5:3 Effect of vacuum aging and display on lipid oxidation of subcutaneous fat and three layers of beef longissimus

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Introduction

The primary mode of fresh beef distribution has changed from shipment of carcasses to that of vacuum packaged primal and subprimal cuts. Minks and Stringer (1972) found that vacuum aged steaks were slightly more desirable in palatability characteristics than those non-vacuum aged. Seideman et al. (1976) noted that degree of vacuum in primal cut storage had no effect on later surface discoloration and palatability characteristics of retail steaks.

Allen et al. (1976) suggested that channeling grass fed beef into vacuumized boxed beef sales could improve its display color, tenderness and flavor. Recently, Gutowski (1977) found that 21 days vacuum aging improved taste panel tenderness, juiciness and flavor scores of steaks from grass and short fed cattle but flavor scores were less desirable after 5 days of display. Allen et al. (1976) stated that 5 days display after 21 days vacuum aging reduced acceptability of beef primarily through flavor deterioration. If differences in taste panel characteristics were experienced in vacuum aged beef cuts displayed as long as 5 days, this may be important to the meat industry.

Light accelerates oxidative changes including meat discoloration (Watts, 1954). Heme pigments have been suggested by many to have a role in meat oxidation. Igene et al. (1979) more specifically identified non-heme iron as having an important effect on lipid autoxidation.

This study examined the effects of vacuum and display on muscle and adipose tissue lipid deterioration measured by thiobarbituric acid (TBA) analyses of beef from cattle finished on four nutritional regimens.

Experimental

Two hundred and sixty-six conventionally trimmed 2.5 cm thick. M. longissiumus steaks from 32 cattle finished on four feeding regimens were used in this study. There were eight cattle per feeding regimen and these were:

1. Grass fed; finished on bromegrass and bluestem pasture for 133 days.
2. Short fed; finished 49 days on 80% concentrate 20% roughage (ration 2, Table 1).
3. Long fed; finished 98 days on 80% concentrate 20% roughage (ration 2, Table 1).
4. Forage fed; finished 98 days on 60% roughage 40% concentrate ration (ration 1, Table 1).

One side of each carcass was chilled at 3°C until 48 hours postmortem; the other was conditioned at 13°C until 8 hours postmortem and chilled at 3°C for the balance of the 48 hours. M. longissimus muscle from the wholesale loin from each side was removed and equally divided transversely into 2 halves. Two 2.5 cm steaks were obtained from approximately first and second lumbar vertebral location. One was immediately vacuum packaged and stored at -26°C for further analysis and designated as "fresh pre display". The other, designated as "fresh post display" was allowed to oxygenate for 15 minutes before packaging with polyvinyl chloride (PVC) film and 5 days continuous display at 3°C under 1076 lux (100 foot candles) of Deluxe Warm White light. Lighting consisted of two 40 watt tubes 126 cm from the steak surface. The posterior half of the M. longissimus was vacuum packaged in a Cryovac B barrier bag and aged at 1°C for 21 days. Two 2.5 cm steaks were treated as above and are designated as "vacuum pre display" and "vacuum post display", respectively. Each post display sample was separated into 3 layers of equal thickness, vacuum packaged and freezer (-26°C) stored until analyzed.

Subcutaneous fat of all steaks was separated along the natural seam into outer and inner layers. Samples were individually vacuum packaged and stored at -26°C until TBA analysis.

Thiobarbituric acid analysis.

Steak and subcutaneous fat samples were subjected to thiobarbituric acid analysis (TBA) by a fluorometric procedure developed in our laboratory (Kuntapanit et al., 1978). A modified extraction procedure, similar to that of Witte et al. (1970) but utilizing 7.2% perchloric acid was used. Frozen steaks were partially thawed at 3°C for 8 to 12 hours before cutting into approximately 0.2 to 0.5 cm cubes. Samples of 10 g were weighed into cold Virtis jars (tall type). Fifteen ml of 7.2% perchloric acid was added and the mixture weight brought up to 50 g with distilled water. The mixture was blended at approximately 45,000 rpm (high speed) for 15 seconds. The slurry was filtered through Whatman no. 2 filter paper. Five ml of filtrate was pipetted into 5 ml of cold TBA reagent in a 25 ml test tube. The tube was sealed with paraffin film, mixed and held at room temperature in the dark. After 15 hours, 3 ml of the pink solution was transferred to a culture tube for measurement of fluorescence in a Turner spectrofluorometer using 490 nm for excitation and 550 nm for measuring emission.

