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Summary

Aged (8 and 29 days) strip loins, from cross-bred steers fed dry-rolled corn-based finishing diets containing 0 or 30% wet distillers grains with a synthetic antioxidant blend (AGRADO®PLUS) were packaged in high oxygen modified atmosphere packages (80% O_2:20% CO_2) and studied for decreased tenderness compared to steaks packaged in oxygen-permeable film during retail display. Steaks aged longer and packaged in high oxygen modified atmosphere packages decreased in tenderness, likely due to increased protein oxidation (more carbonyls and less free thiols), during retail display. Feeding AGRADO PLUS tended to decrease tenderness and increased protein oxidation during retail display under high oxygen conditions.

Introduction

High oxygen modified atmosphere packages (HiOx-MAP) are widely used in fresh beef retail markets to sustain the cherry-red color of meat. Steaks packaged in HiOx-MAP decrease remarkably in tenderness compared to steaks in oxygen-permeable (PVC-OW) packages (*2010 Nebraska Beef Cattle Report,* pp. 99-101; *2011 Nebraska Beef Cattle Report*, pp. 100-102).

Antioxidant supplementation (ethoxyquin and tertiary butyl hydroquinone; AGRADO PLUS; AG) helps to minimize oxidation of color and lipids of beef (*2011 Nebraska Beef Cattle Report,* pp 100 – 102).

Therefore, this study was conducted to investigate the mechanism of declining beef tenderness due to HiOx-MAP and to study effects of dietary antioxidant (AG) supplementation as a control measure for the problem.

Procedure

Cross-bred (British × Continental) yearling steers were randomly assigned to one of four dry-rolled corn-based feedlot diets, containing 0 or 30% (DM) wet distillers grains plus solubles (WDGS) with or without AG (AG; 150 ppm/steer/day for 145-160 days). After slaughter and chilling for 48 hours, both short loins from a total of 80 USDA Choice carcasses (20 from each dietary treatment) were obtained and aged for either 8 or 29 days at 36°F.

Each strip loin (*m. longissimus lumborum*) was cut into 1-inch-thick steaks from the anterior to the posterior. The first (for protein oxidation; 0 days retail displayed), and fourth (for shear force; 0 days retail displayed) steaks were immediately vacuum-packaged and stored at -4°F. The second and third steaks were split into halves and assigned for 4 and 7 day protein oxidation analysis either under PVC-OW or HiOx-MAP (80% O_2:20% CO_2) packaging systems. The fifth and sixth steaks were allotted for 7 day retail display shear force analysis under both packaging systems. Packaged steaks were placed on a table in a cooler at 32 ± 36°F and exposed to continuous 1,000-1,800 lux warm white fluorescence lighting to provide simulated retail display conditions. Steaks assigned for 4 and 7 days of retail display were removed from tables accordingly for protein oxidation, and shear force analysis, immediately vacuum-packaged and stored at -4°F.

Protein oxidation of steaks was determined by measuring carbonyl and free thiol (sulfhydryl) contents per mg of myofibrillar proteins. More carbonyls and fewer sulfhydryls indicate more protein oxidation of steaks. The change (delta; Δ; 4/7 day– 0 day) in carbonyls and free thiols were calculated. Instrumental tenderness testing of steaks was performed using Warner-Bratzler shear force test (WBSF). Steaks were cooked to an internal temperature of 160 °F and stored in a cooler for overnight. Six cores with 0.5 in diameter were removed from a steak parallel to the muscle fiber arrangement using a drill press. Cores were sheared on a table-top WBSF analyzer with a triangular Warner-Bratzler shear attachment. An average of the peak shear force (lb) of six cores for each steak was used for statistical analysis (higher WBSF values indicate less tender).

Data were analyzed by ANOVA in the GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, N.C.) as a split-split-split-plot design with dietary treatments as the whole-plot treatment, aging period as the first split-plot treatment, packaging systems as the second split-plot treatment and retail display time (repeated measures) as the third split-plot treatment with the animal as the experimental unit. Separation of means was conducted using LSMEANS procedure with PDIFF and SLICEDIFF options at $P \leq 0.05$. In addition, the CONTRAST statements in SAS were used to compare the effects of feeding Corn *vs.* WDGS, Corn *vs.* Corn+AG, WDGS *vs.* WDGS+AG, and No AG *vs.* AG.

