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Evaluating Ground Beef Formulated with Different Fat Sources[1]

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Abstract: Objectives were to evaluate effects of fat source and formulated fat percentage on fatty acid composition, lean color stability, lipid oxidation, and aerobic microbial load during simulated retail display of ground beef patties. In Experiment 1 beef carcasses ($n = 30$) were chilled for 2 d and then fabricated. *M. semimembranosus* muscles were removed along with 2 fat sources (kidney and pelvic = KP and subcutaneous = S from the same carcass) and ground to achieve 75 and 95% lean. Fatty acid profile and thiobarbituric acid reactive substances (TBARS) were determined over a 7 d simulated retail display period. Saturated fat differed ($P = 0.0004$) by fat source, with KP having a higher percentage than S. Thiobarbituric acid reactive substances were higher for ($P < 0.05$) for patties made with S compared to KP. In Experiment 2 beef carcasses ($n = 20$), were fabricated and blended into ground beef as described in Experiment 1. After designated display time patties were removed and instrumental color measurement, myoglobin concentration, TBARS, and aerobic plate counts (APC) were collected. Oxymyoglobin (OMb) percentage decreased ($P < 0.0001$) by storage day and had source x formulated fat percentage interaction ($P = 0.011$). Inversely, storage day increased metmyoglobin (MMb) percentage ($P < 0.0001$) where d 1 < d 3 < d 5 < d 7, respectively. Changes in myoglobin form contributed to decreased a* values ($P < 0.0001$) over time. However, APC did not differ ($P > 0.05$) for d, fat source, or fat percentage. Discoloration in ground beef over 7 d of retail display was more a function of muscle pigment oxidation (OMb to MMb) than aerobic microbial spoilage.

Keywords: beef, color, myoglobin, shelf life
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Introduction

Consumer purchasing intent is driven by color (Lynch et al., 1986) and flavor is an important driver in beef palatability (Neely et al., 1998). Lipid oxidation can lead to the conversion of oxymyoglobin (OMb) to metmyoglobin (MMb), which produces off-colors and off-odors typically related to spoiled meat (Martin et al., 2013; Faustman and Cassens, 1990). Turk and Smith (2009) and Kerth et al. (2015) found that various fat depots in a beef carcass have different fatty acid profiles, thus resulting in different lipid oxidation potentials. Changing or mixing different types of fat from the same animal could alter shelf life in retail ground beef products. Moreover, some commercial beef processors are removing the kidney, pelvic and heart fat (KPH) on the slaughter floor. This fat is presumably destined for rendering at a reduced value compared with other carcass fat depots (Boykin et al., 2017). The average KPH in U.S. finished beef is 1.9% with a mean hot carcass weight of 390.3 kg resulting in 7.41 kg of KPH per carcass (Boykin et al., 2017). Therefore, 7.41 kg of carcass weight might have added value if KPH fat is blended into ground beef versus the rendering value.

Multiple studies have shown that microbial growth is an important factor in controlling the spoilage of meat (Lavieri and Williams, 2014; Martin et al., 2013; Brooks et al., 2008). Shortened shelf-life

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can be exacerbated by spoilage microbes present in meat products that tend to increase and interact with other oxidation products over time during refrigerated storage. Lavieri and Williams (2014) reported aerobic plate counts (APC) increased with storage time up to 25 d at 1°C regardless of treatment. Therefore, our hypothesis is that discoloration in ground beef over time is due to a combination of lipid oxidation and aerobic microbial spoilage. Consumers have long associated undesirable color changes in meat with spoilage. The objective of this research was to determine the effect of fat source and formulated fat percentage on shelf life, defined as discoloration and lipid oxidation, of ground beef out to 7 d of simulated retail storage

Materials and Methods

Experiment 1

Sample selection and preparation. Crossbred beef steers (n = 30) finished on a corn silage ration were slaughtered in groups of 6 cattle per day at the University of Missouri red meats abattoir under USDA/FSIS inspection. Carcasses were chilled at 1 to 2°C for 48 h followed by fabrication where *M. semimembranosus* muscles (IMPS # 168; USDA, 2014) from one side of the carcass, also commonly referred to as top or inside round, were removed and closely trimmed to no visible external fat (IMPS #169A, USDA, 2014). Within each carcass, 2 sources of fat (kidney and pelvic from the carcass = KP, subcutaneous from the top round = S) were sourced to blend with the top round to achieve either 75 or 95% lean ground beef. Trim and fat blocks were individually ground through a coarse 10 mm plate (#8 Meat Grinder.35 HP, LEM Products, West Chester, Ohio). Final meat blocks (862 g lean and 45 g of fat) for 95% lean product and (680 g of meat and 227 g of fat) for 75% lean product were blended by hand and finely ground through a 4.5 mm plate. The 907 g of product from each treatment within animal was then used to create four, 115 g patties at each fat percentage (n = 60) to be used for the simulated retail display study. Each patty was formed using a patty press (Non-stick adjustable burger press, Model # 934, LEM Products, West Chester, OH). Patties were placed on 13.5 × 13.5 cm Styrofoam trays and overwrapped with oxygen permeable, polyvinyl chloride (UltraWrap Stretch Product #7021860 PVC #3, Anchor Packaging, St. Louis, MO; 15.500 to 16.275 cm^3/m^2 per 24 h oxygen transmission rate at 23°C) and placed in simulated refrigerated retail storage (4°C; fluorescent bulbs; 48T21CWHO, Slyvania, Wilmington, MA; with a color temperature of 4200 K and a CRI of 60 for 24 h/d) where lipid oxidation was measured on d 1, 3, 5, and 7 of the study. The additional 447 g of sample was placed in a 11.4 × 22.9 cm Whirl-pak bag (Whirl-pak # S-16552, Nasco, Fort Atkinson, WI), stored at 4°C, and used for fat and moisture determination and fatty acid analysis. All laboratory analyses were conducted on fresh, never frozen ground beef samples to simulate the majority of ground beef sold at retail.