Table 1. Ration ingredients and proximate analysis

Ingredient	International reference no.	Ration 1	Ration 2
Corn silage, %	3-02-824	40.0	0.0
Alfalfa haylage, %	3-08-151	20.0	20.0
Cracked corn, %	4-02-932	36.0	75.2
Supplement[a], %		4.0	4.8
	Approximate ration composition, dry matter basis[b]		
Dry matter, %		60.0	81.2
Crude protein, %		13.0	13.0
Metabolizable energy, mcal/kg		2.84	3.11

[a] Soybean meal (ref. no. 5-04-604) supplement plus calcium, phosphorus, vitamin A and chlortetracycline. Block salt and a mixture of 1/3 loose salt, 1/3 limestone and 1/3 dicalcium phosphate were also available (free access).
[b] Nutrient composition based on tabular values (N.R.C., 1963) supplemented with limited proximate analysis.

Subcutaneous fat (outer and inner layers) were treated in a similar manner as muscle samples but 2 g samples were used. Fat samples were hard frozen in liquid nitrogen and pulverized before weighing to prevent further autoxidation and to assure sample blending.

Standard. In order to minimize day to day error, known standard concentrations of 1, 1, 3, 3-tetraethoxypropane (TEP) were used for every set of determinations. The TEP in an acid condition yields malonaldehyde which subsequently condenses with TBA reagent to form the fluorophore. The range of TEP standard was predetermined by a trial of authenic samples and TEP. Excess 0.02 M TBA reagent was added to insure sufficient TBA. Mild heat was used to completely dissolve TBA and TBA reagent was freshly prepared prior to each determination run. Standard curves were fitted and TBA calculated by regression on a programming calculator. Final TBA was expressed as ug per g muscle sample or subcutaneous fat sample.

Statistical Analysis

Data were analyzed by analysis of variance (Snedecor and Cochran, 1967). Mean separation was determined by least significant difference (Cochran and Cox, 1966).

Results and Discussion

Kuntapanit et al. (1978) had reported average recovery of malonaldehyde, added as tetraethoxypropane to meat samples, to average 78% by spectrophotometry and 98% by fluorometry. The fluorometric method was simple, convenient and since no heat was used, artifact malonaldehyde changes should be minimal. Williams et al. (1983) reported results by four TBA procedures, including our fluorometric procedure with differing results from each method and no clear suggestion of a superior method.

Mean TBA numbers of M. longissimus from cattle finished on four feeding regimens as influenced by packaging (fresh and vacuum) at pre and post display are presented in Table 2. At pre display, no difference ($P>.05$) occurred between fresh and vacuum held muscles of grass, long and forage fed groups. In muscle from short fed beef, however, vacuum samples had higher ($P<.05$) TBA value (0.134) than fresh samples (0.070). This indicates more oxidation of intramuscular lipid for cattle fed only 49 days on high concentrate rations. However, all TBA numbers for pre display muscle were very low and should not present a flavor problem since Watts (1962) reported that off-odor become detectable at TBA numbers of approximately 1.0. Although vacuum-held samples were stored 21 days more than fresh samples, the conditions of 1°C and vacuum apparently held down lipid autoxidation.

After 5 days of continuous display, 24 hours/day, at 3°C under 1076 lux (100 foot candle) lighting, muscles previously vacuum-held 21 days at 3°C had a higher ($P<.05$) TBA value (average 0.435) than those cut fresh at 48 hours postmortem (average 0.231). Perhaps lipid autoxidation was slightly more advanced in vacuum-held samples prior to display, i.e. higher initial peroxide formation leads to higher malonadldehyde formation. Perhaps the slight trends toward higher TBA values in those vacuum samples before display accounted for accelerated autoxidation compared to fresh samples when placed under the autoxidation encouraging conditions of display (TBA numbers increased to 2 to 5 times higher than pre display). In vacuum pre display samples, although heme pigments were present in the exudate, light was excluded so autoxidation was minimal. More heme pigment may have solubilized with the 21 days longer holding time so for post display samples, a greater heme pigment catalysis under light display could have accelerated autoxidation rate for vacuum samples. Lundberg (1962) stated that biological catalysts which included hemoglobin, hematin compounds and photochemical pigments acted as accelerators of autoxidation in the presence of light. Higher TBA value at post display was expected due to pro-oxidative conditions of display since Lundberg (1962) found that light in the visible wavelength region can markedly accelerate autoxidation rate in lipid containing materials.