Results

Dietary treatments significantly (Figure 1a: $P = 0.02$) affected WBSF values. Steaks from AG-fed cattle had significantly (contrast $P = 0.04$;

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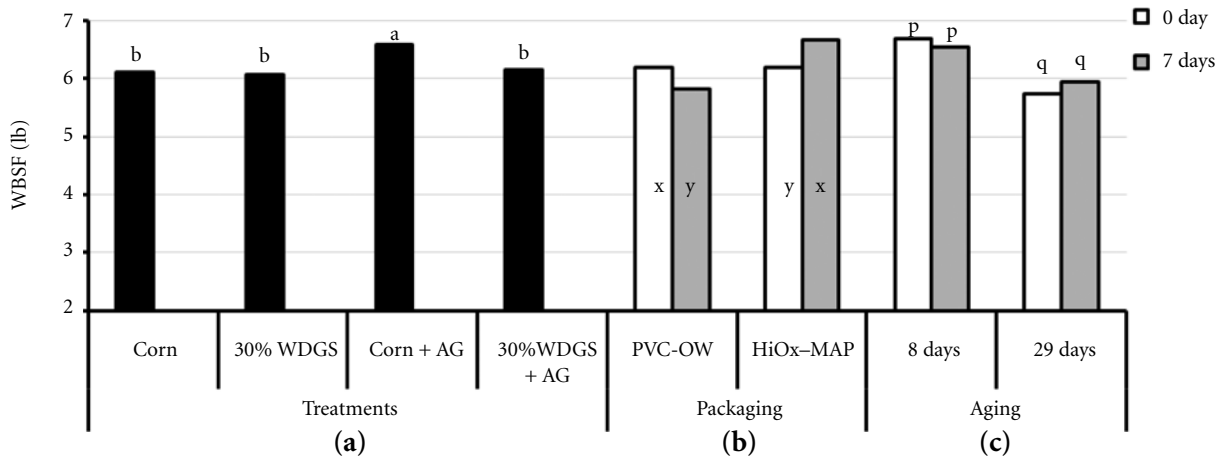

Figure1a. Means of Warner-Bratzler Shear force (WBSF) values of strip loin steaks from different diets (Diet, $P = 0.02$). b. Means of WBSF values of steaks in PVC-overwrapped (PVC-OW) and high oxygen modified atmospheric (HiOx-MAP) packages during retail display period (Packaging × d, $P < 0.0001$). c. Means of WBSF values of steaks aged 8 and 29 days during retail display period (Aging × days, $P = 0.03$). [a-b, x-y, or p-q] Comparison within each category, means lacking a common superscript were significantly different at $P < 0.05$. d = retail display days; WDGS = Wet distillers grains plus solubles; AG = AGRADO PLUS.

Table 1. Means of carbonyls of 8 and 29 day aged strip loin steaks in PVC-overwrapped (PVC-OW) and high oxygen modified atmospheric (HiOx-MAP) packages during 7 days of retail display (Diet × aging × d, $P = 0.0044$).

		Dietary Treatments				Contrast P values			
Aging	Day[1]	Corn + AG[1]	30% WDGS[1] + AG	Corn	30% WDGS	Corn vs Corn +AG	WDGS vs WDGS + AG	No AG vs AG	Corn vs WDGS
	0	2.04^{Bb}	1.82^{Bb}	1.71^{Bb}	2.44^{A}	0.05	0.0002	0.23	0.01
8	4	2.08^{b}	2.33^{a}	2.10^{a}	2.36	0.89	0.88	0.83	0.03
	7	2.46^{Aa}	2.30^{ABa}	1.99^{Bb}	2.22^{AB}	0.005	0.63	0.01	0.80
	0	2.16^{Ac}	1.68^{Bc}	1.71^{Bc}	1.98^{ABc}	0.0003	0.09	0.44	0.17
29	4	2.50^{Ab}	2.31^{ABb}	2.09^{Bb}	2.63^{Ab}	0.007	0.04	0.63	0.10
	7	2.98^{Aa}	2.81^{BCa}	2.55^{Ca}	3.28^{Aa}	0.06	0.03	0.80	0.06