Fat and moisture determination. Extractable fat and moisture determination was performed on the day the meat was ground on each source and fat percentage combination (6 samples per animal) according to Keeton et al. (2003) using a CEM Moisture/Solids Analyzer and Smart Trac Rapid Fat Analysis system (CEM Corp., Matthews, NC). Briefly, 2 CEM square sample pads were placed into the moisture/solids analyzer, dried, and tared. After taring, 3.5 to 4.5 g of ground beef sample was then smeared across one of the pads. The second pad was placed on top of the sample, sandwiching the ground beef sample between the two sample pads, and moisture percentage was determined by weight using the CEM moisture/solids analyzer. Once the moisture analysis was completed, the dried sample and pads were rolled in Trac paper, placed into a CEM Trac tube, and packed tightly at the bottom. The tube was then placed in the CEM rapid fat analyzer where fat percentage was determined on dry basis using nuclear magnetic resonance and converted to wet basis. Each sample was run in triplicate and averaged for statistical analysis.

Fatty acid analysis. Methodology utilized for fatty acid quantification was an adaptation of the methods used by Folch et al. (1957) and Morrison and Smith (1964). All reagents were purchased from Fisher Scientific (Pittsburgh, PA). One g of sample was homogenized in a chloroform:methanol solution (CHCL3:CH3OH, 2:1, v/v) to extract the lipids. The sample was then filtered through a sintered glass filter funnel fitted with a Whatman 2.4 cm GF/C filter and 8 mL a solution of 0.74% KCl was added to the tube. The sample was allowed to sit for 2 h to separate the phases and then the upper phase was removed and discarded. The lower phase was then evaporated to dryness with nitrogen gas in a heated water bath at 70°C using a Meyer N-Evap Analytical Evaporator (Organomation Associates Inc., Berlin, MA). One mL of 0.5 N KOH in CH_3OH was added to the sample and the tube was placed in a water bath at 70°C for 10 min. Then, 1 mL of 14% boron trifluoride (BF3) in CH_3OH was added to the tube, flushed with nitrogen, loosely capped and

placed in a water bath at 70°C for 30 min. After 30 min, the sample was cooled to room temperature and 2 mL of HPLC grade hexane and 2 mL of saturated NaCl was added. Next, the upper layer was removed and placed in a glass tube with approximately 800 mg of Na_2SO_4 to remove moisture from the sample. Following this, 2 mL of hexane was added to the tube with saturated NaCl and once more, the upper layer was removed and placed in the same tube with Na_2SO_4. The liquid portion was then transferred to a scintillation vial which was placed in a water bath at 70°C and the sample was evaporated with nitrogen. A Varian 420 gas chromatograph (Varian, Pala Alto, CA) was used to analyze fatty acid methyl esters; samples were injected onto a fused silica capillary column (SP– 2560; 100 m × 0.25 mm × 0.2 µm film thickness; Supelco, Bellefonte, PA). The temperature of the injector and of the flame-ionization detector was held constant at 240 and 260°C, respectively. Helium was used as the carrier gas at a constant pressure of 37 psi and the oven was operated at 140°C for 5 min (temperature programmed 2.5°C/min to 240°C and held for 16 min). Fatty acids were normalized meaning that the area of each peak was represented as a percentage of the total area. Iodine value (IV) was determined based on the equation described by AOCS (2017) Cd 1c-85 (2017): IV = (0.95 × C16:1) + (0.86 × C18:1n9) + (1.732 × C18:2n6) + (2.616 × C18:3n3) + (0.785 × C20:1). Iodine value is a measurement to estimate the amount of unsaturation of fatty acids found in carcass fat (DeRouchey et al., 2011). An internal standard fatty acid methyl ester was used and all fatty acid values are expressed as the percentage of fatty acids detected.