Table 2. TBA number[a] of beef longissimus[b] steaks from cattle finished on 4 nutritional regimens as influenced by packaging[b] at pre and post display[c].

Processing	Nutritional Regimen				
	Grass	Short	Long	Forage	Avg
Pre display					
Fresh	0.046	0.070[e]	0.096	0.102	0.078
Vacuum	0.054	0.134[d]	0.110	0.102	0.100
Post display					
Fresh	0.072[e]	0.272[e]	0.295[e]	0.286[e]	0.231[e]
Vacuum	0.191[d]	0.429[d]	0.593[d]	0.525[d]	0.435[d]

[a] TBA number: ug malonaldehyde/g muscle (av. of 3 layers).
[b] Packaging: Fresh-sample collected after 48 hr chill, or Vacuum-sample taken after additional vacuum aging at 1°C for 21 days.
[c] Display of steaks wrapped in PVC film continuously displayed 5 days at 3°C under 1076 lux (100 foot candles) Deluxe Warm White light.
[de] Means in same column within pre or post display with different superscript letters are different ($P<.05$).

Grass fed product cut after 48 hours at 3°C exhibited no post display difference between the 3 layers in TBA number (Table 3). M. longissimus samples from grass fed beef had a mean % ether extract of 0.92 which contained 8.82% polyunsaturated fatty acids. In the relatively short time of pre display and display, the low fat content of grass fed muscle resulted in very low TBA numbers. The % ether extract was 1.04, 4.27 and 2.31, respectively, for longissimus from short, long, and forage-fed cattle and the % polyunsaturated of total lipid was 2.21, 2.51 and 1.40. However, both long and forage fed groups showed lowest post display values for the middle layer and highest TBA numbers for the bottom layer. The combined effect of light reflected from the bottom of the display case and of catalysis by heme pigments or non-heme iron in the likely greater amount of exudate in the bottom layers was greater than that of light alone on the top layer. Igene et al. (1979) suggested that heme pigments catalyze autoxidation although follow-up studies indicated non-heme iron to be mainly responsible. Muscles that were vacuum-held prior to display exhibited an order where the top layer was highest ($P<.05$) in TBA number followed by the bottom layer ($P<.05$) for every nutritional regimen. Watts (1954) concluded that light accelerates all oxidative changes in meats. The steak surface directly exposed to display light exhibited greatest autoxidation as evidenced by highest TBA number. The bottom layer, indirectly exposed to display light and coupled with non-heme iron and heme pigments in exudate, had undergone

Table 3. Post-display[a] TBA number[b] of beef longissimus steaks from cattle finished on 4 nutritional regimens as influenced by packaging[c].

Processing	Steak layer	Nutritional Regimen Grass	Short	Long	Forage	Avg
Post display						
Fresh	Top	0.094	0.332^{d}	0.280^{e}	0.291^{e}	0.250^{d}
	Middle	0.060	0.153^{e}	0.180^{f}	0.224^{f}	0.154^{e}
	Bottom	0.064	0.332^{d}	0.427^{d}	0.342^{d}	0.291^{d}
Vacuum	Top	0.255^{d}	0.544^{d}	0.746^{d}	0.663^{d}	0.552^{d}
	Middle	0.124^{e}	0.281^{f}	0.334^{e}	0.320^{e}	0.265^{e}
	Bottom	0.194^{e}	0.461^{e}	0.699^{e}	0.591^{e}	0.486^{e}

[a]Display in PVC film continuously for 5 days at 3°C under 1076 lux (100 foot candles) Deluxe Warm White light.
[b]TBA number: ug malonadlehyde/g muscle.
[c]Packaging: Fresh-sample collected after 48 hr chill, or Vacuum-sample taken after additional vacuum aging at 1°C for 21 days.
[def]Means in same column within either Fresh or Vacuum with same or no superscript letters are not different (P>.05).

a lesser but still pronounced autoxidative change. The middle layer had the lowest TBA number since it was not exposed to display light, thus autoxidation was minimal. The layer effect should be even more pronounced if a very thin layer of muscle from the top and bottom surfaces had been sampled. Our 8 mm thick samples from the top and bottom steak surface contained lipids in varying stages of autoxidation and our determinations are an average. The differences among steak layer TBA numbers are important from the research sampling standpoint. Since wide variation in autoxidation among layers of displayed steaks was well illustrated, one should take precautions in sampling for muscle food research involving autoxidation and display. However, our 5 days continuous display is a severe treatment and one not often encountered commercially in the U.S.A.