[A-C]Comparison within rows among treatments, means lacking a common superscript were significantly different at $P < 0.05$.
[a-c]Comparison along columns within same treatment, means lacking a common superscript were significantly different at $P < 0.05$.
[1]d = retail display days; WDGS = Wet distillers grains plus solubles; AG = AGRADO®PLUS.

data not shown) higher WBSF values (less tender) compared to steaks from cattle fed non-AG supplemented diets. Overall, steaks from corn plus AG-fed cattle had the highest WBSF vales (Figure 1a) than steaks from cattle fed other diets. Perhaps AG interferes with proteolytic enzyme activity needed for postmortem meat tenderization.

During retail display, steaks in PVC-OW improved in tenderness while those in HiOx-MAP decreased in tenderness (Figure 1b; $P < 0.0001$). In addition, 29-day aged steaks were more tender than 8 dayaged steaks (Figure1c; $P = 0.03$). However, 29-day aged steaks tended to decrease in tenderness (higher WBSF values) during retail display (Figure1c; $P = 0.06$).

The high oxygen (80% O_2) level in MAP packages likely oxidized muscle proteins, especially myofibrillar proteins and proteolytic enzymes, consequently causing myofibrillar protein to cross-link (aggregate) and major proteolytic enzymes (calpains) to inactivate. An increase in carbonyls and a decrease in sulfhydryl (free thiol) groups in protein molecules are indicative of protein oxidation. Therefore, in this study carbonyls and free thiols were spectrophotometrically quantified.

Eight and 29 day aged steaks from corn plus AG diets had significantly (Table 1; $P < 0.05$) more carbonyls (more protein oxidation) than steaks from non-AG supplemented corn diets. There is no clear explanation for more carbonyls in steaks from cattle fed corn plus AG diets. Carbonyls (Δ) of all 29 day aged steaks increased during retail display (Figure 2a; $P = 0.0002$) as well as steaks in HiOx-MAP (Figure 2b; $P = 0.06$) indicating more proteins were oxidized when steaks were aged longer (29 days) or packaged in HiO_2-MAP system. These results explain the increase in WBSF values of steaks aged longer or packaged in HiOx-MAP during retail display period.

Free thiols decreased (more protein oxidation) during aging (Table 2; $P < 0.05$) and during retail display

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Table 2. Means of free thiols (sulfhydryls) 8- and 29-day aged strip loin steaks in PVC-overwrapped (PVC-OW) and high oxygen modified atmospheric (HiOx-MAP) packages during 7 days of retail display (Diet × aging × days, $P < 0.0001$).

		Dietary Treatments				Contrast P values			
Aging	Day[1]	Corn + AG[1]	30% WDGS1 + AG	Corn	30% WDGS	Corn vs Corn +AG	WDGS vs WDGS + AG	No AG vs AG	Corn vs WDGS
	0	73.52^{Bab}	84.30^{Aa}	80.63^{Aa}	69.57^{Bab}	0.005	<0.0001	0.043	0.996
8	4	75.22^{Aa}	71.94^{ABb}	68.81^{Bb}	73.30^{Aa}	0.005	0.511	0.109	0.673
	7	70.44^{b}	66.93^{c}	70.80^{b}	67.47^{b}	0.843	0.785	0.739	0.058
	0	80.41^{Aa}	78.05^{ABa}	74.63^{Ba}	74.69^{Ba}	0.004	0.130	0.002	0.449
29	4	70.15^{b}	66.48^{b}	69.92^{b}	66.52^{b}	0.991	0.940	0.965	0.010
	7	64.69^{c}	66.31^{b}	66.48^{b}	65.50^{b}	0.296	0.572	0.722	0.909

[A-B]Means along rows among treatments with different superscripts are significantly different at $P < 0.05$.
[a-c]Means along columns within treatments with different superscripts are significantly different at $P < 0.05$.
[1]d = retail display days; WDGS = Wet distillers grains plus solubles; AG = AGRADO®PLUS.