Lipid oxidation determination. Lipid oxidation products were determined using the thiobarbituric acid reactive substances (TBARS) extraction method described by Pegg (2001). One of the 4 patties from each fat source and fat percentage combination of each animal was removed on d 1, 3, 5, and 7 of the shelf-life study to be used immediately (fresh) for lipid oxidation products determination. All reagents were purchased from Fisher Scientific (Pittsburgh, PA). Briefly, 5 g of each ground beef sample was weighed out and placed in a sample cup. 2.5 mL of an antioxidant solution, 50 mL of ice-cold TCA reagent, and 50 mL of distilled water was added to the sample in the cup and then homogenized for 3 min using a 2-speed Handheld Blender (Hamilton-Beach Model # 59760, Southern Pines, NC). The sample was then filtered and a 5-mL aliquot of sample solution was added along with 5 mL of thiobarbituric acid to a 50 mL conical centrifuge tube. The tubes were capped, vortexed, and heated in a boiling water bath for 35 min. The tubes were removed after 35 min from the water bath (Isotemp 110, Fisher Scientific), chilled in ice for 5 min to stop the reaction, and then the sample was transferred to an acrylic cuvette with a visible spectral range of 340 to 750 nm. Absorbance was measured at 532 nm using a spectrophotometer (Thermo spectronic Genesys 20 4001/4, Fisher Scientific). The TBARS values were obtained from the absorbance as described by Pegg (2001) and showed mg of malonaldehyde/kg of sample. Each sample was ran in duplicate and averaged for statistical analysis.

Statistical analysis. Statistical Analysis for proximate analysis and fatty acid profiles was performed using the MIXED procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) to obtain least square means and standard error estimates. The model included the fixed effects of fat source (KP and S) and fat percentage (5 and 25%) and all possible interactions. Objective color and TBARS were analyzed using the MIXED procedure of SAS and the model included the fixed effects of fat source, fat percentage, day (1, 3, 5, or 7), and all possible interactions. Significance was determined at $P < 0.05$.

Experiment 2

Sample selection and preparation. Crossbred beef steers (n = 20) finished on a corn silage ration were slaughtered in groups of four cattle per day at the University of Missouri red meats abattoir under USDA/FSIS inspection. Carcasses were chilled at 1 to 2°C for 48 h followed by fabrication where *M. semimembranosus* muscles (IMPS #168, USDA, 2014) were removed and closely trimmed to no visible external fat (IMPS #169A, USDA, 2014). Within each carcass, 2 sources of fat, KP and S, were sourced to and processed and stored as described in Experiment 1 with the end result being final meat blocks of 95% lean product (969.6 g of meat and 51.0 g of fat) and 75% lean product (765.4 g of meat and 255.2 g of fat). The 1,020.6 g of product from each treatment within animal was then used to create eight, 113.4 g patties to be used for the shelf life study where samples were evaluated on d 1, 3, 5, and 7 to collect an instrumental measurement of color (L*, a*, b*), calculated myoglobin concentration, trained sensory panel, and TBARS. Aerobic plate counts were performed on the d 1 and 7 patties. The additional 113.4 g of sample was placed in a Whirl-pak bag (Whirl-pak # S-16552, Nasco), stored at 2°C, and used for fat and moisture determination. All laboratory analyses were conducted on fresh ground beef samples to simulate the majority of ground beef sold at retail.

Fat and moisture percentage determination. Fat and moisture percentage determination was performed in triplicate as described in Experiment 1.

Objective color determination. Surface color measurements (L*, a*, b*) of the ground beef patties were acquired utilizing a HunterLab MiniScan model 4500L (Hunter Associates Laboratory, Reston, VA) with a D65 light source, 1.27 cm aperture, geometry 45°/0° and physical standard was used to calibrate the MiniScan each day. Color coordinates were recorded on each sample's specific day of removal from simulated retail display. On the first day of simulated retail display, all of the d 1 patties were measured prior to removal from the case for further analysis. This was continued on d 3, 5, and 7 of the study. Samples were evaluated in triplicate and averaged to achieve a more accurate representation of each ground beef patty.

Myoglobin concentration determination. Myoglobin concentrations of the ground beef samples were calculated via selected wavelengths described in the Meat Color Measurements Guidelines (American Meat Science Association; 2012). The reflectance was measured at the isobestic wavelengths 470, 530, 570, and 700 nm using HunterLab MiniScan model 45/0 LAV (Hunter Associates Laboratory, VA) in triplicate on d 1, 3, 5, and 7 of the study. The reflectance (*R*) was converted to reflex attenuance (A) using Eq. [1]. The A-values were then inserted into Eq. [2] to calculate MMb and into Eq. [3] to calculate deoxymyoglobin (DMb). Oxymyoglobin was then calculated using Eq. [4]:

$$A = \log(1/R) \quad [1]$$

$$\%MMb = \{1.395 - [(A570 - A700) / (A530-- A700)]\} \times 100 \quad [2]$$

$$\%DMb = \{2.375x - [1 - (A470 - A700) / (A525 - A700)]\} \times 100 \quad [3]$$

$$\%OMb = 100 - (\%MMb + \%DMb) \quad [4]$$

Hue angle (HA), saturation index (SI), and a*/b* ratios were determined according to American Meat Science Association (2012). HA = [arctangent (b*/a*)] with larger values indicating less red, more MMb, and a more well-done cooked color (American Meat Science Association, 2012). Hue angle is very useful to indicate shifts in color over time toward discoloration. $SI = (a^{*2} + b^{*2})^{1/2}$ with larger values indicating more saturation of the principal hue of the sample. Saturation index is very useful to indicate intensity of the hue is the product. Larger ratios of a*/b* indicate more redness and less discoloration (American Meat Science Association, 2012).