Mean TBA numbers of beef subcutaneous fat over longissimus steaks cut fresh versus those cut after 21 days vacuum aging are presented in Table 4. Before display, vacuum samples showed higher (P<.05) TBA numbers for every nutritional regimen and for their averages (0.153 and 0.460 for fresh and vacuum samples, respectively). TBA numbers of vacuum-aged fat samples were approximately 2 to 4 times greater than for fresh samples, i.e., TBA value was 0.134 for fresh and 0.581 for vacuum in short fed group. Post display samples exhibited a similar pattern but at higher magnitude with average TBA number of vacuum-held samples approximately 2 to 5 times greater than fresh samples. Schupp et al. (1976) stated that higher TBA number for subcutaneous fat did not explain the flavor differences among forage and grain fed cattle, but steaks in that study were not exposed to the severe conditions of our study, i.e. the extended display.

Table 4. TBA number[a] of subcutaneous fat over beef longissimus steaks from cattle finished on 4 nutritional regimens as influenced by packaging[b] and display[c].

Display and packaging	Nutritional Regimen Grass	Short	Long	Forage	Avg
Pre display					
Fresh	0.086^{e}	0.134^{e}	0.182^{e}	0.209^{e}	0.153^{e}
Vacuum	0.194^{d}	0.581^{d}	0.560^{d}	0.504^{d}	0.460^{d}
Post display					
Fresh	0.200^{e}	0.241^{e}	0.203^{e}	0.245^{e}	0.222^{e}
Vacuum	0.364^{d}	1.240^{d}	0.752^{d}	0.760^{d}	0.779^{d}

[a]TBA number: ug malonaldehyde per g.
[b]Packaging: Fresh-sample collected after 48 hr chill, or Vacuum-sample taken after additional vacuum aging at 1°C for 21 days.
[c]Display in PVC film continously for 5 days at 3°C under 1076 lux (100 foot candles) Deluxe White light.
[de]Means in same column within pre or post display with different superscript letters are different (P<.05).

Table 5 presents TBA of subcutaneous fat layers (outer and inner) over the longissimus after continuous display for 5 days. No difference (P>.05) was found between layers for both fresh and vacuum samples except averages for vacuum aged samples where inner layer TBA value (0.662) was higher (P<.05) than outer layer (0.576) for pooled means from four feeding regimens. Numerous individual samples of subcutaneous samples showed TBA numbers close to or above reported threshold numbers 1 to 1.5. Thus, depending on proportion of fat consumed from a steak, the subcutaneous fat could contribute to a flavor problem.

Table 5. TBA number[a] of subcutaneous fat over beef longissimus steaks from cattle finished on 4 nutritional regimens at post display[b] as influenced by steak packaging[c].

Packaging	Nutritional Regimen Grass	Short	Long	Forage	Avg
Fresh					
layer					
outer	0.143	0.173	0.191	0.230	0.185
inner	0.143	0.202	0.194	0.224	0.191
Vacuum					
outer	0.264	0.818	0.620	0.603	0.576^{e}
inner	0.293	1.002	0.691	0.661	0.662^{d}

[a]TBA number: ug malonaldehyde/g.
[b]Display in PVC film continously for 5 days at 3°C under 1076 lux (100 foot candles) Deluxe Warm White light.
[c]Packaging either Fresh with sample collected after 48 hr chill, or Vacuum-sample taken after additional vacuum aging at 1°C for 21 days.
[de]Means in same column within either Fresh or Vacuum with different superscript letters are different (P<.05).

In summary, vacuum aging of beef cuts contributed to a higher TBA number of intramuscular and subcutaneous lipids of post display beef samples, with display in oxygen permeable film. Average TBA numbers suggest no flavor or odor problems, but localized areas of oxidation could present these problems.

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