$) by vitamin E supplementation. Microbial counts increased ($P < .01$) as retail display progressed. Differences in microbial counts for steaks from subprimals stored for different periods of time throughout the retail-display period are shown in Figure 7. A count of > 10^7 cfu/g is considered indicative of spoilage (Ayres, 1960; Branen, 1978). Microbial counts for steaks from 40S and 60S did not reach or exceed 10^7 cfu/g until d 7 of retail-display; however, microbial counts for steaks from 80S and 100S were > 10^7 cfu/g on approximately d 3 of retail-display. Extension of vacuum-packaged storage from 40 to 80 d allowed increased microbial growth, but subprimals that were stored for 100 d exhibited less microbial growth than subprimals that had been in vacuum-packaged storage for 80 d.

Respondents (n = 10,941) to the Japanese survey indicated that their most important consideration when selecting beef was fresh meat color (69.4%). Fat color (16.1%), amount of marbling (4.7%), price (3.1%), and amount of drip loss or purge (2.4%) were also listed as important selection criteria by Japanese purveyors, restaurateurs, and retailers. A second question asked was, "Can you detect a difference between the products displayed in the retail case (i.e., quality/appearance)"? Of the 10,138 survey participants, 87% responded yes that a detectable difference could be noticed between the various beef retail cuts. Of the participants who could detect differences, 79% responded that these differences were due to bright red lean color.

Implications

Currently, the United States exports approximately $4.5 billion worth of beef annually. As a result of the extended refrigerated storage-life (minimum of 30 to 50 d) required for U.S. beef items to be transported to most international ports, many quality shortcomings are frequently observed. The present study demonstrates that supplementing the diets of cattle with vitamin E is an effective tool for improving appearance of beef cuts following extended (40 d or greater) periods of vacuum-packaged storage.

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