Trained sensory panel. A total of 15 trained panelists participated in the ground beef sensory panel to determine odor and visual characteristics throughout shelf life of ground beef patties by methods described by Rhee et al. (1997) and Ohman et al. (2015). On d 1, 3, 5, and 7 of retail display, 6 to 8 panelists were asked to evaluate 16 patties. Patties were placed in a 15.24 cm diameter glass Petri dish and covered with a plastic watch glass. Each panelist evaluated all patties on retail display day they were present for. The patties were allowed to sit at room temperature (21°C) for 30 min, to develop and trap odor volatiles. To evaluate odor volatiles, panelist briefly lifted the watch glass and sniffed the patties. Off-odors and the intensity of the odor was immediately recorded, as described by Rhee et al. (1997). Putrid, sour, and fruity/sweet were the off-odor descriptors used on an eight point intensity scale (0 = no off-odor and 7 = extreme off-odor). Panelists completed a training session prior to the first evaluation that provided examples of odors and intensity levels. Strawberry yogurt was used as a reference for fruity/sweet odor and buttermilk was used to describe the sour off-odor. Odor intensity was described to panelists with the use of eight vials of increasing concentration of vanilla to distilled water (0 = 0% vanilla, 100% distilled water, 7 = 100% vanilla, 0% distilled water; Ohman et al., 2015). Odor and intensity references were provided at each evaluation. After odor analysis, watch glasses were removed and the patties were placed under a MacBeth light apparatus (Model EBX-22; 60W Incandescent bulb; Kollmorgan Corporation, Newburgh, NY) for evaluation of lean color and percent discoloration. Panelists were provided with references for lean color and percent discoloration at each evaluation. Lean color was determined using a scale as described by Montgomery et al. (2003), where 1 = dark brownish– greenish gray, 2 = light brownish– greenish gray, 3 = light gray, 4 = moderately dark red, 5 = slightly dark red, 6 = cherry red, 7 = moderately light cherry red, 8 = very light cherry red. Percent discoloration was determined on an 8 point scale (0 = no discoloration, 7 = complete discoloration). Procedures were approved by the University of Missouri Institutional Review Board.

Lipid oxidation determination. Lipid oxidation products were determined using the TBARS extraction method in duplicate as described in Experiment 1.

Aerobic plate count. Samples from each treatment were processed APC on d 1 and d 7. Duplicate 25 g portions of ground beef were weighed from 2 patties from each treatment for a total of four replications per treatment. Each 25-g sample was placed in separate sterile stomacher bag (Whirl-pak, Nasco) and homogenized in 225 mL of sterile 0.1% peptone water (BD Difco, Fisher Scientific) for 2 min (Seward Stomacher Model # 400C, Fisher Scientific, Pittsburgh, PA). Samples were serially diluted in sterile 0.1% peptone water at dilutions of 10^{-1} to 10^{-4} and 1 mL of each dilution was pour plated in approximately 20 mL of plate count agar. Plates were incubated at 5°C for 7 d. After the incubation period, colonies were manually counted, recorded, averaged and expressed as colony forming unit (CFU)/g. The minimum detection limit for determining APC was 10 CFU/g or 1 colony at 10^{-1}.

Statistical analysis. Statistical analysis for fat and moisture determination was performed using the MIXED procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) to obtain LS means and SE estimates. The model included the fixed effects of fat source (KP and S) and fat percentage (5 and 25%) and all possible interactions. Furthermore, statistical analysis for TBARS values, myoglobin percentages, color scores and sensory panel scores was performed using the repeated measures option in the MIXED procedure of SAS and the model included the fixed effects of fat source, fat percentage, d (1, 3, 5, or 7), and all possible interactions. Significance was determined at $P < 0.05$.

Results

Experiment 1

Ground beef made with S fat had a greater percentage of fat, monounsaturated fatty acids (MUFA), greater IV and TBARS values than ground beef made with KP fat ($P < 0.05$, Table 1). However, ground beef made with KP fat had a greater percentage ($P < 0.05$) of saturated fatty acids (SFA) and an elevated ($P < 0.05$) SFA to polyunsaturated fatty acid (PUFA) ratio. There were no differences due to fat source ($P > 0.05$) for percent moisture, PUFA, n-3, or n-6 fatty acids. Fat source also did not affect ($P > 0.05$) n-3 to n-6 ratio.

Fat and moisture percentages differed ($P < 0.05$) for ground beef formulated to 5 and 25% (Table 2). Ground beef formulated to 5% fat had a greater in percentage ($P < 0.05$) of MUFA, PUFA and n-6 fatty acids; in addition, IV was greater ($P < 0.05$) when compared to ground beef with 25% fat. Whereas ground beef formulated to 25% fat was greater ($P < 0.05$) in SFA percentage and had an elevated ($P < 0.05$) SFA:PUFA ratio compared to ground beef formulated to 5% fat. Fat percentage did not affect ($P > 0.05$) percentage of n-3 fatty acids, n-3 to n-6 ratio, or TBARS values. However, TBARS values increased ($P < 0.05$) from storage d 3 to 7 for all ground beef products (Table 3).