(Figure 3b; $P = 0.0002$). Before retail display (0 days), steaks from AG-fed cattle had higher free thiols (Table 2; $P < 0.05$; less protein oxidation) than steak from non-AG-fed cattle. Following retail display, steaks from cattle fed AG supplemented diets had greater decrease in free thiols ($P < 0.05$; data not shown) than steaks from non-AG-fed cattle; however, there was no clear pattern during retail display attributable to different dietary treatments. Steaks in HiOx-MAP tended to have fewer free thiols (Figure 3a; $P = 0.09$; more protein oxidation) compared to steaks in PVC-OW during retail display.

Overall results indicate steaks aged longer and packaged in HiO_2-MAP had more protein oxidation and reduced tenderness during retail display. Feeding AGRADO®PLUS tends to increase protein oxidation and decrease tenderness during retail display.

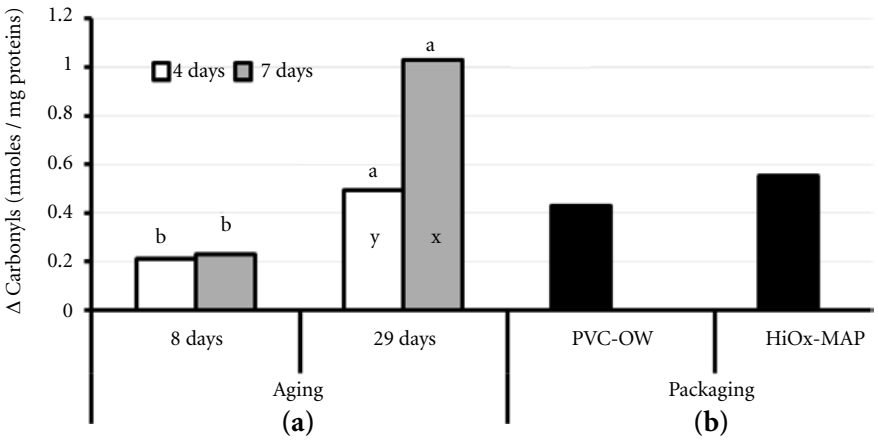

Figure 2a. Means of change (Δ; from 0 days to 4 or 7 days) in carbonyls of steaks aged 8 and 29 days during retail display period (Aging × day, $P = 0.0002$). b. Means of Δ in carbonyls of steaks packaged in PVC-overwrapped (PVC-OW) and high oxygen modified atmospheric (HiOx-MAP) packages (Packaging, $P = 0.06$). [a-b or x-y]Comparison within each category, means lacking a common superscript were significantly different at $P < 0.05$.

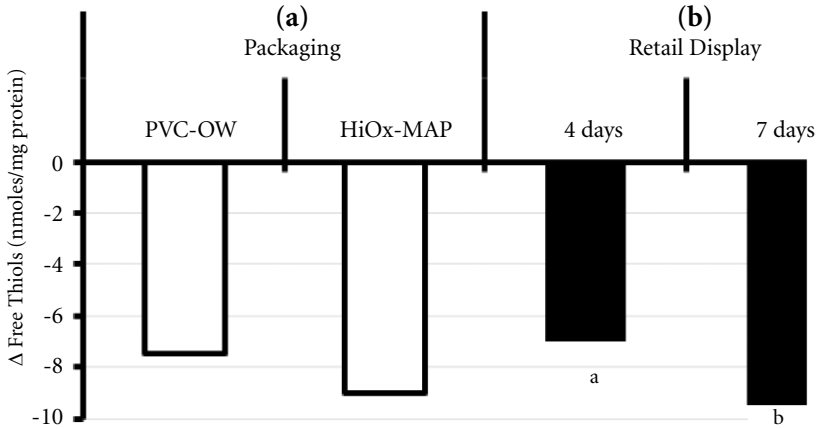

Figure 3a. Means of change (Δ; from 0 days to 4 or 7 days) in free thiols of steaks packaged in PVC-overwrapped (PVC-OW) and high oxygen modified atmospheric (HiO2-MAP) packages (Packaging, $P = 0.09$). b. Means of Δ in free thiols of steaks during retail display (Day, $P = 0.0002$). [a-b] Comparison among retail display d, means lacking a common superscript were significantly different at $P < 0.05$.

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