Table 1. Effect of fat source[1] on ground beef fatty acid composition[2] and lipid oxidation

Item	S ($n = 60$)	KP ($n = 60$)	SEM	*P*-value
Fat, %	15.69[a]	14.80[b]	0.28	0.0284
Moisture, %	63.26	63.82	0.23	0.0888
SFA, %	52.17[b]	57.96[a]	1.08	0.0002
MUFA, %	37.59[a]	33.12[b]	1.16	0.0076
PUFA, %	5.50	5.15	0.23	0.2833
SFA/PUFA	10.80[b]	15.52[a]	1.22	0.0074
n-3[3], %	0.32	0.26	0.04	0.2273
n-6[4], %	4.53	4.26	0.22	0.4027
n-3/n-6	0.10	0.10	0.02	0.9337
IV[5]	38.32[a]	33.95[b]	1.03	0.0032
TBARS[6]	0.117[a]	0.107[b]	0.007	0.0101

[a,b]Data within a row lacking a common superscript differ $P < 0.05$.

[1]S = subcutaneous fat; KP = kidney and pelvic fat.

[2]Percent of total fatty acids detected.

[3]Omega-3 fatty acids.

[4]Omega-6 fatty acids.

[5]Calculated iodine value.

[6]Thiobarbituric acid reactive substances expressed as mg malonaldehyde/kg sample; $n = 240$ for each fat type.

Table 2. Effect of formulated fat percentage on ground beef fatty acid composition[1] and lipid oxidation

Item	5% ($n = 60$)	25% ($n = 60$)	SEM	*P*-value
Fat, %	6.89[b]	23.61[a]	0.28	< 0.0001
Moisture, %	69.98[b]	57.10[a]	0.23	< 0.0001
SFA, %	50.70[b]	59.44[a]	1.08	< 0.0001
MUFA, %	38.75[a]	31.97[b]	1.16	< 0.0001
PUFA, %	5.96[a]	4.69[b]	0.23	0.0002
SFA/PUFA	10.02[b]	16.31[a]	1.22	0.0004
n-3[2], %	0.31	0.27	0.04	0.4473
n-6[3], %	5.08[a]	3.71[b]	0.22	< 0.0001
n-3/n-6	0.07	0.12	0.02	0.0600
IV[4]	39.95[a]	32.32[b]	1.03	< 0.0001
TBARS[5]	0.112	0.111	0.007	0.7848

[a,b]Data within a row lacking a common superscript differ $P < 0.05$.

[1]Percent of total fatty acids detected.

[2]Omega-3 fatty acids.

[3]Omega-6 fatty acids.

[4]Calculated iodine value.

[5]Thiobarbituric acid reactive substances expressed as mg malonaldehyde/kg sample; $n = 240$ for each pat percentage.

Table 3. Effect of storage day on ground beef lipid oxidation (n = 480)

Item	1	3	5	7	SEM	P-value
TBARS[1]	0.104[b]	0.087[c]	0.113[b]	0.143[a]	0.008	< .0001

[a–c]Data within a row lacking a common superscript differ $P < 0.05$.

[1]Thiobarbituric acid reactive substances expressed as mg malonaldehyde/kg sample.

Experiment 2

Ground beef made with S had higher ($P < 0.05$) L* values than ground beef made with KP (Table 4). Conversely, ground beef made with KP had higher ($P < 0.05$) b* and saturation index values and a greater percentage of oxymyoglobin. Fat source had no effect ($P > 0.05$) on fat and moisture percentage, TBARS values, a*, a/b, or hue angle values. In addition, fat source did not affect ($P > 0.05$) any of the sensory attributes.

Increasing percent of added fat from 5 to 25% increased ($P < 0.05$) fat percentage and oxymyoglobin percentage; L*, b*, and hue angle values; and discoloration and color score (Table 5). In addition, increasing added fat decreased ($P < 0.05$) percentage moisture, a* value, a/b, and fruity/sweet odor scores. Fat percentage did not affect ($P > 0.05$) TBARS and saturation values or sour and putrid odor scores.

As storage day increased, a*, b*, a/b, and saturation index values decreased ($P < 0.05$; Table 6) but hue angle values increased ($P < 0.05$). In addition, percentages of DMb and OMb decreased ($P < 0.05$) while MMb increased ($P < 0.05$) with increasing storage day. Both fruity/sweet and sour odor scores decreased at d 3 ($P < 0.05$) but it is important to keep in mind that the average scores are at the bottom of the scale used for evaluating odor indicating low levels of odor detection throughout the study. Storage day had no effect on TBARS and L* values or the remaining sensory attributes.

An interaction between fat source and added fat percentage ($P < 0.05$) was reported for percentage MMb and DMb (Table 7). There was a higher ($P < 0.05$) percentage of DMb in lower fat ground round than higher fat products; the interaction is driven by the higher added fat products with the 25% added S fat had greater ($P < 0.05$) DMb than the 25% added KP fat.

APC data revealed very low levels of aerobic bacterial ranged from 10^2 CFU/g on d 1 to 10^3 CFU/g on d 7 of simulated retail display (data not presented in tabular form). Fat source did not affect ($P > 0.05$) APC in this study. Patties made with 25% fat had higher plate counts ($P < 0.05$) compared to patties with 5% fat (2.31 vs. 1.93 Log CFU/g, respectively). In addition, more bacterial growth ($P > 0.05$) was detected at display d 7 compared to d 0 (2.62 vs. 1.61 Log CFU/g, respectively).

Table 4. Effect of fat source[1] on lipid oxidation, color and odor of ground beef

Item	S (n = 160)	KP (n = 160)	SEM	P-value
Fat[2], %	14.7	14.5	0.30	0.6033
Moisture[2], %	63.8	63.6	0.33	0.7677
TBARS[3]	0.113	0.119	0.007	0.3502
	Objective color measurements[4]			
L*	44.82[a]	44.41[b]	0.35	0.0403
a*	16.60	16.72	0.24	0.2276
b*	18.89[b]	19.28[a]	0.14	0.0021
a/b	0.88	0.87	0.01	0.2013
SI	25.27[a]	25.38[b]	0.23	0.0098
HA	48.99	49.20	0.33	0.1899
OMb, %	51.87[b]	52.33[a]	0.21	0.0009
	Sensory evaluation[5]			
Fruity/sweet	0.9	1.0	0.04	0.1696
Sour	0.9	1.0	0.05	0.6707
Putrid	0.3	0.4	0.05	0.6264
Discoloration	0.4	0.4	0.07	0.3978
Color	5.3	5.3	0.09	0.5947

[a,b]Data within a row lacking a common superscript differ $P < 0.05$.

[1]S = subcutaneous fat; KP = kidney and pelvic fat.

[2]n = 40 for each fat type.

[3]Thiobarbituric acid reactive substances expressed as mg malonaldehyde/kg sample.

[4]SI = saturation index; HA = hue angle; OMb = oxymyoglobin.

[5]Fruity/sweet, sour, putrid 0 = no odor and 7 = extreme odor; discoloration 0 = no discoloration and 7 = complete discoloration; color 1 = dark brownish– greenish gray, 2 = light brownish– greenish gray, 3 = light gray, 4 = moderately dark red, 5 = slightly dark red, 6 = cherry red, 7 = moderately light cherry red, 8 = very light cherry red.

Discussion

Fat source

Research has shown that various fat depots in the beef carcass have different fatty acid profiles, thus resulting in different subjectivity to lipid oxidation and shelf life in retail ground beef products (Aldai et al., 2007). Kidney and pelvic fat contained the highest concentrations of SFA, as well as the lowest concentrations of MUFA and the lowest iodine value. All of these factors tend to decrease oxidation rate over time. Lipid oxidation is largely affected by PUFA, because they contain multiple double bonds that possess especially reactive hydrogens that dissociate from the carbon chain at lower energy compared to single bonds. Differences in TBARS values due to fat source were detected ($P < 0.05$) in Experiment 1, but not

Table 5. Effect of formulated fat percentage on lipid oxidation, color and odor of ground beef

Item	5% (n = 160)	25% (n = 160)	SEM	P-value
Fat[1], %	6.5[b]	22.7[a]	0.30	< 0.0001
Moisture[1], %	70.1[a]	57.4[b]	0.33	< 0.0001
TBARS[2]	0.122	0.109	0.007	0.1199
Objective color measurements[3]				
L*	39.37[b]	49.86[a]	0.35	< 0.0001
a*	17.65[a]	15.68[b]	0.24	< 0.0001
b*	18.37[b]	19.90[a]	0.14	< 0.0001
a/b	0.96[a]	0.79[b]	0.01	< 0.0001
SI	25.49	25.36	0.23	0.2599
HA	46.27[b]	51.91[a]	0.33	< 0.0001
OMb, %	49.74[b]	54.46[a]	0.23	< 0.0001
Sensory evaluation[4]				
Fruity/sweet	1.1[a]	0.8[b]	0.04	< 0.0001
Sour	1.0	0.9	0.05	0.5994
Putrid	0.3	0.4	0.05	0.2569
Discoloration	0.2[b]	0.6[a]	0.07	< 0.0001
Color	4.5[b]	6.1[a]	0.09	< 0.0001

[a,b]Data within a row lacking a common superscript differ $P < 0.05$.

[1]n = 40 for each fat percentage.

[2]Thiobarbituric acid reactive substances expressed as mg malonaldehyde/kg sample.

[3]SI = saturation index; HA = hue angle; OMb = oxymyoglobin.

[4]Fruity/sweet, sour, putrid 0 = no odor and 7 = extreme odor; discoloration 0 = no discoloration and 7 = complete discoloration; color 1 = dark brownish– greenish gray, 2 = light brownish– greenish gray, 3 = light gray, 4 = moderately dark red, 5 = slightly dark red, 6 = cherry red, 7 = moderately light cherry red, 8 = very light cherry red.

Experiment 2. The variation in lipid oxidation could also be attributed to the variation in unidentified fatty acid content of the lean sources used in each formulation (Martin et al., 2013). However, because each treatment was compared within the same muscle of the same animal where the muscle was mixed throughout the ground beef there should be little variation due to the lean source. In Experiment 1, fatty acid profiles indicated KP would have less oxidation compared to S because it showed a greater SFA:PUFA ratio and a lower IV. The higher the IV is, the more unsaturated the fat. Due to the fact that unsaturated fatty acids cause fat to be more prone to oxidation and become softer, IV can be used as an indirect indicator of lipid oxidation and carcass fat firmness (DeRouchey et al., 2011). Removing the S fat and replacing it with KP fat in ground beef blends could decrease oxidation, leading to increased shelf life of the product.

Formulated fat percentage

Results also showed that the 2 formulated fat percentage treatments differed ($P < 0.0001$) in moisture percentage, as expected and in agreement with results reported by Cannell et al. (1989), Troutt et al. (1992), and Martin et al. (2013). This is due to the fact that fat contains roughly 20% water while muscle can contain from 70 to 80% water depending on the type (Nurnberg et al., 1998). As fat percentage increases in ground beef a higher percentage of the product contains less water leading to decreased moisture percentage when considering that all components of proximate analysis add to 100%. In disagreement with our findings, Cannell et al. (1989) reported that MUFA increased and SFA decreases ($P < 0.05$) as fat percentage increased from 5 to 25%; these differences could be due to fatty acid quantification method used, that number and amount of unidentified components, or diet fed to the cattle.

Patties with 5% fat, on average, were between moderately dark red and slightly dark red; whereas, patties containing 25% fat were most commonly called cherry red which agrees with Troutt et al. (1992). Troutt et al. (1992) and Martin et al. (2013) both reported higher L* values for ground beef containing higher percentages of fat. Consumer rejection of meat products begins when surface MMb reaches 40% (Greene et al., 1971). No differences in lean color or percent discoloration were found in this study which contradicts previous studies investigating the retail display of ground beef (Jimenez-Villareal et al., 2003; Pietrasik et al., 2016).

Storage day

Our data showed TBARS values fluctuating over the simulated retail display, this has been reported before by McMillin et al. (1991) who reported a decrease in TBARS over a 4 d sampling time. It is well documented that oxidation increases as storage time lengthens (Kerth et al., 2015; Martin et al., 2013; Hoyle Parks et al., 2012). Hoyle Parks et al. (2012) showed a steady increase in TBARS values of fresh ground beef from 0 to 84 h. Greene and Cumuze (1981) reported that oxidized flavor in beef was detected over a range of TBARS values from 0.6 to 2.0 mg malonaldehyde/kg and that the general population would not detect until 2.0 mg malonaldehyde/kg.

Many reviews have suggested the link between lipid oxidation and myoglobin oxidation (Baron and Andersen 2002; Monahan et al., 2005; Faustman et al., 2010; Yin et al., 2011). The products and secondary products derived from lipid oxidation that have been suggested as contributing to myoglobin oxidation include peroxides and aldehydes (Monahan et al., 2005). Yin et al. (2011) pointed specifically to 4-hydroxy-

Table 6. Effect of simulated retail display day on lipid oxidation, color and odor of ground beef

Item	1 (n = 40)	3 (n = 40)	5 (n = 40)	7 (n = 40)	SEM	P-value
TBARS[1]	0.119	0.098	0.127	0.116	0.009	0.0713
			Objective color measurements[2]			
L*	44.72	44.47	44.50	44.077	0.38	0.6565
a*	19.34[a]	16.71[b]	15.66[c]	14.95[d]	0.25	< 0.0001
b*	20.79[a]	19.09[b]	18.58[c]	18.08[d]	0.15	< 0.0001
a/b	0.93[a]	0.88[b]	0.85[c]	0.83[d]	0.01	< 0.0001
SI	28.44[a]	25.41[b]	24.34[c]	23.51[d]	0.25	< 0.0001
HA	47.12[d]	48.85[c]	49.95[b]	50.45[a]	0.35	< 0.0001
MMb, %	35.9[d]	39.4[c]	41.2[b]	42.2[a]	0.32	< 0.0001
DMb, %	9.1[a]	8.0[b]	8.0[b]	7.9[b]	0.29	< 0.0001
OMb, %	55.0[a]	52.6[b]	50.8[c]	50.0[d]	0.21	< 0.0001
			Sensory evaluation[3]			
Fruity/sweet	1.0[a]	1.0[a]	0.8[b]	0.9[ab]	0.05	0.0354
Sour	1.0[a]	1.1[a]	0.9[b]	0.8[b]	0.06	< 0.0001
Putrid	0.4	0.4	0.3	0.3	0.06	0.0768
Discoloration	0.3	0.5	0.4	0.4	0.08	0.1877
Color	5.3	5.2	5.3	5.3	0.1	0.4136

[a–d]Data within a row lacking a common superscript differ $P < 0.05$.

[1]Thiobarbituric acid reactive substances expressed as mg malonaldehyde/kg sample.

[2]SI = saturation index; HA = hue angle; MMb = metmyoglobin; DMb = deoxymyoglobin; OMb = oxymyoglobin.

[3]Fruity/sweet, sour, putrid 0 = no odor and 7 = extreme odor; discoloration 0 = no discoloration and 7 = complete discoloration; color 1 = dark brownish– greenish gray, 2 = light brownish– greenish gray, 3 = light gray, 4 = moderately dark red, 5 = slightly dark red, 6 = cherry red, 7 = moderately light cherry red, 8 = very light cherry red.

2-nonenal causing OMb oxidation in 7 meat producing species. The ability for these lipid oxidation products to cause OMb oxidation has been reported to be pH dependent, and more likely at the ultimate pH of meat than physiological pH (Baron and Andersen, 2002).

We found no difference in L* value due to storage day or sensory panel color score. Similar findings showed no difference ($P > 0.05$) for L* from 0 to 84 h; however, L* did tend to increase ($P = 0.072$) from 24 to 84 h (Hoyle Parks et al., 2012). This means ground beef patties become lighter as days in refrigerated retail display increase. The results from this study could have been affected by the storage conditions; specifically temperature and light intensity.

Redness (a* values) decreased over retail display ($P < 0.0001$), where 7 d < 5 d < 3 d < 1 d. Troutt et al. (1992) reported decreased a* values from d 0 to 3 of retail display. Jimenez-Villarreal et al. (2003) and Raines et al. (2010) also reported that a* values decreased during refrigerated retail display out to 7 and 4 d, respectively.

Day also effected ($P < 0.0001$) b* values where d 1 > d 3 > d 5 > d 7 which agrees with Jimenez-Villarreal et al. (2003) and Pietrasik et al. (2016). Troutt et al. (1992) reported decreases in b* values out to 3 d of display while Raines et al. (2010) reported b* values decreased during refrigerated retail display, out to 4 d.

Metmyoglobin concentrations increased ($P = < 0.0001$) with days on retail display where d 1 < d 3 < d 5 < d 7 which agrees with the findings of Jimenes-Villarreal et al. (2003) who reported an increase in percent discoloration of ground beef patties over a seven day period. There was a proportional drop in DMb and OMb ($P < 0.05$) in this study but no change in discoloration as determined by sensory panel.

Data from this study reports slight changes in fruity/sweet and sour odors over time. However it should be noted the magnitude of the sensory scores is very low. This contradicts other researchers (McMillin et al., 1991; Rhee et al., 1997; Jimenez-Villarreal et al., 2003; Pietrasik et al., 2016) who showed an increase in off-odors over time. Fruity/sweet odor as well as putrid odor are the result of spoilage (Ohman et al., 2015).

Table 7. Effect of fat source[1] and formulated fat percentage on myoglobin state of ground beef

	S		KP			
Item[2]	5 (n = 80)	25 (n = 80)	5 (n = 80)	25 (n = 80)	SEM	P-value
MMb, %	39.7[b]	39.5[b]	39.5[b]	40.0[a]	0.32	< .0001
DMb, %	10.8[a]	6.3[b]	10.5[a]	5.3[c]	0.29	0.0057

[a–c]Data within a row lacking a common superscript differ $P < 0.05$.

[1]S = subcutaneous fat; KP = kidney and pelvic fat.

[2]MMb = metmyogloblin; DMb = deoxymyoglobin.

Ismail et al. (2009) also found volatile compounds associated with spoilage increased over 7 d storage at high and low fat percentage ground beef. The lack of odor throughout the study can be contributed to the storage temperature (2°C) of the patties inhibiting microbial growth. Ayres (1960) shows bacterial numbers of 10^7 and 10^8 CFU/cm^2 can cause a noticeable increase in off-odor. Our microbial data (data not presented in tabular form) supports that there was no sufficient microbial growth to cause the formation of off-odors.

Pietrasik et al. (2016) found increases in APC corresponding with increases in spoilage odor, decreases in a* and b* values corresponding with increases in discoloration, and increased TBARS over 3 d of storage at 4°C in high and low fat ground beef patties. Rogers et al. (2014), using ground beef patties and a combination of 2 and 10°C storage temperatures, reported decreases in red lean color which corresponded with decreases in a* and b* values. In addition, increases with off odors which corresponded to increases in TBARS, and increases in APC were also reported (Rogers et al., 2014) . The low APC numbers from this study indicate that decreased retail display quality, as measured by color and TBARS, was not due to growth of aerobic bacteria.

Conclusion

The use of KP as a fat source in ground beef may decrease lipid oxidation without affecting color because it has higher SFA levels compared to S. Discoloration in ground beef over 7 d of retail display was more a function of muscle pigment oxidation (OMb to MMb) than aerobic microbial spoilage. This research confirmed that desirable color in ground beef decreases over storage day. However, other contradicting results between the two experiments and cited literature were most likely due to low storage temperatures in the simulated retail display.

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